Proceedings of

34th WESTERN POULTRY DISEASE CONFERENCE

plus an

AVIAN PARAMYXOVIRUS WORKSHOP

MARCH 3-6, 1985

COOPERATIVE EXTENSION AND
SCHOOL OF VETERINARY MEDICINE
UNIVERSITY OF CALIFORNIA, DAVIS

SPECIAL ACKNOWLEDGEMENT

The Western Poultry Disease Conference has established a SUSTAINING MEMBERSHIP to recognize substantial, direct financial assistance to the "WPDC Speakers' Travel Fund". The major contributions of sustaining members and the support of organizations that send speakers to the Conference have made for excellent programs. This financial support enables us to defray expenses of outstanding scientists who otherwise could not travel to present papers and to participate in the Conferences. To these contributors, to our speakers, and to our guests, many thanks.

Our distinguished SUSTAINING MEMBERS are listed on the facing page. SPECIAL DONOR contributions (over $500) are particularly noteworthy. To all - many thanks.

The WESTERN POULTRY DISEASE CONFERENCE is grateful for the continuing support of the AMERICAN ASSOCIATION OF AVIAN PATHOLOGISTS through generous travel grants.

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Thanks too, to organizations, some of whom are also sustaining members which sent speakers at no expnse to the Western Poultry Disease Conference.

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11th ANECA Congress - Puerto Vallarta, Mexico

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ANNUAL MEETING and MINUTES OF 34th WESTERN POULTRY DISEASE CONFERENCE

The annual business meeting of the Western Poultry Disease Conference was held in Davis, California, Wednesday afternoon March 5, 1985. The session was called to order by Dr. Gregg Cutler, President and the minutes of the meeting as printed in the proceedings of the 33rd WPDC were approved. A brief report indicated that the Sustaining and Donor member contribution for the current (1985) conference totalled slightly more than ten thousand dollars from forty-three donors and sustaining members. These contributions include the annual support of the American Association of Avian Pathologists in the amount of $1,600 and in part are due to the exceptional interest in the pigeon paramyxovirus workshop and discussions. The desirability of completing the organization of the conference with its constitution and completion of the by-laws was mentioned as was the need for organized planning and more general input from the participating members, especially within the Western region.

The invitation tendered in 1984 to hold the 1986 Western Poultry Disease Conference in conjuction with the 1986 convention of ANECA was again extended by Dr. Mario Padron. After some discussion, questions and answers concerning both the time and place of the proposed joint conference and convention the invitation was heartily and enthusiastically accepted by the group. The convention will be held in Puerto Vallarta in the state of Jalisco in Mexico with registration planned for Tuesday, April 29 and the conferences and presentations for April 30, May 1 and May 2. "Cena Baile" is planned for Friday night May 2. The proposed site of the conference will be the Posada Vallarta Hotel.

The officers and group for 1985-86 as chosen were: Dr. Donald Waldrip, President; Dr. Duncan McMartin, Program Chairman; Dr. Marcus Jensen, (Provo, Utah), Program Chairman-Elect and A.S. Rosenwald, Secretary-Treasurer. After a rousing vote of thanks for the program chairman for 1985, Dr. Don Waldrip the meeting adjourned.

Since that time joint meetings of some of the officers of ANECA together with officers and representatives from WPDC have discussed the 1986 conference which was mentioned above. The planning is moving ahead with the call for papers to go out in the very near future: those interested in presenting material at the 35th WPDC - 11th ANECA meeting should contact either Dr. McMartin or Dr. Padron, who's addresses are given. It seems most feasible that those from the United States, Canada, Europe and Asia send their proposed titles and a brief informal description of the material which is to be presented to Dr. McMartin at the University of California in Davis. Those people in Latin America should submit their titles and the information to Dr. Padron. Presentations will be in either English or Spanish with simultaneous translation from the language used by the speaker to the alternate language for the benefit of the conferees who are not bilingual.

More details will be sent shortly, but this is a summary of the planning to date. Early registration to insure room reservations and advance room payments will probably be necessary. Specifics concerning this will be sent out along with or shortly after the call for papers.

35th WESTERN POULTRY DISEASE CONFERENCE (WPDC)
held jointly with the 11th Convention of
the Asociacion Nacional de Especialistas en Ciencias Avicolas (ANECA)
April 29 - May 2, 1986
Puerto Vallarta, Jalisco, MEXICO

For information or to submit titles, contact:

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1980-1983 CALIFORNIA COMMERCIAL EGG INDUSTRY FLOCK SURVEY, "REARING AND LAY FLOCK LOSSES"

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Introduction

Flock losses, whether due to actual mortality or culling, can have profound effects on flock profitability. All growers and producers expect some losses. The difficulty arises when for some reason a particular flock experiences a loss rate or pattern different from what was expected. Management many times does not know what went wrong or what went right to cause this deviation from his expectations. Further, it is diffucult sometimes for a manager to know if he has a mortality problem.

An industry-wide survey was conducted by the University of California Cooperative Extension aimed at identifying some of the management factors which may influence flock losses as well as many other performance and economic characteristics.

Methods

Beginning in January, 1980 and continuing until 1983 Cooperative Extension conducted a large scale survey of management practices and performance characteristics of the California Egg Industry. The survey reviewed data on pullets from one week of age through their productive life in the lay house. Data collected were body weight, mortality and feed consumption during the grow period (0 - 20 weeks of age); and body weight, mortality, feed consumption, egg production, egg weight, shell thickness and egg specific gravity during the lay period (21 - 100 weeks of age). Mortality, feed consumption and egg production data were taken from Chilson-Type (Chilson Management Systems) records maintained by individual growers and producers. No attempt was made to identify the type or cause of mortality. Therefore, these data include total losses due to culling, cannibalism and disease.

The analysis of mortality data included 71 and 93 individual flock records of a single strain during the rearing and lay phases, respectively. Data were separated by season of hatch, genetic strain and individual producer and statistically analyzed using the analysis of varience and Duncan's Multiple Rance Techniques.

Results - Grow Phase

Neither genetic strain or season of hatch significantly affected flock losses from 0 - 20 weeks of age (Tables 1 and 2).

Table 1

Total Flock Loss To 20 Weeks By Strain

Strain	No.	Losses (% of H.H.)
A	20	6.09 N.S. *
B	17	6.33
C	16	6.12

* N.S. - No statistical difference between means (P>0.05)

Table 2

Total Flock Loss To 20 Weeks By Season Of Hatch

Season	No.	Losses (% of H.H.)
Spring	21	6.30 N.S. *
Summer	12	5.22
Fall	15	5.54
Winter	21	6.31
All	69	5.95

* N.S. - No statistical difference between means (P>0.05)

Table 3 shows that average weekly loss rates during the early grow period (0 - 5 weeks) are higher than those during the latter period (16 - 20 weeks). This was true for the three strains represented.

Table 3

Average Weekly Loss Rates For The 5 Week Periods Of Grow

Weeks	Average Weekly Loss Strains A %	B %	C %
0 - 5	.47a	.56a	.57a
6 - 10	.32ab	.30b	.25b
11 - 15	.24b	.31b	.25b
16 - 20	.20b	.17c	.19b

Means within columns with different letters are statistically different ($P \leq 0.05$)

Excessively higher losses occurring between 0 and 5 weeks of age is not surprising since there may be significant culling due to sexing errors and weak chicks during this time period. By the time pullets are 16 - 20 weeks of age, most culling and stress causing management practices (vaccination and beak trimming) have been accomplished. The major stress factor associated with the 16 - 20 week period is cage density. The 6 - 15 week period is the time when frequent vaccinations occure in addition to the major beak trimming between 10 - 12 weeks of age.

The greatest source of variability in 0 - 20 week flock loss was due to individual growers. Individual grower losses ranged from 4.36 to 9.05 percent of the pullets housed. The average of all flocks surveyed was 5.95 percent.

Results - Lay Phase

Unlike the grow out period, significant strain differences were detected for over-all hen-housed flock losses between 21 and 60 weeks of age (Table 4). Strain also influenced pattern of loss rate during the lay cycle (Table 5).

Table 4

Effect Of Strain On Adult Hen-Housed Losses

Strain	No.	% Loss (21 - 60 Wks) %
A	18	8.89a *
B	29	8.70ab
C	14	7.11b

* Means are statistically different ($P \leq 0.05$)

Table 5

Effect Of Strain On Loss Pattern During Lay Period (21 - 60 Wks)

	Average Weekly Loss (%) Strain A	Strain B	Strain C
Early (21 - 40 wks)	.236a *	.271a	.173a
Late (41 - 60 wks)	.216b	.196b	.196a
Avg.	.240	.233	.185

* Means within columns with different letters are statistically different ($P \leq 0.05$)

Strains A and B both exhibit a higher "early" loss rate pattern, while strain C did not. This difference in pattern could potentially give strain C an economic advantage since these layers have the potential for laying more eggs.

Season of hatch also plays a role in determining loss rates as well as when losses occure (Tables 6, 7, 8 and 9).

Table 6

Effect Of Season Of Hatch On Lay Flock Losses (21 - 60 wks)

Season Of Hatch	Average Weekly Loss %	Total Loss (H.H.)
Spring	.211	7.99b *
Summer	.296	11.01a
Fall	.228	8.66b
Winter	.238	9.02ab

* Different letters indicate significant differences ($P \leq 0.05$)

Table 7

Effect Of Season Of Hatch On Early Lay Cycle Loss Rate (21 - 40) Weeks

Season Of Hatch	Month Of Peak Production	Weekly Loss %
Spring	Oct - Jan	.211b *
Summer	Jan - Apr	.346a
Fall	Apr - Jul	.254b
Winter	Jul - Oct	.262ab

* Different letters indicate significant differences ($P \leq 0.05$)

Table 8

Effect Of Season Of Hatch On Late Lay Cycle
Loss Rate (41 - 60 Weeks)

Season Of Hatch	Month Of Peak Production	Weekly Loss %
Spring	Oct - Jan	.210b *
Summer	Jan - Apr	.246a
Fall	Apr - Jul	.200b
Winter	Jul - Oct	.213b

* Different letters indicate significant differences ($P \leq 0.05$)

Table 9

Effect Of Season Of Hatch On Loss Pattern
During Lay Period (21 - 60 Weeks)

	Average Weekly Loss (%)			
	Spring	Summer	Fall	Winter
Early (21 - 40 wks)	.211a *	.346a *	.254a *	.262a *
Late (41 - 60 wks)	.210a	.246a	.200b	.213b

* Different letters indicate significant differences ($P \leq 0.05$)

Spring and Fall hatched chicks suffered lower lay flock losses overall (Table 6). Early flock losses (21 - 40 weeks) were exaggerated by Summer hatched flocks (Table 7). Our observations indicate a much higher incidence of uterine prolapse in Summer hatched birds which might be explained by the time of year they are coming into production coupled with their higher body weights. Late flock loss (41 - 60 weeks) was also greatest for summer hatched birds (Table 8). This response may be due to the fact that this period occures during the hottest time of the year. Table 9 shows that Spring hatched flock losses were uniformly low while Summer hatch flock losses were uniformly high. Fall and Winter hatched flocks both exhibit a pattern of higher early loss rates.

Table 10

Effect Of Rearing Method On Adult Mortality (21 - 60 Weeks)

Rearing Method	H.H. Loss %
All Floor	10.39 N.S. *
Floor to Cage	9.15
All Cage	9.19

* N.S. - No statistical difference between means ($P > 0.05$)

Comparisons were made between flocks reared entirely on the floor, brooded on the floor and reared in cages, and brooded and reared entirely in cages. The method of rearing did not significantly impact on total adult losses (Table 10).

As was the case in the rearing comparisons, management was the most significant factor in influencing adult flock loss rates. An almost 3 fold difference (6.83 to 19.37 percent) was observed between individual producers. The survey average loss to 60 weeks of age was 9.1 percent per hen-housed which corresponded to an average weekly loss rate of .23 percent.

Summary

This study generally agrees with an earlier study involving 206 flocks reported by Bell in 1975. Althouth in his report differences in loss rates between individual growers and producers were not identified, differences due to genetic strain were evaluated. As was the case with our 1980 study no significant differences in loss rates during the grow-out period were found due to strain; however significant loss patterns in differences were detected during the first production year (21 - 60 weeks of age).

Obviously, there are many factors which can affect flock losses. This study has shown that genetics and season of hatch probably play minor roles compared to management during the gorw period. Presumably factors such as beak trimming, vaccinations, moving, culling, growding, environmental control, nutrition and disease all play a role. Unfortunately, these factors are very difficult to quantitate in a large scale survey such as the one being reported on.

Genetics and season of hatch have been shown to play a more significant role in the lay period. These factors not only affect amounts of losses but just as importantly, they affect when these losses occure. The age at which hens die or stop producing eggs directly impacts on the total numbers of eggs produced and so is of economic importance to the producer. As during the grow period, management plays a very significant role in loss rates during the lay cycle. It is important to remember that management factors such as nutrition, environment, lighting, crowding and disease all work together to create what we refer to as a management effect.

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A NEW APPROACH AND POSSIBILITIES FOR POULTRY HOUSE ENVIRONMENTAL CONTROL

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Introduction

What started out as a project, in 1974, to develop a least-cost approach to broiler growing has many aspects that now appear to allow average to superior performance in existing housing, with dramatic reductions in labor, energy costs, and new housing requirements. While the methods described here are quite different in many respects, the sound principles of poultry husbandry are being adhered to but by radically different methods, which when understood and followed, allow a 75 per cent reduction in labor, 70 to 90 % or more reduction in fuel costs, and generally improved performance in the areas of uniformity, grade, livability, feed conversion, and disease prevention.

The project has, through its eleven year development period, of course, had its stumbling blocks, but now the numbers, understanding, and results are starting to be very encouraging. In retrospect, the problems have generally been in the area of "the positive effort giving the negative effect." Originally, it was anticipated that some decreases in production efficiency would have to be accepted, but now the overall performance appears potentially superior.

The basic problems (opportunity areas) of the project that were considered were labor, energy, and housing costs.

Labor factors that were considered include:

-cost
-availability
-effectiveness
-reliability

A system is needed that takes the hand work out of poultry rearing, expands the effectiveness of as few as possible (preferably one person per farm regardless of size) and is less demanding on hand labor, or put another way, allows labor to be less critical to the performance of the bird. So, special considerations were given to the equipment to be used and/or developed, lay-out of the equipment, and just how much equipment is really necessary.

Energy factors that were considered include:

-Effectiveness of utilization (efficiency).
-Develop a system with absolutely minimum consumption requirements.
-Fully develop or utilize resident or free energy available to its greatest capability.

A system is needed that is simple to manage, reliable, and totally effective in maintaining an appropriate temperature in a dry (dust free) environment with the least possible utilization of purchased energy, ie; make bird, litter, and sun heat do the majority of the heating, and through minimum inputs and ventilation to realize the necessary environmental conditions-warm, even, draft-free, dry environmental conditions.

Housing factors to be considered:

-What is necessary and unnecessary.
-What is effective and ineffective.
-Cost-benefit tradeoffs.
-Be realistic and practical.

Many times, environmental conditions in the curtain sidewall poultry house are "perfect" without the slightest effort on the part of the grower. The goal was to look at the practical least-cost situation and develop it to its fullest potential, utilizing the simplest methods and determining what was really necessary and what was not necessary.

Results

A new patented fresh air-tempered air machine (Enviro-opti-mizer TM) and system for environmental (climate) control of the poultry house which causes improved control and conditions (lowered ammonia, moisture, and odor virtually eliminated) is described. Energy consumption is reduced by 60-70% or better as compared to conventional methods.

On a regular and specified basis a series of fans destratify the house air and initiate a horizontal or laminar circulation. The machine then introduces fresh or fresh-tempered (newer warm or hot air) into the house circulation causing the expulsion of excess heat and moisture at the end or ends of the house away from the machine. Pulsing occurs 24 hours a day.

In addition, a new method of brooding was developed, which virtually eliminates labor, and allows a good start in the smallest possible area so as to allow the bird heat to be utilized at the earliest possible age with the system in lieu of brooder stoves, etc. With this brooding method conventional heat sources can be off by 2-10 days depending on outside temperature with improved liveability, uniformity and house conditions. The drug selection and level of the feed is critical, however as fully 30% more feed and water can be consumed in the first week as compared to conventional brooding.

For the first time bird heat can be shown to be effective and utilized from an early age to market because of the ability of the machine and system to heat and dry in the winter, and cool and dry in the summer. The environment is improved though virtual elimination of ammonia (less the 5ppm) and moisture in the litter (including cake), humidity in the air, and an even uniform desired temperature can be maintained throughout the house. Outside variations in temperature of 20° generally cause only 3-4° change inside.

In addition to reduced fuel consumption and improved (healthy) environmental conditions all phases of performance are generally improved, labor is reduced (one man can care for up to 250,000 birds without any assistance whatsoever.) Net income for the farmer has been shown to be improved by 20-30%

Litter E coli counts of as few as 2 colonies per gram of litter are documented and large numbers of birds have been grown with no coccidostat (live oocysts cannot be found in the litter). The system has the potential of providing a natural "Microbiological Handle" on the space under control.

The machine and method of environmental control has further applications in other areas (greenhouses, large buildings, i.e. warehouses, factories, etc.) as an effective large space environmental control at greatly reduced energy, installation cost, and results.

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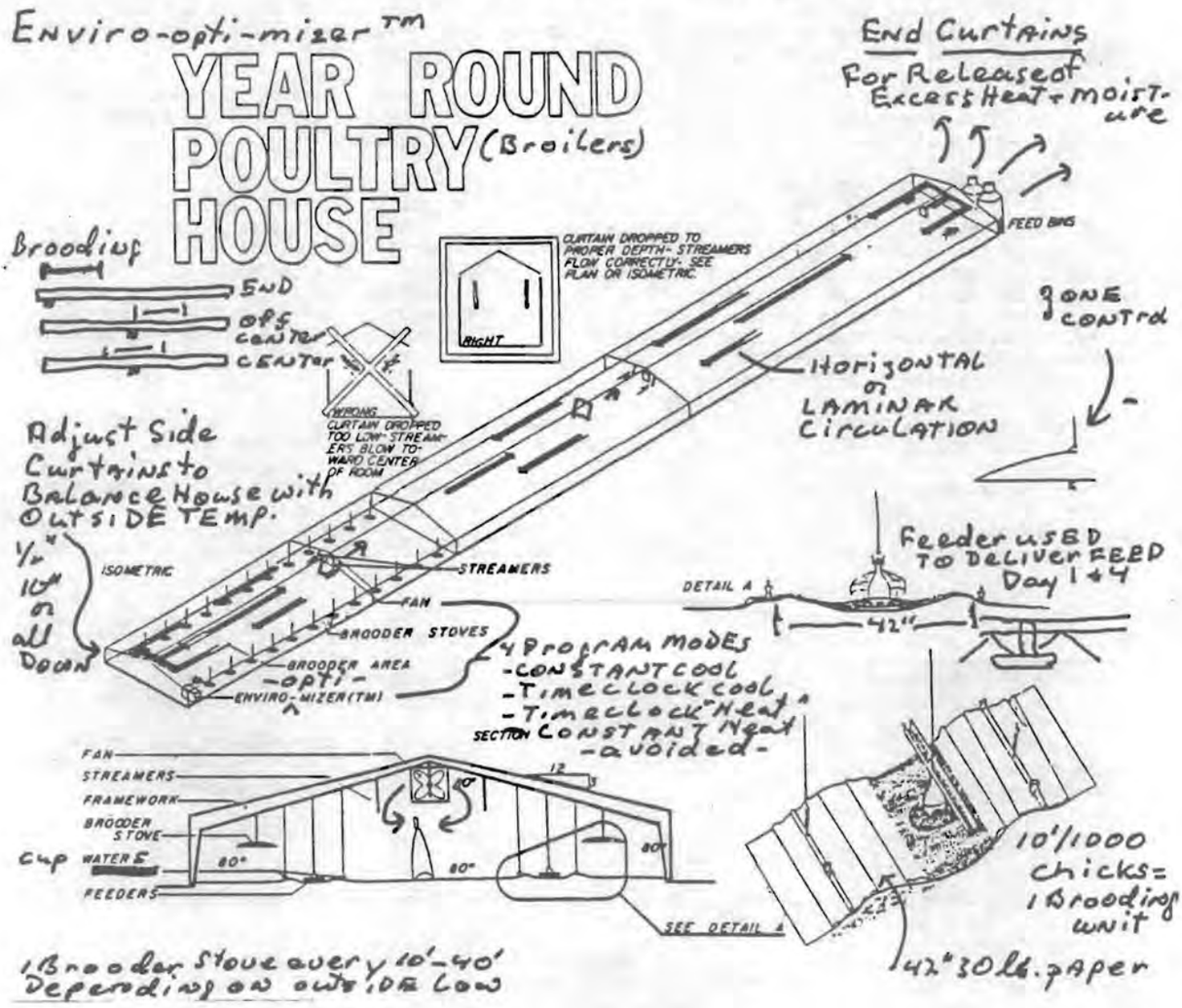

VISCERAL GOUT - AN INTERESTING ODDITY?

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Visceral gout was the subject of one of our Service Tips in 1976. We ended the title with a question mark to indicate we questioned whether this in fact was an oddity or did it actually occur more frequently than anyone realized.

More recently people, such as Dr. Mallinson who gave the condition the somewhat more sophisticated name of urolithiasis, and Dr. Halvorson who reproduced it with excess calcium in the growing period, have at least focused some attention on what can be an annoying and frustrating problem.

There is still very little information about this condition which has been called acute toxic nephritis, uremic poisoning, nutritional gout and uremia or uremia poisoning and now urolithiasis. Historically gout has appeared as an individual problem in a few birds and less frequently as a fairly general flock problem. Recently more general flock problems have been reported. This is not one of the more rewarding conditions to deal with to say the least. Any condition involving the kidneys in any species presents not only diagnostic challenges, but also therapeutic challenges as you well know.

The list of factors producing kidney lesions is quite long, including infectious bursal disease, infectious bronchitis, avian nephritis virus and probably other viruses. Mycotoxins may logically be involved as well as vitamin excesses or deficiencies. Calcium, sodium, bicarbonate and potassium may be involved. Protein in excess as well as simple water deprivation could also lead to kidney damage. Bacterial infections can also be involved.

Needless to say, we have a complex etiology with perhaps multiple factors involved. In the case of layers, the true cause or inciting factors has probably long passed by the time the pathology is completed. The reserve capacity of the kidney is great enough to sustain life even though a significant amount of kidney damage has occurred, possibly at a very young age. As the metabolic demands of production increase, the impaired kidneys eventually become overloaded and we see gout.

My purpose is not to get into kidney function in the bird as I don't profess to know much about this - for those interested in a short, easily read description of this as well as other comments on urolithiasis, I would refer you to Dr. Wideman's article in the September, 1983 issue of Poultry Digest.

Many people do not know what we are talking about when we say visceral gout, but I will guarantee that once you see this condition you will not easily forget the spectacular lesions you see. Upon first viewing the interior of the plueral and peritoneal cavity, you will surely be amazed by the stark whiteness of the uric acid that covers nearly every serosal surface including the heart. I'm sure this has been mistakenly diagnosed as air sac disease more often than not. The covering is white in gout as contrasted to the more yellow pustular appearance noted in true CRD. The uric acid can even be found in joints. Severity of this lesion pretty well runs the gamut; in some birds that are sacrificed early, the viscera will have little if any white film, but rather you will see white concretions in the ureters of at least one and sometimes both kidneys. Kidney architecture is quite readily noted, fluid may or may not appear to be retained.

Remarkable as it may seem, a bird showing these dramatic lesions will usually have a complete egg in the oviduct and be in apparently good production. How this can be is a wonderment to me. An acute toxic death with no outward symptoms or deterioration appears to be the end result. In other species with urinary problems, there are usually symptoms of some sort, obvious frequent urination, apparent distress and straining in the tomcat and the characteristic stance and tail wringing of the calculi steer, but nothing that I'm aware of in the bird. At times I have felt the excessive staining of the vent feathers might be a tip off and logically it should, but I no longer feel confident in using this method.

Therapy once gout is detected is very iffy and certainly not a confidence builder. Many medications have been tried over the years with little consistent success. Treatment should be directed to (1) relieve added strain on the kidney and (2) assist in elimination of urine. The first thing one thinks of is, of course, to reduce crude protein which is easily accomplished, but remember there is a fine line here. Since most flocks are in good production, reduce crude protein by small increments. Obtain the best raw ingredients; grain should be as free of mold and mold toxins as possible. If excessive calcium is being used, reduce it slightly. Stimulate urine flow with products that tend to cause flushing. Black strap molasses is excellent for this. Fifty to 100 pounds per ton of feed for five days will usually do the job. Dry molasses will work, but somewhat less effectively.

Other diuretics would probably work, but they would be quite expensive and to the best of my knowledge are not labelled for such use in birds.

Extra vitamins and electrolytes may be given in the drinking water, however, unless there is some flushing prior to their use, their effeciency will be in doubt.

Muriate of potash (potassium chloride) can also be used at the rate of one pound per 25 gallons of water or in the feed at the rate of 1.5% for a week as a flush. Check the salt level in the feed and add up to ten pounds per ton for a few days as another alternative.

Be absolutely sure birds have access to plenty of clean water; cooling water in the summer may be of benefit.

Prevention is almost impossible unless a precise cause can be found. Keeping good growing period records to track down layer house problems is a must. Tightening up of feed quality control is essential; it is wise to have a feed sample of each and every delivery just in case a layer feed was delivered or at least too much calcium was added.

Prognosis of these cases is difficult. Egg laying will continue at a slightly lower rate than expected and mortality will probably not be alarmingly high during any one period, but will likely be somewhere around two percent per month.

I can't say this any better than Dr. Wideman did in his article and I quote, "Unfortunately, we can only provide the following answers. First the condition is called urolithiasis because the urinary tract ("uro") contains stones or mineralized concretions ("lithiasis") similar to the kidney stones that occur in humans.

" Second, the cause of urolithiasis is not known.

" Third, we do not know how to prevent urolithiasis nor can we recommend any specific treatment for an afflicted flock."

IMMUNITY INDUCED BY A SOLUBLE ANTIGEN OF PASTEURELLA MULTOCIDA AGAINST FOWL CHOLERA IN TURKEYS

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We have previously shown that a protective antigen of a molecular weight of 4×10^6 can be purified from a saline extract of P. multocida, type 3. A similar antigen has been purified from a type 1 strain. The antigen protected turkeys when given in two 10 microgram protein doses. The purified antigen from a type 1 strain was antigenically distinct from a similar antigen purified from a type 3 strain, and there was no significant protection in turkeys between the two antigens.

An enzyme-linked immunosorbent assay (ELISA) was used to assess the correlation between protection against the challenge exposure and antibody titers at the time of infection. The type 3 purified antigen was attached to microtiter plates, and enzyme-conjugated rabbit anti-turkey IgG was used to quantitate antibodies. Eight-week-old turkeys were injected with the purified type 3 antigen in 10 or 100 microgram/dose in either an oil or aluminum hydroxide adjuvant. The second injection was done 31 days after the initial vaccination. Four or eight weeks after the second vaccination the birds were inoculated with the type 3 organisms. Those birds having ELISA titer higher than 1:1,000 were generally protected. The majority of the birds showing less than 1:400 titer was not protected. Unvaccinated turkeys consistently showed titers of less than 1:10.

These results suggest that the type of antigen may be useful for the prevention of fowl cholera.

AN OVERVIEW OF AVIAN CORONAVIRUSES (INFECTIOUS BRONCHITIS) AND THEIR ANTIGENIC ASSESSMENT WITH MONOCLONAL ANTIBODIES

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Infectious bronchitis virus (IBV) is one of the major causes of respiratory disease in chicken flocks throughout the world. In the United States, the disease is estimated to cost the industry about 150 million dollars annually. It manifests itself as an acute, highly contagious entity and often affects young chicks seriously, causing respiratory signs. The severity of these signs will vary, however, depending upon the age of the bird, immune status and serotype or strain of IBV involved. Mortality may reach 90% in susceptible chicks and those recovering may suffer from occult urogenital damage. Later this is manifested by a failure to lay. In older birds and layer flocks, IBV is associated with a dramatic drop in egg production and/or impaired egg quality because of overt oviduct damage.

Some nephrotropic strains of this virus, notably the Australian T and Holland strains, have a predilection for the kidney causing nephrosis-nephritis, which may ultimately lead to death. Recovered chickens may become carriers and are suspected sources of cycling, recurrent infections within the flock. Isolation of IBV from the cecal tonsils and cloacal swabs is being effected with increased frequency. Whether these agents represent viruses with a new trophism is not presently known.

Outbreaks of IB occur in vaccinated as well as unvaccinated flocks making control of the disease difficult, frustrating and costly. These breaks are attributed in part to the spontaneous emergence of new antigenic variants of IBV and to the introduction of geographically exotic serotypes of the virus for which available vaccines do not confer protection.

Infectious bronchitis is caused by positive-stranded RNA virus which has a large genome ($5.4\text{-}6.9 \times 10^6$ Daltons; 48-58 S). The naked genome is infectious, contains the message and functions directly as mRNA without being transcribed. It contains between 16,000 to 21,000 nucleotides which allows for considerable genetically translatable potential and also a similar potential for genetic variation through spontaneous mutations or antigenic drift.

The morphology of the virus is mostly spherical but pleomorphic in shape with club-like projections (peplomers) uniformly distributed on its surface. These projections impart a crown-like appearance or corona, hence, the classification name Coronavirus. IBV is the prototype of the Family Coronaviridae. There are 3-6 major proteins or polypeptides associated with the virion. Some of these are found in the peplomers and they may be the antigens responsible for the neutralizing characteristics of the various serotypes.

The identification and characterization of IBV serotypes/strains is a time consuming task due to the large number of strains described, and the need for adaptation of field isolates to laboratory substrates such as embryonating chicken eggs and kidney cell cultures. Tracheal organ cultures, using tracheal rings from SPF chicks, has proven to be very useful for isolation and identification of serotypes by VN assays. Since the trachea is one of the natural habitats of IBV, no previous adaptation of the virus is required. We have used this system over the years in our laboratory characterizing several hundred IBV isolates.

Except for the Delmarva Peninsula, where an active surveillance program has been in effect, little is known about the strains of IBV which are responsible for the disease in poultry producing areas of the United States. Limited data available suggest that each region may have its own indigenous or common serotypes/strains.

Based upon work by other investigators and our own reciprocal VN data collected over a twelve year period, there are at least 16 distinguishable IBV serotypes present in the United States. They are Mass, CONN, JMK, MD-27, MD-31, I-97, I-609, Holte, Clark-33, SE-17, Florida, ARK-99, ARK-155, Maine 209, 212, and 246. However, there is not total agreement as to the actual number. For example, Stuart Hopkins proposed seven serotypes from data derived from cell culture plaque reduction tests with cloned virus. He proposed Mass, CONN (with Florida, Clark-33 and ARK-99 as subtypes) Georgia (SE-17) Delaware (with JMK, Holte and Gray viruses as subtypes) Iowa-97, I-609 and New Hampshire. We did not test the New Hampshire serotype and Hopkins did not test the three Maine serotypes, ARK-155, MD-27 and MD-31.

The techniques used to study the serotypes probably accounts for differences in their number and classification. Until procedures and reagents are standardized this will remain controversial and argumentive. Even conventional or polyclonal antisera are difficult to standardize because they have inherently variable biological properties.

These antisera are impossible to duplicate since their population is constantly changing both quantitatively and qualitatively, even in animals of the same species. This problem can be circumvented by making antibodies in the laboratory employing hybridoma technology. Cloned hybridomas produce antibodies with a single, defined specificity. Once the specificity is known these antibodies become powerful analytical probes which are useful for the rapid identification of IBV isolates.

In our definitive ELISA assays, the highly specific MCA is first placed in a well of an ELISA plate. This bound MCA will then capture and thus identify, after completion of the assay, the virus which has been propagated in cell cultures or embryonated eggs. This specific MCA can also be used to detect the presence of virus directly in infected tissues at post-mortem examination.

We have prepared numerous MCA's against the IBV serotypes indigenous to the Delmarva Peninsula and have selected MCA probes capable of differentiating serotypes and identifying viral proteins present, which might be responsible for these differences.

After propagation of the cloned hybrids, the supernatants were screened by ELISA for specific antibody activity against varying concentrations of nine IBV serotypes, Newcastle disease virus and normal allantoic fluid. The titer of each antigen preparation, as defined by each MCA, was calculated. Five different MCA's were tested against the Mass-41, CONN, JMK, ARK-99, Holte, MD-27, I-609, I-97, SE-17 and NH serotypes or strains, and control preparations of NDV and host material.

One MCA-LAS-I is specific for the Mass-41 serotype and does not react with the other serotypes or control antigens. Conversely, MCA-N reacts with all IBV serotypes but not control antigens and is therefore group specific.

MCA-8G, like MCA-LAS-I, reacts preferentially with the Mass-41 serotype but it also reacts minimally with five other IBV serotypes.

MCA's -26 and -7 appear to be mirror images of each other in that when MCA-26 reacts at high titer levels with the Mass-41 serotype, MCA-7 reacts at relatively low titer levels with it. Conversely, in reactions with the CONN serotype, MCA-7 reacts much more intensely than does MCA-26.

The latter two MCA's were also used to analyze IBV serotypes by means of an antibody index (AI), i.e. test antigen titer defined by MCA-26 divided by test antigen titer defined by MCA-7. This analysis yielded two distinct antigenic groups. Group I had computed AI's greater than 1 and is comprised of the Mass-41, JMK, Holte, MD-27, ARK-99 and I-609 serotypes. Group II defined by having AI's of less than 1.0 included the I-97, SE-17, CONN and NH serotypes.

These same antibodies were used to analyze strains within the Mass serotype and it was found that a further separation could be made. The Mass-41 serotype was assigned to Group I because it reacts solely with the MCA-LAS-I. Those not reacting with LAS-I but having AI's greater than 1.0 were assigned to Group II and include the Mass Connaught and Beaudette strains. Group III is comprised of the Mass Holland and Mild Mass strains. These do not react with LAS-I and have AI's of less than one.

The reactivity of these antibodies was mapped by Western Blot assay, coupled with ELISA. These studies indicate that antigenic variation exists on all three of the major proteins of IBV. Antigenic differences between serotypes appear to reside at the level of the peplomer (E_2) and the membrane protein (E_1), which is also common and shared by all serotypes studied. The antigenic differences between strains within a serotype, however, appear to reside at the level of the nucleocapsid.

We are in the process of developing group and strain specific MCA-based antigen detection systems to directly identify IBV antigens in unpurified, infective allantoic fluid. We are adding new MCA's to our collection which we expect will expedite the serotypic identification of IBV field isolants, cutting the turnaround time from months to a few days.

Another goal is to eliminate the need for virus isolation entirely by detecting and identifying the virus directly in infected tissues. This technique should prove to be valuable as a rapid, precise diagnostic tool which will save both time and money.

EVALUATION OF STENOROL , COBAN , and AVATEC UNDER FLOOR PEN RESEARCH CONDITIONS

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Synopsis

This long-term pen study evaluated the efficacy of Stenorol^{R} (halofuginone), Coban (monensin), and Avatec (lasalocid) under floor pen research conditions. The parameters measured -- body weight, feed efficiency, mortality and parasitology evaluations -- demonstrated that all coccidiostats performed equally well in controlling coccidiosis when exposed to a moderate challenge in most growouts. Body weights were heavier for halofuginone fed broilers and feed efficiency was better for monensin fed broilers.

Test Design

Compounds Tested:	Stenorol^{R} (halofuginone) 3 ppm Coban (monensin) 121.2 ppm and 99.2 ppm 3-Nitro (roxarsone) 49.6 ppm Avatec (lasalocid) 133.3 ppm All treatments contained Flavomycin^{R}(bambermycins) (starter and finisher 1.1 ppm , withdrawal 2.2ppm)
Dates:	
Dates:	November 17, 1981 to November 23, 1982
Housing:	A controlled environment floor pen house containing 28 pens on each side of a 6-foot aisle.
Test Animals:	7,680 commercial broilers were used in each growout in the pen house.
Allotment:	Forth-eight (48) pens of 160 broilers (80 of each sex) in each pen. There were eight replications of each treatment in a randomized complete block design. Treatment assignment to each pen remained the same for the six growouts.

Results and Discussion

The six treatments were the same in growouts 1,2 and 3; treatments 4,5 and 6 were changed in growouts 4,5 and 6. The body weights were heavier when halofuginone was in the treatment. The average weight of the broilers fed treatment 3 was the heaviest of all six treatments. Treatment 2 (monensin fed broilers for 6 continuous growouts) body weights were the lightest of all treatments.

Broilers fed monensin at 90 or 110 g/ton (Treatment 2 - Growouts 1-6 and Treatment 4 - Growouts 4-6) had the best feed efficiency. There were no differences in mortality among the six treatments. The performance data indicated good coccidiosis control by all coccidiostats. Lesion scores and fecal oocyst counts tended to be higher when monensin was in the treatment.

Conclusions

Halofuginone, monensin and lasalocid were evaluated for performance and coccidiosis efficacy in a series of six growouts in a floor pen research house. All coccidiostats performed equally well in controlling coccidiosis in the presence of a moderate challenge. In the treatments containing halofugimone, the broilers were heavier. Monensin treatments had better feed efficiency.

Treatment Allocations

GROWOUTS	TREATMENTS 1	2	3	4	5	6
1	H 3-N	M 121.2 3-N	H	H 3-N	H 3-N	H 3-N
2	H 3-N	M 121.2 3-N	H	H 3-N	H 3-N	H 3-N
3	H 3-N	M 121.2 3-N	H	H 3-N	H 3-N	H 3-N
4	H 3-N	M 121.2 3-N	H	M 121.2 3-N	M 99.2 3-N	L 133.3 3-N
5	H 3-N	M 121.2 3-N	H	M 121.2 3-N	M 99.2 3-N	L 133.3 3-N
6	H 3-N	M121.2 3-N	H	M 121.2 3-N	H 3-N	H 3-N

H HALOFUGINONE @ 3 PPM
M MONENSIN @ 121.2 PPM AND 99.2 PPM
L LASALOCID @ 133.3 PPM
3-N ROXARSONE @ 49.6 PPM

ALL TREATMENTS CONTAIN FLAVOMYCIN: (STARTER AND FINISHER 1.1 PPM, WITHDRAWAL 2.2 PPM)

Summary of Weights and Feed Efficiency

AVERAGE BODY WEIGHT: LBS

Pooled Data From:	TREATMENTS 1	2	3	4	5	6
Growouts 1-3*	4.15^{a}	3.93^{b}	4.17^{a}	4.14^{a}	4.14^{a}	4.16^{a}
Growouts 4-6*	$4.02^{a,c}$	3.91^{b}	4.12^{d}	3.99^{c}	4.03^{a}	4.04^{a}
Growouts 1-6*	4.09^{a}	3.92^{b}	4.15^{c}	4.06^{d}	4.08^{a}	4.10^{a}

AVERAGE FEED EFFICIENCY:

Pooled Data From:	TREATMENTS 1	2	3	4	5	6
Growouts 1-3*	1.95^{a}	1.89^{b}	1.95^{a}	1.95^{a}	1.95^{a}	1.95^{a}
Growouts 4-6*	1.97^{a}	1.94^{b}	1.99^{a}	1.94^{b}	1.96^{a}	1.97^{a}
Growouts 1-6*	1.96^{a}	1.91^{b}	1.97^{a}	1.94^{d}	$1.95^{a,d}$	1.96^{a}

*Means with different superscripts are significantly different ($P<.05$).

RECENT OUTBREAKS OF MYCOPLASMA GALLISEPTICUM IN TURKEYS DIAGNOSED AT THE TURLOCK POULTRY PATHOLOGY LABORATORY

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During the period of October 1, 1984 to January 2, 1985 an outbreak of Mycoplasma gallisepticum (MG) occured in turkeys in the Turlock area. The cases were diagnosed at the Turlock Poultry Pathology Laboratory from accessions submitted to the lab by local poultry producers. Two owners were involved. The first owner had two ranches on which MG was diagnosed while the second owner had three ranches involved. Both owners have their own separate breeder stock and all turkeys on these ranches were grown for meat. The birds ranged in ages from 9 - 15 weeks old. The purpose of this paper is to present an epidemiological description of the outbreak in an attempt to identify possible causes and sources of MG.

The initial complaint and clinical sign was sinusitis. To confirm a diagnosis of MG one or more of the following criteria must have been met: 1) positive serology--rapid plate test and/or hemagglutination inhibition (HI) according to the NPIP standards, 2) isolation and identification via direct fluorescent antibody technique, or 3) isolation and identification via biochemical characteristics (Dr. Al DaMassa, University of California, Davis).

A case in this study was defined as each house on any ranch that contained birds diagnosed as having MG according to the criteria previously described. This is in contrast to some investigators who believe that if a house is infected with MG then the whole ranch must be infected. The reasons for the definition used in this study are 1) on one ranch only three of five houses were positively diagnosed with MG birds and 2) on another ranch, one house was vaccinated with a bacterin in the face of an outbreak while the other two houses were not vaccinated.

MG had never been experienced on any of the ranches reported in this investigation. Thus, the normal endemic level was zero and any cases of MG observed on these ranches would then be considered an outbreak. Producers reported a 5 - 50% morbidity rate.

Figure 1 represents the spot map of MG infected flocks during this outbreak. Two owners, A and B, with two and three ranches infected, respectively, were involved. The wind pattern is primarily out of the northwest.

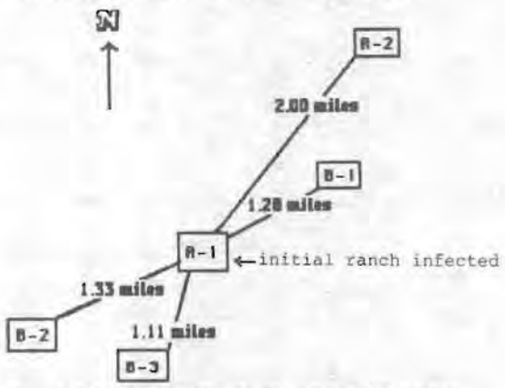

Figure 1. **Spot Map of MG Infected Flocks**

Figure 2 represents the epidemic curve for this investigation. The total number of new cases for each month is seen in Figure 2A. The month was then divided into two-week segments and each house (new case) diagnosed with MG is represented in Figure 2B. No cases of MG in turkeys were reported or diagnosed at the lab prior to the outbreak. Five cases were diagnosed in October, four in both November and

December, and two in January. No new cases have been reported since then (Figure 2A). The time sequence for houses infected with MG can be seen in Figure 2B. Each box represents a different house on that numbered ranch. Hence, it took at most one month for the disease to spread or stop. Note that not all houses on a ranch were infected, e.g., ranch B-1 had three house infected while there are five houses present on that ranch. It is unknown why only certain houses were infected since all the houses were managed equally and no special precautions were taken. In addition, the last house labeled A-1 (Figure 2B) that became MG positive had been vaccinated with an MG bacterin vaccine. The first two houses became infected simultaneously at which time the third house was vaccinated. It is interesting to note that the vaccine did not prevent the disease but only delayed the onset of clinical signs and, according to the producer, decreased the severity of the disease. Condemnation rates were not evaluated because the producer experienced a concurrent infection of cranial cholera in the flock which indubitably caused an increased rate (one house experienced an 81% condemnation rate).

The occurence of this disease outbreak did have some common host determinants. It can be seen from the spot map (Figure 1) that all ranches affected were in close proximity to one another and appeared to center around the ranch that experienced the initial outbreak. Many wild birds, such as starlings, sparrows and crows, were observed flying in and around the houses. One producer reported that some starlings made nests in the roof of some of the houses. Though it appears that wild birds do not transmit MG, possibly they are carriers via nesting material or any such type of fomite. In addition to wild birds, there are many chicken ranches in the area. Many MG-positive layer and broiler operations are located in the Turlock and surrounding areas.

Another common host determinant was the feed mill. Both producers utilized the same feed mill. Though both owners had their own feed trucks, only one would disinfect its truck prior to entering the feed mill. Once MG was diagnosed on their ranches, the feed mill insisted that the trucks be disinfected prior to entering the feed mill. Later, the feed mill refused entry of the producers' trucks and agreed to deliver the feed to a designated area for transfer of the feed.

The final potential host determinant is the diagnostic lab. Hopefully, producers took the proper precautions before and after bringing cases to the lab to prevent transmission of any diseases to or from their operations.

It appears that this outbreak of MG in meat turkeys in the Turlock area can be classified as a propagated epidemic. Transmission of MG occured over a period of time and appears to have spread from one farm to another. The source of MG is still uncertain though owner A reported finding his now ex-worker was raising "game chickens" in a house on the ranch with the initial Mg outbreak. Likewise, the mode of spread is still uncertain.

In conclusion, MG is still prevalent in meat turkeys in the Turlock area. It appears that vaccination with a bacterin in face of an outbreak will only delay the onset of clinical signs and possibly decrease the severity of the disease. Improvement in management and eradication procedures are still needed to prevent and control another possible outbreak of MG.

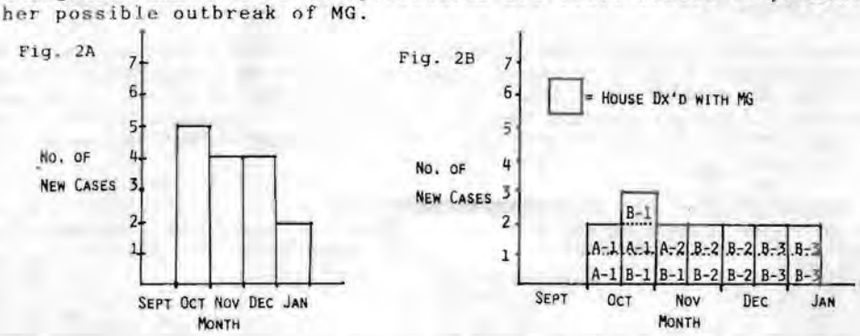

Figure 2. EPIDEMIC CURVE OF MG OUTBREAK IN THE TURLOCK AREA

CASE REPORT:

THE ROLE OF MYCOPLASMAS IN A CASE OF REDUCED HATCHABILITY IN TURKEY BREEDERS IN EUROPE.

P.A. BROWN AND J.A. NEWMAN

CASE HISTORY

The flocks involved were part of one of the largest hatcheries located in Europe. The breeders were obtained from three separate primary breeding companies. They were imported into Europe as eggs and were hatched in company owned hatching facilities.

The problem was reported to be a reproductive failure. It was determined to be a reduced hatchability of fertile eggs. The hatchability was 10 - 20 percent below normal due to late embryo mortality. The abnormalities reported were dwarfing, edematous heads, excess urates and discolored livers and spleens. Mycoplasma iowae (MI) was isolated from the dead in shell and vaginal swabs from the breeder hens.

EPIDEMIOLOGICAL INVESTIGATIONS AND LABORATORY FINDINGS

The hatchability results of the four sampled flocks are summarized in Fig.1. The hatchability of flock A was considered to be normal while that of flocks B, C, and D were below expected levels. Flocks B and D were treated with Tylan^{R} at 41 and 49 weeks of age respectively, which appeared to improve the hatchability slightly. The results of cultures from the dead in shell (D.I.S.) of Flocks A and D are given in Table 1.

The only visible gross lesions observed in the embryos of the normal and affected flocks was considerable stunting and dwarfing of embryos from affected flocks.

The Mycoplasma meleagridis (MM) serological results are presented in Table 2. There was no serology conducted on any of the flocks until 32 weeks of age. Flock C was positive at that time. Flock B and D became positive at 36 and 44 weeks respectively. Flock A did not sero-convert until almost the end of production (46 weeks of age). Flock D was inseminated with semen from MM positive toms. There was no serology conducted on this flock between 34 and 44 weeks.

There was no serological test available for MI. Samples from Flocks A & D have been sent to Liverpool, England where an ELISA test for MI is being developed.

DISCUSSION

MM and MI (formerly sero type I), have both been associated with reduced hatchability. Due to the successfull MM eradication efforts of the major primary breeding companies the incidence of MM at the multiplier level has been greatly reduced. Edson[3] in 1980 reported on an excessive late embryo mortality due to the synergistic effect of MM and a then unidentified mycoplasma. This mycoplasma has since been identified as MI.

MI has been isolated from both chickens and turkeys. It has been demonstrated experimentally to cause late embryo mortality in both species[1,2], with turkeys being more susceptible. Bradbury and McCarthy[2] noted that the numbers of colonies from intestinal isolates of hatched chicks was greater than those from the esophagus, suggesting that the intestinal environment was preferred. This observation is supported by Canadian workers[4] who infected poults orally and demonstrated colonization of the intestinal tract.

Rhoades[5] isolated MI from 10 % of dead turkey embryos (yolk sac) but not cull poults from a commercial hatchery. He pointed out the potential of these organisms for reducing hatchability. A subsequent experimental challenge with for strains of MI substantiated this hypothesis.

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TABLE 1. MI cultural results of dead in shell turkey poults

FLOCK	ESOPHAGUS	AIR SAC
A	1/8[ab]	ND[c]
D	8/8	1/1

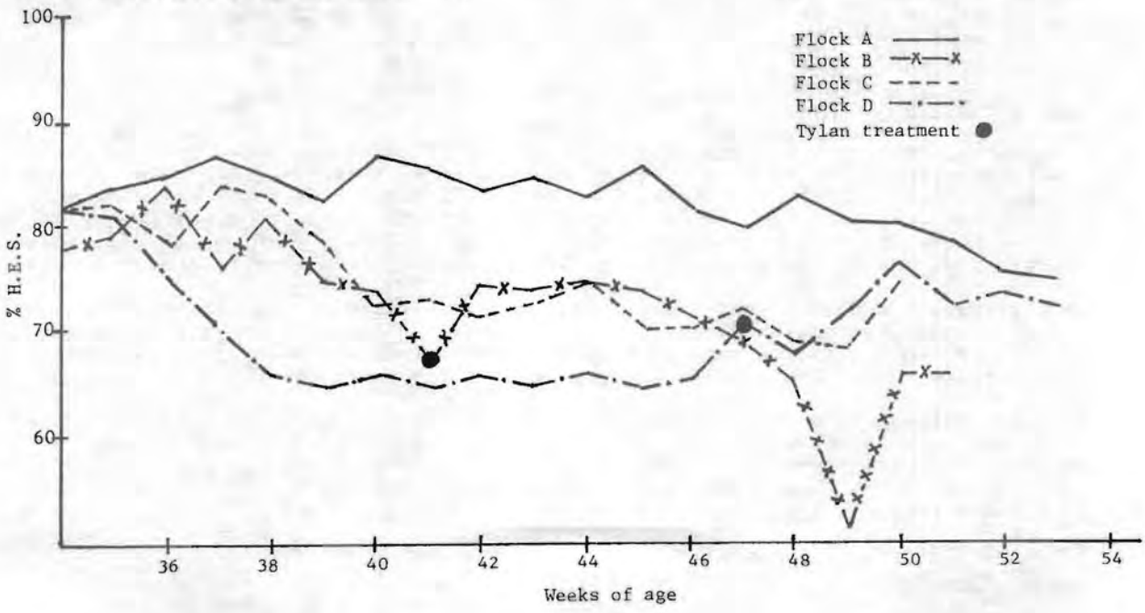
a. number positive/number cultured b. few colonies c. not done

TABLE 2. MM serological results

	WEEKS OF AGE							
FLOCK	32	34	36	38	40	42	44	46
A	0/30[a]	ND[b]	0/30	ND	ND	0/30	ND	48/48
B	0/50	ND	15/48	ND	ND	ND	42/46	ND
C	23/24	ND	10/43	ND	ND	24/29	ND	ND
D	ND	0/50	ND	ND	ND	ND	43/46	ND

a. number positive/number tested b. not done

Fig 1. Hatch of eggs set (H.E.S.)



CASE REPORT - A VARIANT MYCOPLASMA GALLISEPTICUM IN BREEDER TURKEYS

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Introduction

Infectious sinusitis in turkeys was first described in 1905. The causative agent, Mycoplasma gallisepticum (MG), produces a highly contagious respiratory disease which affects the infraorbital sinuses and air sacs of turkeys. The disease results in significant economic loss through condemnation for airsacculitis at processing and increased production cost due to slow growth (5).

Since the mid to late 60's, egg transmitted infection of MG has been controlled in breeding stock and has been seen only on occasion in commercial poults (2). Recently, however, outbreaks have been reported in breeders and this has caused concern in the turkey industry (3).

Diagnosis of MG can be made by the use of serological tests which can be confirmed by culture. The serum plate test (S.P.T.) and the tube test are two basic screening tests for MG serum antibodies. Both of these tests have an acceptable degree of accuracy, and are used to determine the flock status to MG. Non-specific reactions to these tests are usually low, but occasionally high numbers of these reactions do occur, particularly after the use of erysipelas bacterins or when Mycoplasma synoviae (MS) infection is present in the flock (4).

The hemagglutination inhibition test (HI), is both accurate and specific for detecting Mycoplasma gallisepticum antibodies and is commonly used to evaluate serum that reacts in the S.P.T. or the tube test (4).

In this case report, the M. gallisepticum isolated was called a variant because it required an extended (10 days) growth period. It gave non-specific reactions with a S.P.T. and negative reaction with a HI test when using standard antigens. This also was the term used by Dr. B.S. Pomeroy, when reporting similar observations in MG infected turkeys (1).

Case Report - Flock A:

As part of the health monitoring program of this 21 week-old multiplier breeder flock, 385 randomly collected sera were tested for mycoplasmas. At this time, a higher than normal number (133/385) of non-specific or suspicious S.P.T. to MG was reported. A representative sample of the 133 was evaluated by the HI test for MG and gave a negative reaction. Several of the birds were noticed to have a clinical sinusitis and four representative birds were submitted to the laboratory for necropsy and diagnosis.

At necropsy, the turkeys had a severe bilateral swelling of the infraorbital sinuses and a nasal and ocular discharge. The sinuses contained a gelatinous opaque material and the thickened air sacs were cloudy and contained a foamy fluid exudate. Occasional yellow-white plaques were also present on the air sacs.

Representative samples of trachea tissue, sinus and air sac fluid were submitted to bacteriology and to the mycoplasma laboratory for culture. The bacterial report indicated the presence of small numbers of a mixed population of E. coli and Pseudomonas sp., and this was considered to be non-pathogenic contamination. The mycoplasma culture was positive for Mycoplasma meleagridis but negative for MG.

Serological studies were carried out on the four serum samples for MG and MS. The results of the S.P.T. and HI test are reported in Table 1.

Table 1

Serologic Results of Flock A for M. gallisepticum and M. synoviae

Method	MG	MS
S.P.T.	4(s)/4	0/4
HI	0/4	-

(s) - suspicious

Histologically, there was marked hyperplasia of the glandular component of the nasal mucosa. A mild generalized scattering of mononuclear cells and prominent lymphoid hyperplasia was noted in the submucosa of the trachea and parenchyma of the

lungs. The air sacs were thickened due to a mononuclear infiltrate, a lympho-follicular reaction, vascularization and an accumulation of cellular debris.

Additional turkeys were requested by the laboratory and again all findings were similar to the previous submissions.

At this time, since all attempts to demonstrate MG either by culture or serology were unsuccessful, the flock owners decided to allow the flock to start production.

Flock B:

This designation has been given to several small flocks of commercial turkeys on individual farms spread throughout the province. These flocks contained poults derived from breeder Flock A. Numerous submissions from these flocks began arriving at the laboratory in early May, all with a sinusitis problem. The pathologic changes seen in Flock B were similar to those seen in Flock A, so Flock A was immediately withdrawn from production. Tests performed for MG on sera from Flock B gave a similar S.P.T. and HI results as those of Flock A. Submissions to the laboratory for mycoplasma isolation again produced a rapidly growing MM. However, this time, broth cultures which were allowed to incubate beyond 10 days began to turn acid and at this time, an MG was isolated. The identification of an MG isolate was confirmed by a weak reaction to specific fluorescent antibody, a glucose positive test and a gel-diffusion test. The isolate was termed "Guelph MG 6505". The Guelph MG 6505 isolate was cloned three times to produce a pure culture and an antigen was prepared which could be used for HI testing (4). Serological tests for MG performed on convalescent sera from the birds of Flock B, using the standard available antigen and the Guelph MG 6505 antigen, are reported in Table 2.

Table 2

Serologic Results of Flock B for M. gallisepticum

Antigen	Method	MG
Commercial	S.P.T.	11(s)/15
U.S.D.A.	HI	0/11
MG 6505	HI	10/10

(s) - suspicious

The sera titers to MG using the Guelph MG 6505 antigen were between 1/40 and 1/256 and anything greater than 1/40 was considered a positive reaction.

Flock C:

This breeder flock was produced from breeding eggs purchased from a large American based company, and the poults were placed on the same brooder farm as had been used for breeder Flock A. Serological monitoring for mycoplasmas was initiated when the turkeys were nine weeks old and all tests at this time gave normal S.P.T. negative reactions. When the poults were 11 weeks of age, they were moved to the same breeder farm which had housed Flock A. At the time of arrival, several birds had a sinusitis and eight were submitted to the laboratory for necropsy.

All birds had a severe sinusitis and a nasal discharge. The appropriate samples were submitted for mycoplasma isolation. Following the procedure of extend growth time described previously for the isolation of Guelph MG 6505, a Mycoplasma gallisepticum was isolated.

Serology carried out at this time gave suspicious S.P.T. and negative HI to MG with the standard hemagglutinating antigen.

When the poults were 15 weeks old, sera was again submitted for MG evaluation. Twenty-five samples were tested with the now available MG 6505 and the U.S.D.A. hemagglutinating antigen and the results are reported in Table 3.

Table 3

Serologic Results of Flock C for M. gallisepticum

Antigen	Method	MG
Commercial	S.P.T.	23(s)/25
U.S.D.A.	HI	0/10
MG 6505	HI	10/10

(s) - suspicious

It was apparent that Flock C was infected with the variant MG, and it was recommended that the turkeys be slaughtered for meat purposes. Due to the previous experience with the poults from Flock A, the recommendation was accepted and acted on.

Summary

In this case report, the failure to isolate MG from Flock A was likely due to the early rapid growth of an MM which served to mask the presence of the slower growing MG. The failure to detect MG serum antibodies on HI tests would appear to indicate that Guelph MG 6505 is antigenically different from that contained in the U.S.D.A. hemagglutinating antigen. Dr. B.S. Pomeroy suggests that some chickens may be asymptomatic carriers of a variant Mycoplasma gallisepticum which can produce a severe sinusitis in turkeys, which fail to react in HI tests with standard HI antigen (1).

The farm used to brood and raise the breeder turkeys, was owned privately and was not under the control of the flock owner. It was learned much later, that leghorn layers were being kept on the same farm within 300 yards of the turkeys. The chickens may have been the source of the variant MG.

Since the company has changed the location of brooding and raising breeder turkeys all flocks which have passed through the same premises as Flock A, have done so without incident.

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COMPARISON OF EGG YOLK AND SERUM FOR THE DETECTION OF MYCOPLASMA GALLISEPTICUM AND M. SYNOVIAE ANTIBODIES BY HI AND ELISA

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Serum testing has been a common procedure for detecting Mycoplasma gallisepticum (MG) and M. synoviae (MS) infections. However, some disadvantages of this procedure involve the need for a trained staff to collect the blood samples, stressing of birds during handling, and the potential for transmitting disease from one ranch to another. Furthermore, in disease surveys where a large number of samples are to be collected in a short period of time, bleeding of large number of birds becomes impractical.

The primary objective of this study was to evaluate egg yolk testing as an alternative to serum testing to detect antibodies to MG and MS.

Chloroform extracted and saline diluted egg yolk were compared to serum using enzyme linked immunosorbent assay (ELISA), hemagglutination (HI), and serum plate agglutination (SPA). Egg yolk extracted with chloroform was comparable to serum in the MG and MS HI and ELISA tests. Egg yolk diluted in saline gave slightly less sensitive results than chloroform extract. Both preparations were unsatisfactory in the SPA test.

The optimal ELISA screening dilution for MG and MS testing of egg yolk or egg yolk extract was 1:800. The latter procedure has been applied in commercial layers in a large field survey in Southern California.

The complete paper will be published in Avian Diseases.

ELIMINATING *MYCOPLASMA GALLISEPTICUM* ON A MULTI-AGE LAYER RANCH IN THE FACE OF F STRAIN USE

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This report is the conclusion of a previous progress report on a *Mycoplasma gallisepticum* elimination program, given at the 1984 Conference (McMartin, 1984). Starting on a multi-age layer ranch which had used live F-strain MG vaccine (MGF) for several years, a program of eliminating MGF was implemented in February 1983, employing a strategic combination of unvaccinated houses and houses either vaccinated with MG bacterin (Salsbury Laboratories, Charles City, Iowa) or treated with tylosin tartrate to supress MGF excretion. Freedom from MG(F) was assessed by testing 20-25 random blood samples at intervals of 2-4 weeks.

The chronological sequence of house-by-house elimination of MGF is shown in Table 1. The last MGF-vaccinated layers were sold in June 1984, and in February 1985 only 2 bacterin-vaccinated houses still remained (Nos. 7 & 17). On the basis of serology, all of the remaining layers were believed to be MG-free.

Table 1

Sequence of House-by-House Elimination of MGF-vaccinated Layers from a Multi-age Ranch

House No.	HOUSE STATUS* DATES						
	Jan. '83	Feb. '83	June '83	Oct. '83	Feb. '84	June '84	Oct. '84
1	F	F	F	F	F **Sapr	U	U
2	F **Sjan	B	B	B	B	B **Ssep	U
3	F	F **Sapr	U	U	U	U	U
4	F	F	F **Ssep	U	U	U	U
5	F	F	F	F	F **Sapr	U	U
6	F	F	F	F	F **Sapr	U	U
7	F	F **Smay	B	B	B	B	B
8	F	F	F	F	F	F **Sjun	U
9	F	F	F	F	F	F **Sjun	U
10	F	F	F **Ssep	U	U	U	U
11	F	F	F **Ssep	U	U	U	U
12	F **Sjan	U	U	U	U	U **Ssep	U
13	F	F **Smar	B	B	B	B	B
14	F	F **Smar	B	B	B	B	B
15	F **Sjan	U	U	U	U	U **Ssep	U
16	F	F	F	F **Sjan	U	U	U
17	F	F **Smay	B	B	B	B	B
18	F	F	F	F **Sjan	U	U	U

* F = Vaccinated F Strain MG
B = Vaccinated MG bacterin
U = No vaccination

** Sapr = Sold April, *et seq*.

No breakdowns occurred in the course of the 2 year program, but SPF chickens housed with 20 MGF vaccinated layers taken from House 9 in June 1984 became serologically positive. MG(F) was recovered both from the MGF vaccinated and from the SPF infected poultry.

Because the ranch is in an area of southern California in which some 70% of the ranches are either vaccinated with MGF or are serologically positive to MG, serological monitoring will continue indefinitely.

A benefit-cost analysis of the program is in progress. Ranch management believes there has been a reduction in respiratory problems, reduction in mortality, and an increase in egg production.

Important factors in the success of this program are believed to include:

1. Ability of the ranch to rear its own MG-free pullets.
2. Strict attention to preventing movement of chickens from one house to another.

Factors which did not affect the success of this program included:

1. The short distance between the houses (25 ft.).
2. No sanitizing procedures were used by egg-collection and clean-up crews when moving from house to house.
3. Some wild bird access (sparrows, etc.) through the main doors of the laying houses.

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COMMENTS ON AVIAN INFLUENZA

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One of the problems that led to some confusion and controversy on the avian influenza (AI) problem in Pennsylvania was the extension of past experiences with AI in turkeys to the Pennsylvania outbreak. There were significant and important differences that severely limited the amount of extrapolation that could be done.

First, the fowl plague-like character of the disease resulted in decisions to seek its eradication from the poultry industry. This decision made the use of the vaccine approach undesirable in that vaccinated birds could be infected and shed the virulent virus without showing signs of disease. While this feature might be acceptable with milder AI viruses confronted in turkeys in the past, it was the opinion of many that it would have impaired the eradication of the virus that caused the fowl plague-like disease. Vaccine use would have also resulted in positive serology for vaccinated but unexposed and noninfected flocks. This development would remove the usual Agar-Gel Precipitin (AGP) serology as a tool for declaring an area free of AI infection as the eradication effort progressed.

Eradication was selected as the approach to the AI problem because of the fowl plague-like character of the disease. Such a disease is totally unacceptable to most countries of the world and results in severe trade restrictions, including embargos on all poultry and poultry products. Such restrictions did occur on an interstate and international basis. For normal poultry commerce to resume, eradication of the virus from poultry had to occur and in a manner that could be convincing and substantiated. There was no intent or claim that AI would be removed from wild waterfowl. Such a task is totally impossible and highlights the fact that waterfowl are still infected and shedding AI viruses on a global scope. This clearly means that the challenge to the poultry industry is going to be to provide an effective barrier between waterfowl and poultry. It also means that the industry must operate in a manner so that the disease will not spread like wildfire when it does gain access to poultry.

The dramatic and unexpected change that occurred in the pathogenicity of the Pennsylvania AI virus between April and October is a clear signal that we must view AI viruses with caution and respect. To judge the potential of future AI problems based on what has occurred in the past may be a hazardous path. I believe we can expect further surprises from the AI viruses that may be as unpredictable as the Pennsylvania experience.

Finally the term "fowl plague" may still have a place in avian diseases terminology. If it is used to describe the classical disease with the high mortality, swollen heads, combs, wattles, hemorrhages, diarrhea, etc. caused by any AI virus, regardless of subtype, it may be helpful. There should be no single fowl plague virus, rather the term could only be used to describe a clinical syndrome. It is not without risk to assign such a general term to a clinical syndrome but the benefits gained from separating that syndrome from the mild AI-virus induced disease in turkeys may be worth it. From the standpoint of interstate and international commerce, fowl plague is one thing -- mild turkey AI is another. There should be some generally accepted way to distinguish them, especially when they share the same H and N antigen designations (H5N2, for example).

SEROLOGICAL AND CHALLENGE RESPONSE IN CHICKENS VACCINATED WITH INACTIVATED H5N2 AVIAN INFLUENZA VIRUS - A PRELIMINARY REPORT

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Introduction

The decision by the USDA to prohibit the use of a vaccine during the recent epornitic of highly pathogenic avian influenza was considered unfair by some and considered essential by others. Both scientific and political arguments were used in making this decision. While some scientific data on the use of avian influenza vaccines were available, very little information was based on work involving a fowl plague-like disease.

This study, while not yet complete, will provide preliminary evidence of the sequential antibody response to vaccination of chickens with H5N2 virus and the ability of vaccinated birds to survive challenge with a fowl plaque virus.

Experimental Design

Forty 20 week old chickens (20 "field" leghorns and 20 SPAFAS leghorns) were vaccinated with 0.5 cc of avian influenza vaccine H5 killed virus, chicken embryo origin oil emulsion (Maine Biological Laboratories, Waterville, ME), by the intramuscular route. Half of these birds were revaccinated with the same vaccine at 25 weeks of age.

Table 1
Experimental Design

	Field Birds		SPAFAS Birds	
	Group I (10 Birds)	Group II (10 Birds)	Group III (10 Birds)	Group IV (10 Birds)
Vaccinated at 20 wks.	+	+	+	+
Revaccinated at 25 wks.		+		+

All birds were held in separate cages in the same isolation room. Blood samples were collected at various intervals from 0 weeks to 36 weeks post vaccination for determining the presence of agar gel diffusion antibodies and hemagglutination inhibition geometric mean titers.

The procedure for the agar gel diffusion test was that used by the National Veterinary Services Laboratories, (NVSL), Diagnostic Virology Laboratory. They also provided the antigen and control antisera. The hemagglutination inhibition test antigen was TURKEY/WIS 68 (H5N9). The procedure for this test was that used by the USDA Regional Poultry Research Laboratory in Athens, GA.

Virus challenge with highly pathogenic avian influenza (Pennsylvania H5N2) was done approximately 10 months post vaccination at the National Veterinary Services Laboratories, Ames, IA.

Thirty-nine vaccinated chickens were air shipped to Ames, IA, following the lifting of the USDA Pennsylvania quarantine. These birds were avian influenza virus negative on multiple cloacal and tracheal swab tests. In addition, 12 genetically related SPAFAS (related to the SPAFAS vaccinates) and 12 NVSL control leghorns, all *non* exposed and non vaccinated were used as challenge controls. Preliminary challenge trials were done to determine a 70% - 90% lethal dose by the intratracheal route. In addition, virus shed will be determined in recovered birds.

Results

Agar gel diffusion antibodies, in birds vaccinated once were first detected by two weeks post vaccination, reached a peak percent positive (80% in field birds and 100% in SPAFAS birds) by 4 - 5 weeks and then declined. By 23 weeks post-vaccination 22% field birds and 10% SPAFAS birds were positive but all birds were antibody negative at 32 and 36 weeks post vaccination.

In birds vaccinated twice, all field birds were positive by 6 weeks post-primary vaccination. Antibodies were detected in 80% (field) and 100% (SPAFAS) of the birds at 11 weeks but declined to 44% and 33% by 23 weeks post vaccination. All birds were antibody negative at 32 weeks and 36 weeks.

Geometric mean HI titers were first detected at 10 days post vaccination and peaked at 7 weeks in birds vaccinated only once. While there was a rapid decline in GMT's after 7 weeks, HI antibody was detected in most birds at 32 & 36 weeks post vaccination. GMT's were higher in birds vaccinated twice through 23 weeks, after which they declined.

Preliminary challenge results are given in table 2.

Table 2
Virus Challenge with Highly Pathogenic Avian Influenza H5N2

	Vaccination	Mortality
FIELD BIRDS		
Group I (9 birds)	1 X	0
Group II (10 birds)	2 X	0
SPAFAS		
Group III (10 birds)	1 X	0
Group IV (10 birds)	2 X	0
CONTROLS (12 SPAFAS birds)	0 X	9
(12 NVSL SPAFAS birds)	0 X	9

The final mortality figures will not be available until later. These data represent mortality through 10 days post challenge.

Summary

Vaccination of chickens with the killed H5N2 oil emulsion product used in this trial results in a rapid onset of HI antibodies by 10 days post vaccination and of agar gel diffusion (AGD) antibodies by 14 days. While the HI antibody is detectable in most birds at 32 weeks post vaccination, the AGD antibody is no longer detectable at this time. This is in contrast to what we observed in naturally infected birds. Classified as "low path avian influenza infected", that were permitted to live for longer periods of time, these flocks had a persistent 20-30% positive reaction rate.

Chickens vaccinated only once were protected against challenge with a highly pathogenic avian influenza virus dose that killed 75% of the non-vaccinated controls. It is notable that this protection was present more than 10 months post vaccination.

Two very important questions remain to be answered. Do vaccinated challenged birds shed virus, and if so for how long? These studies should be completed very soon and that data and the final mortality data will be published as soon as possible.

Acknowledgements

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Avian Influenza Outbreaks in Turkey Breeder Flocks From 1979 to 1981

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Introduction

Between January of 1979 and February of 1981 a series of avian influenza (AI) outbreaks in California turkey breeders were investigated. Investigations were carried out by on-farm visitations or via serologic testing of blood samples. In some cases dead turkeys from breeder flocks experiencing a drop in egg production or elevated mortality were obtained for diagnostic work-up and virus isolation. Diagnostic procedures were conducted by the California State Livestock and Poultry Pathology Laboratories (located in Turlock and Petaluma), University of California, Davis, School of Veterinary Medicine; and at Nicholas Turkey Breeding Farms laboratory, Sonoma, California. Virus identification and characterization was carried out by the University of California, Davis, School of Veterinary Medicine; and the National Animal Disease Center, Ames, Iowa.

1979

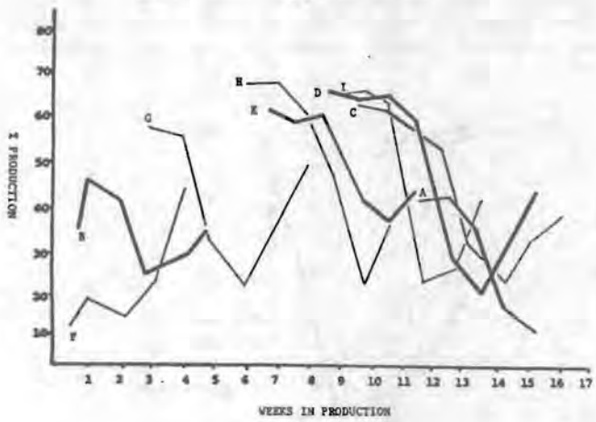
Between January 1979 and May 1979, there were 11 known outbreaks of avian influenza caused by serotype H_5N_3 in turkey breeder flocks producing eggs in outdoor facilities for four different companies, A, B, C, and D. Nine of these outbreaks affecting breeder flocks were in the San Joaquin Valley (Central California). Two outbreaks were in breeder flocks located 65 miles west of Turlock in the Santa Cruz County close to the California coast. (Fig. 1.)

1. Nicholas Turkey Breeding Farms, Sonoma, California. 2. University of California, School of Veterinary Medicine, Davis, California. 3. Poultry Health Laboratory, Davis, California.

The initial outbreak was diagnosed in a heavy pure strain male line flock. The flock was marketed because of a dramatic drop in egg production. The outbreak started in the broody pen area and within a few days extended to four production pens in the same complex. The flock appeared droopy, inactive, and with clinically apparent respiratory signs (dyspnea, rales). Dead birds presented severe pulmonary edema, congestion, and in some cases ruptured enlarged livers with internal bleeding. The kidneys and spleens were enlarged and congested. The developing and mature ova were severely congested. Superficial pectoral muscles appeared pale, apparently due to hypovolemic shock and visceral congestion. Yolk peritonitis was a common finding. Histopathology supported the clinical findings.

The affected flocks ranged from 33 weeks (one week in production) to 46 weeks of age. There were eight commercial breeder flocks and three primary breeder flocks. The overall mortality was not significant in these outbreaks but severe drops in egg production (20% to 50%) occurred in breeder flocks within 1-3 weeks (Fig. 2). There was some recovery in egg production post infection, but this never reached the initially projected level. Serological surveillance in naturally infected and recovered flocks had HI titers that ranged between zero to 1:128 at 10 weeks and 100% of the samples were agar gel and precipitins (AGP) antibody positive at 10 weeks post infection.

FIG. 2. EFFECT OF H_5N_3 ON TURKEY BREEDERS



An oil emulsion vaccine was produced from the H_5N_3 virus isolated from the lung and tracheal swabs of the initial outbreak. Once the infection was confirmed, all non-affected breeder flocks in the region were vaccinated twice prior to production. The vaccination program was carried through the following year (1980). A serologic surveillance conducted three weeks after the first dose of vaccine indicated a low HI titer (1:16) and only 47% of breeders responding. However, there were higher (1:128) HI titers post second dose of vaccine. No investigation was made in meat turkey flocks in the region in 1979 to demonstrate if avian influenza was present in commercial turkey flocks.

1980

Between January 1980 to March 1980, there were five known outbreaks of avian influenza caused by serotype H_9N_2 in turkey breeder flocks in outdoor facilities producing eggs for three different companies (A, C, and D). The premises in companies C and D were the same as those affected in 1979. Company A had three outbreaks of H_9N_2 on different farms in 1980 from those premises which suffered from H_5N_3 in 1979. The initial outbreak occurred in a flock of turkey breeder hens prior to production. A total of 20 turkeys of 4500 breeder candidates died at about 29 weeks of age. Severe pulmonary congestion and enlarged and congested livers and spleens were a common finding. Subsequent breaks occurred in flocks in production. All turkey flocks in the region, including those above, had been vaccinated for H_5N_3. Mortality was insignificant and drop in production was not too dramatic except in one flock in Company D where there was a 15% drop in egg production at the onset of production. Because of the mildness of the disease, a suggestion at that time was made that there may have been a slight cross protection by the H_5N_3 vaccination program that accounted for the less dramatic effect of H_9N_2 in 1980 outbreaks. An oil emulsion vaccine was produced with H_9N_2 isolate which was recovered from the lung of a dead turkey from company C (initial outbreak). The later flock went through a normal production cycle because of the immunity induced by a natural outbreak of an early age. Serologic surveillance of a naturally infected and recovered flock (company C) demonstrated HI titers of 0 to 1:128 six weeks after the initial outbreak. Eighty percent of breeder hens in company A (other than those broke with H_9N_2) demonstrated HI titers of 1:8 to 1:128 four weeks after the second vaccination.

Field investigations of the 1979 and 1980 outbreaks revealed that the same commercial service crew that carried out artificial inseminations and sometimes vaccinations were employed and used by all companies when the flocks were in egg production. The service crew had 7 automatic semen tube filler machines and 5 separate groups of people, each with one crew supervisor. Crew members were interchanged between groups from time to time. The use of insemination tubes exposed to dust and dander from infected farms on non-infected flocks was a common practice even though a reasonable attempt at sanitation was carried out on insemination machines and vehicles.

1981

In February of 1981, an outbreak of avian influenza serotype H_6N_1 occurred in a semi-confinement primary breeder flock located north of San Francisco, California. The outbreak started in one pen and spread within 5-10 days through the 4 pens of breeder hens. The source of infection was suspected to be free flying waterfowl that contaminated the new water reservoir which served as the source of drinking water for the infected flock. Wild ducks had been noticed in the area. However, three ducks examined after the outbreak took place did not yield H_6N_1 virus. The mortality in this flock was significant because the outbreak coincided with aovarian development (i.e. follicular hierarchy of first clutch was fully developed) and the inefficient air sac function presumably due to the large ovarian mass apparently predisposed this flock to pulmonary failure. The mortality reached 13.2% (603 hens) within 4 weeks. However, by the second week of the outbreak there was a mortality of 9%. Clinically, the flock appeared droopy and quiet. Examination of the dead birds showed severe congestion of the internal organs. On recovery, the flock went through egg production with a high peak and a long duration (27 weeks). Loss of the projected production, the cost of growing 603 hens up to 29-30 weeks of age and the loss of primary breeder salvage resulted in an estimated $55,825 loss.

Serologically, the birds were agar gel precipitin (AGP) positive 100% for 21 weeks post infection. HI titers of 1:256 at 7 weeks post infection remained as high as 1:32 at 21 weeks post infection. (Table 1.). Virus isolated from lung tissues propagated in turkey embryos failed to cause morbidity or mortality in four and a half week old turkey poults when CA fluid was inoculated by intravenous and intranasal routes. However, a slight and transient droopiness was observed in some of the inoculated turkeys. Serum samples from inoculated poults were positive to avian influenza by AGP and HI titers as high as 1:16 were detected at 12 days post inoculation. Eggs collected from 20 hens each week between 33 and 36 weeks of age were tested after incubation for 7 days but no hemagglutinating agent could be isolated. Infection did not spread to other breeder turkey flocks in the region. This was probably so because the commercial service crew working with the affected flock did not handle other breeders in the region.

TABLE 1.
SEROLOGICAL SURVEILLANCE ON A NATURALLY INFECTED TURKEY BREEDER FLOCK[1] WITH H_6N_1 (1981)

	WEEKS POST INFECTION						
HI TITERS	1	2	4	7	11	15	21
0	4	4	0	0	1	1	5
4	0	0	0	0	0	3	3
8	0	6	0	2	3	7	2
16	1	4	8	7	8	6	4
32	-	4	7	5	5	3	1
64	-	2	3	3	3	0	0
128	-	0	2	2	0	0	0
256	-	0	0	1	0	0	0
TOTAL	5	20	20	20	20	20	15
AGP	5	16	20	20	20	20	15

[1]PRIMARY BREEDER FLOCK (CO, E)

Conclusion

Seventeen outbreaks of avian influenza in selected California turkey breeders caused by serotypes H_5N_3, H_9N_2 and H_6N_1 were investigated between January of 1979 and February of 1981. Significant economic losses due to drops in egg production were experienced. Mortality was not a serious consideration in these outbreaks. No reports of avian influenza type H_5N_3, H_9N_2 and H_6N_1 outbreaks were made in the progeny produced from all infected and recovered breeder flocks. The initial source of virus for these outbreaks was suspected to be waterfowl migrating through the foothills of the Sierra Mountains. The spread of infection onto the different unrelated breeder premises strongly implicated transmission by a commercial insemination service crew.

AVIAN INFLUENZA EPIDEMIC IN CENTRAL CALIFORNIA, 1984

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Introduction

Avian Influenza has been seen in California many times since 1964. It has caused serious economic losses in turkey breeding flocks and has spread extensively between ranches. These losses have been mainly due to reduction in egg production. Pathogenicity has been variable but death losses have been typically low. An epidemic of Avian Influenza occured in 1983-84 in Pennsylvania and Virginia affecting both chickens and turkeys. The virus was serotyped H5 N2 and many isolates were pathotyped as highly pathogenic. The 1984 H5 N3 Avian Influenza outbreak in California was similar to previous outbreaks. The mortality was low, serious losses in egg production were seen and the disease spread between ranches using common service persons.

The Initial Outbreak

On February 28, 1985 a flock of 7400 turkey hens in Stanislaus County (Stanislaus No. 1) percent production. They were first inseminated on the 19th. Because of unsatisfactory semen production from the toms they were inseminated on the 25 & 27 with semen from another Stanislaus County breeding flock. (Stanislaus #2). Eight hens and one tom died on February 28th in the Stanislaus #1 flock. The next day fifteen hens and five toms died, egg production was down one hundred eggs. On March first egg production was down one thousand eggs and it was apparent that there were many sick birds. During the next week egg production fell from 3500 to 476 and one hundred sixty five birds died. On necropsy air sacculitis, peritionitis, swollen livers and some exudate in the trachea was seen. Most of the flock was depressed, off feed, and when they moved appeared to be very sore. Many of the eggs showed poor shell quality.

Highly Exposed Poultry

At this time three ofther flocks were being inseminated by the same crew as the Stanislaus #1 flock in Mariposa County (Mariposa #1) and 2 flocks in Santa Cruz County. The owner of the Stanislaus #1 flock also owned the Stanislaus #2 flock. There was also a young flock on the Marinosa County Ranch (Mariposa #2). These five were classified as being exposed. Surveillance increased and strict biosecurity measures were initated in these flocks.

Other Exposed Poultry

Eighty percent of the 20 million turkeys raised in California are raised in the northern and central San Joaquin Valley. Most of the broilers and many chicken hatching eggs and table eggs are also raised here. Surveillance and biosecurity measures were also initiated in these flocks.

Poultry Industry Action

Meetins were held in Sacramento and Modesto during the first week of March. During that week typing at Ames determined the virus to be serotyped H5 N3. It was decided to treat the disease as an emergency and destroy the Stanislaus No. 1 flock. A fund was collected from the members of the poultry industry and on March 11, 1985 the flock was destroyed and buried on the ranch. On Friday March 15th the older flock on the ranch in Marinosa County was found to be infected. It was destroyed the following Sunday and buried on the ranch. There was no indemnity paid for this flock and it was destroyed and buried largely with company labor. An H5 N3 virus was isolated from a dead bird from the Santa Cruz #1 Ranch during the first week of April. These birds were destroyed and buried on the ranch. An idemnity was paid with money the state legislature had appropriated. After the first week in April the vaccine became available. The Santa Cruz #2 and Marinosa #2 flock were vaccinated. Stanislaus #2 flock was serologically positive but no virus was isolated. Two hundred eighty five thousand doses of the H5 N3 vaccine were produced and used in other breeding turkeys in the area. About this time the pathotyping at Aimes showed the virus to be non pathogenic to chickens. On April 20th the virus was isolated from a dead bird picked up at the Santa Cruz #2 Ranch. This flock was not slaughtered. They had been vaccinated, they were very isolated, attempts to grow the virus failed, it would have been impossible to bury the flock on the premises necessating a trip to a landfill. Probably the most important factor in deciding not to slaughter the flock was the fact that most of the turkeys which were considered to be at risk had been vaccinated. No further serologically or virologically positive cases were seen.

Source of the Infection

It is suspected that the source of the infection for the Stanislaus #1 flock was semen from the toms at the Stanislaus #2 flock. This flock was infected early, before egg production began but did have some death loss and was serologically positive. There was an earlier flock on the Oakdale Ranch which ended its lay early in February which may have been infected with Avian Influenza.

Testing

Early in the epidemic when it appeared that the first case was at the Stanislaus No. 1, Ranch we tested a few dozen local ducks and duck eggs for Avian Influenza virus the results were all negative. All of the ranches had wild waterfowl in the area. A group of laying hens were innoculated with the virus at Davis and did not appear to loose egg production. A number of eggs were sampled from the Stanislaus No. 1 flock, both egg contents and swabs of the shells showed no influenza virus. I realize that Avian Influenza virus has been found on and in eggs but clinical experience indicates that it may not be a great factor in the spread of the disease. Serological testing and virus isolation attempts were made on the remaining flocks. 300 birds each in the Stanislaus No. 2, Mariposa No. 2 and the Santa Cruz No. 2 flocks were swabbed at least twice with no more virus isolated. There appears to be very little virus shed after the clinical signs abate in a flock.

Conclusion

The 1984 H5 N3 Avian Influenza epidemic was in many ways a typical epidemic if any epidemic of a disease as variable as Avian Influenza can be described as typical. One flock experienced a very dramatic disease, others a milder disease and in at least one almost no signs were seen. The unusual aspect of the outbreak was the decision to control it by destruction and burial of the infected flocks. There is no doubt that the Pennsylvania outbreak and the severity of the first case influenced this decision. The fact that the outbreak was limited to the community of flocks serviced by a single inseminator indicates that this was the mode of spread. I am encouraged by the ability of the California Industry to respond quickly and effectively to an emergency situation and by the level of biosecurity the poultry industry is able to provide.

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DISEASE CONTROL FOR THE EIGHTIES

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For the past 30 years, my students and I have been engaged in research that has direct application to the control of animal disease and we have participated in activities here and abroad that test the practicality of these applications.

I am not happy about much of what I have learned or about what the future promises. The public thinks that science is an Aladdin lamp which gives instantaneous answers and administators expect scientific discoveries to be translated into benefits that will show up in the next months balance sheet. When budgets are tight, administrators, legislators, and the public believe maintenance can be deferred and programs slashed across the board. Many of our problems are created by those unrealistic expectations and by failures to make hard and discriminatory decisions on budget matters.

It is also frightening to poke around government agencies trying to find out how things work, how they could work better and why some things fail and encounter everywhere fences erected by current administrators and past administrations to separate agencies, subagencies, and even units within subagencies. Whatever administrative rationale these barriers have, they stifle communication required to solve problems, they foster wasteful competition and increase costs of government. One hears that it is administratively impossible to get professionals to work in teams, to get various units to collaborate and to get staff to participate in planning and decision making. Don't tell this to someone from a University where, as a departmental chairman, I reported to three deans and to two chancellors, each of whom paid part of my salary. Relating to them was not always easy but the important fact is that throughout the University collaboration exists among researchers, instructors, and extension people. Who has guaranteed that administration should be easy?

Recently I have participated in a National Academy of Science study (1) of the foreign animal disease program of USDA and in the preparation of material for the USDA FY 1986 budget hearing, and in collaboration with my wife, I have written a textbook (2) on the institutional problems of disease control. As a result, I see some major changes on the horizon in the way we will do research, diagnosis and control animal disease in this country in the future. I see the possibility of deregulation of animal disease control and discussion of the possibility enables me to bring out a number of problems. Not only is there a climate in the government for deregulation but there are instruments to be used if deregulation occurs.

Dividing the costs of disease into two categories, on-site costs and off-site costs helps one to understand the controversies over disease control. The loss of an animal to disease, the costs of drugs to treat disease or vaccines to prevent it, the increased outlay for feed required to nurse animals back to market weight, the extra labor needed to clean up animal pens and shelters, the remodelling outlays required to avoid introduction or spread of disease within units, and the loss at market time from downgrading or missed deadlines are all on-site costs of disease. They are all measurable and it is in the selfish interest of any wise owner to reduce them. Off-site costs of disease are the losses that result in another herd or flock as the result of negligence, the sale of a carrier animal to the owner of that herd or flock, the trespass of an animal or unconfined run-off onto a pasture or yard. Efforts taken to prevent losses from occurring on adjacent properties bring no direct benefit to the owner of the animals from which the disease spread into other herds and flocks.

It is the off-site costs of disease that has forced the government to act as a regulatory agency by seeking and implementing laws and regulations to protect property owners. Now some authorities say that if the seller of a diseased animal knew that he would be liable for off-site costs -- the losses that result from introducing a disease carrier into a susceptible herd -- it is unlikely that diseased animals would be sold. These legal actions are possible now but this recourse has not occurred often enough to deter the spread of disease. So what can make the future different? It will be changing attitudes and the availability of new scientific tools as illustrated by two developments.

One development has been the success of class action suits such as the one by hundreds of mink growers against several meat packers who sold them stilbestrol ladden poultry by-products. The other development was the recent acceptance in court of evidence based on advanced biotechnology. In that case my laboratory provided evidence that was used to convince a jury in a Miami federal court, that two virus isolates were identical based on fingerprints of their nucleic acids. This meant that the two birds from which the viruses were isolated had been kept at one time in the same facility. Since this could have happened only if one of the birds had been stolen from a quarantine station, the defendants were convicted of theft of birds.

As a result of changes occurring, diagnosis must become more defensive. By this I mean better able to meet legal challenges, and more relevant to disease problems in the field. A diagnostician must understand the term "line of evidence", which means the documentation of the location of the diagnostic specimen from the time it arrives in the laboratory until the diagnosis is completed. To do this one must be able to present log books in which all activities are entered, dated, and initialled. If it can be established by these documents that the specimen was always properly identified and its whereabouts known, then the diagnostician must provide evidence that the diagnostic methods used were appropriate and met standards used by recognized experts in the field.

In research laboratories serious problems have arisen because of a failure to segregate unrelated activities. Such segregation is required, and rightly so, of any laboratory producing a licensed vaccine. Unfortunately, many diagnostic and research laboratories are not designed to adequately segregate steps in the isolation and identification of pathogens. Until some director tells his agency that budget cuts and deteriorating facilities will result in restrictions of the kinds of diagnostic services available and in the number of specimens that can be processed, or a client who has suffered economic loss from a resulting misdiagnosis sues the government for redress, the situation is unlikely to improve.

I have discussed the possibility of deregulation of animal disease control and its impact on diagnostic laboratories because it forces us to reexamine our ideas of the cost effectiveness of disease control and the practicality of alterative restraint systems. Regulation always lags behind new developments in epidemology and diagnostic technology and the new developments in animal management and marketing. However, regulations are applied fairly uniformly to all producers and processors irrespective of size, and consequently, it is possible to predict with some confidence the consequences of most actions. Legal redress can utilize the new information on any salient aspect of the case, but legal action is expensive and even when affordable, it is time consuming, and the outcome is never sure. However, proponents argue that substitutions of legal redress for regulation frees the marketplace from restraints that greatly increase the cost of doing business, permits rapid change to take place in production and marketing and switches the cost of disease control from the taxpayer to the producer and user of the goods that were formerly regulated. However, when most taxpayers are either producers or users, shifting the cost is subbordinate to the size of the cost inherent in either of the two alteratives. I suspect that the cost of the untested legal approach to disease control will be far greater for the producer and consumer but not necessarily for the government.

The Natural Academy in its report to USDA, identified serious problems in the animal health facilities of USDA. The two major laboratories which have been in operation for about 20 years in one case and 30 years in the other, have not been kept up to the state-of-the-art for biocontainment. One of these laboratories can be remodeled at reasonable cost but remodeling the other is a questionable investment. The Academy recommended that USDA schedule replacement or remodelling of all laboratories to avoid future crises.

Recently I visited a large laboratory in a third world country that was sophisticated in its design, well engineered, and constructed to meet current standards for biosafety. It was superior to our two major USDA animal disease laboratories. It appears that the United States is lagging behind its international competitors in its program to defend its supply of meat, milk and eggs. We are allowing our laboratories to deteriorate and reducing our research and control programs. Is this really what the American public wants?

A recent letter to Science (3) took that magazine to task for major error of fact. A news item reporting the development of bioengineered malarial vaccine touted it as a breakthrough, saying there never has been a vaccine for a parasitic disease. Yet, vaccines for animal hemoparasites such as babesiois, anaplasmosis, and theileriosis and even for a lung worm nematod of sheep have been used very successfully in veterinary practice over twenty years. The public assumption, shared by editors and administrators, that human medicine is more innovative than animal medicine and that agriculture lags behind other branches of science persists in the face of countless examples of the contrary. As a result, funding for animal disease research and diagnosis remains a low priority item in government budgets. We are partly at fault as we have failed to document in a scientific fashion the costs of animal disease and the relative costs of alternative methods of control.

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QT35 CELL CULTURE FOR AVIAN VIRUS PROPAGATION

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Introduction

In 1977, Moscovici et al. (3) reported on the establishment of several continuous tissue culture cell lines from chemically induced tumors of Japanese quail (Coturnix coturnix japonica). They reported that most of these established cell lines were susceptible to subgroup A avian sarcoma viruses, but were relatively resistant to subgroup C, E and F viruses in comparison to normal quail embryo fibroblasts. Cho (1) found that the T and CS strains of reticuloendotheliosis virus (REV) could induce focus formation and cytonecrotic changes in one of the above described cell lines designated QT35.

Our interest in the QT35 cell line stemmed from several years search to locate a continuous cell line which would support the growth of a wide variety of avian viruses. We have evaluated a number of mammalian continuous cell lines (e.g. BHK-21, VERO, PK-15) with limited success. The ability to propagate a number of the more commonly encountered avian viruses in a continuous cell line would make virus and antibody quantitation less arduous and easier to evaluate. Additionally, it would be a valuable resource for laboratories with limited access to SPF embryos and chicks. The QT35 cell line may be a potential substrate for the biologics industry.

The mammalian virus evaluation was included to determine the sensitivity spectrum of this cell line.

Materials and Methods

QT35 cell culture - a continuous avian cell line derived from a methylcholanthrene-induced fibrosarcoma of Japanese quail. It is free of avian retroviruses and mycoplasma and the cells are predominantly of fusiform-fibroblastic morphology. The QT35 cells (60-85 passage level) were grown in 199-F10 medium (1) and subcultured (1:3 split; 2 times/week) by trypsin-versene disaggregation.

Primary and secondary CEF cell culture - these cell cultures were prepared by standard procedures from SPF chicken embryos and grown in medium 199 (2).

Avian and mammalian viruses - representative strains or serotypes of the following virus families were examined for their ability to replicate in QT35 cell culture: adenoviridae, coronaviridae, herpesviridae, orthomyxoviridae, paramyxoviridae, parvoviridae, picornaviridae, poxviridae, reoviridae, retroviridae and togaviridae.

Assays - avian and mammalian virus replication in QT35 cell culture was assayed by observing for cytopathic effect (CPE) in unstained cultures and direct immunofluorescent (DIF) reaction. Viruses were given 3 to 5 blind passages in this culture before being discontinued. Viruses and antibody were quantified by standard microtiter procedures.

Results

Inoculation of QT cell culture with high inputs of one or more embryo or cell culture (primary and continuous) adapted virus strains or serotypes per virus family resulted in 7 of 9 avian virus families (coronaviridae, herpesviridae, orthomyxoviridae, paramyxoviridae, poxviridae, reoviridae and retroviridae) and 1 of 8 mammalian virus families (paramyxoviridae) producing a cytopathic change. Of those virus strains found to produce CPE, the majority (66%) exhibited this effect on the first blind passage. If CPE didn't appear by the third blind passage, it wasn't found in any instance thereafter. The QT35 cell line appears to have greater sensitivity for avian viruses than mammalian and to be particularly responsive to the viruses of paramyxoviridae, poxviridae and reoviridae families. Cytopathic changes exhibited by tested viruses included focus, round-cell and syncytial formation. Additionally, avian reoviruses (only ones tested to date) were found to plaque under agar.

In growth comparison studies of avian reo and IBD viruses in CEF versus QT35 cell culture, it was found that these viruses produced comparable titers (10^5-$10^7 TCID_{50}$/0.05 ml) in both culture systems. Because of the respectable titers produced by the reo- and IBD viruses, microtiter virus neutralization (VN) tests were developed using 300,000 QT35 cells per well, 2-fold serum dilutions and 100 $TCID_{50}$ of virus. A comparison of IBD microtiter VN tests performed with CEF and QT35 cells revealed comparable antibody titers (no more than 4-fold titer differences) in both culture systems. We found antibody endpoints easier to determine and therefore more accurate using the QT35 cell culture primarily because of its stability.

Recently, we were able to demonstrate QT35 cell attachment to Lux and Flow Laboratory microcarriers. Cells were seen to uniformly cover the beads.

Summary

The QT35 continuous cell line was found to be relatively easy to manage and the cells were found to attach to microcarriers.

Representative strains or serotypes of 7 of 9 avian and 1 of 8 mammalian virus families were observed to produce CPE in 1 to 3 blind passages. Cytopathic changes exhibited by examined viruses included focus, round-cell and syncytial formation.

Avian reovirus and IBDV strains were found to replicate to equivalent titers in CEF and QT35 cell culture. IBDV antibody titers were comparable in the same.

The results of this study indicate a usefulness for the QT35 cell line in poultry diagnostic and research laboratories.

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RIBOFLAVIN DEFICIENCY IN BROILER CHICKS

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Summary:

Ten-day old broiler chicks from two farms exhibited signs of ataxia/paralysis in October 1984. The birds signs varied from birds walking on their intratarsal joints with toes curled medially, to birds in sternal recumbancy with their legs extended out behind their body. Approximately 15% of the birds in the affected houses showed these signs. Differential diagnoses included: Coban^{R} toxicity, chloride deficiency, B-vitamin deficiency, and arsenic toxicity. Post mortem examination revealed no gross lesions. Microscopically, the sciatic nerves showed vacuolar change, and mild perineural edema. Levels of arsenic and Coban^{R} in the feed were within normal ranges. Birds with mild ataxia recovered when supplemented with B-vitamins in their water or via injection, while severely affected birds did not. All evidence pointed to a riboflavin deficiency in the affected flock, even though it could not be substantiated by feed analysis or a bioassay on suspect feed.

History:

On October 12, Farm #1 called to report that their birds were down on their bellies with their legs sticking straight out behind them. The birds were from a 15,000 bird house, and were 10 days of age. They were vaccinated at day 1 with Marek's vaccine. Mortality in the flock was normal. The affected building was cool, approximately 65°F. The birds were confined with cardboard fencing to one-third of the house. Approximately 10-15% of the birds were ataxic. There were two forms of ataxia: One group (2/3 of the affected) were weak in the legs and walking on their hocks (intratarsal joint)-they had tightly curled toes; the other group were in sternal recumbancy with their legs extended behind them. The ataxia was evenly distributed throughout the house. The birds walking on their hocks were bright and alert, while those on their sternums were depressed. The litter was dry. Feed pans were filled with litter and wet, caked feed containing many insects. The waterers were 3 inches off the ground and functional. The grower felt that the birds progressed from mild ataxia to complete paralysis within a 24 hour period. A second barn on the farm showed no signs of disease-they were four days younger. Differential diagnoses included: Coban^{R} toxicity, B-vitamin deficiency, chloride deficiency, or arsenic toxicity. Recommendations given to the grower included: warm the building, lower the drinking cups to the floor, add vitamins to the water (both barns), and obtain a new batch of feed. Feed samples were sent to: Elanco to check Coban^{R} levels, Washington State University Diagnostic Laboratory for Coban^{R} and arsenic levels, and to Woodson-Tenant Laboratory for riboflavin content. Several birds were necropsied and tissues sent for histopathologic study.

On October 21, a grower in the same general area as Farm #1, reported that he had birds that were walking on their hocks. The birds were not related to those on Farm #1. They were 10 days old, and were vaccinated for Marek's Disease at a day of age. Both Farm #1 and #2 were fed from the same feed

company, and both received a load of pre-starter in the same week. The birds were immediately put on water soluble vitamins. Five days later the farm was visited because they had not responded to medication. The affected building contained 30,000 birds. The building was warm (80°F), and there was fresh food and water available. Approximately 20% of the birds were walking on their hocks, had tightly curled toes, and were weak on their legs. Necropsies of several birds were performed. The vitamin supplement was changed. Twelve affected birds were brought to the laboratory and half were injected intramuscularly with 0.5 ml of a B-vitamin complex (1mg riboflavin (5' phosphate sodium), 6 mg Thiamine, 50 mg Niacinamide, 5 mg Dexpanthenol, 2.5 mg Pyridoxine, 2.5 mcg Cyanocobalamin). Feed samples were sent for Coban^{R} analysis.

In January of 1985, fifteen day-old broiler chicks were subjected to a feeding trial. Eight were fed pre-starter from Farm #1 and seven were given feed supplied by the diagnostic laboratory. Birds were kept in batteries and supplied with heat, food, and water.

Results:

Necropsy of affected birds revealed no gross lesions in any organ system. On Farm #2, it was noticed that leg muscles of affected birds were flaccid. Brains, sciatic nerves, lumbar spinal cords, and skeletal muscles were submitted for histopathological examination. Microscopically, the sciatic nerves showed vacuolar change, and mild perineural edema.

Levels of Coban^{R}, arsenic, and B-vitamins (riboflavin) in the feed sample from Farm #1 were normal. Coban^{R} level in the feed from Farm #2 was in the normal range.

Farm #1: Mortality increased from 20 to 63 one day after initial outbreak, but decreased back to 20 after three days. On the fourth day, the grower indicated that it was difficult to tell that anything had been wrong with his birds. The second barn did not show signs of disease. The vitamin supplement was then discontinued.

Farm #2: After five days on vitamins (different product than what was used on Farm #1*) the birds appeared not to be improving. The vitamin product was changed. Three days later there was a marked decrease in the number of affected birds with no increase in morality. After one week it was difficult to pick out any affected birds.

Two of the twelve birds brought to the lab and injected with B-vitamins died within 24 hours. Necropsy of the dead revealed severe dehydration, and flaccid skeletal musculature. Four of the six treated birds showed full recovery after 36 hours, while the untreated (6 birds) showed no improvement after 48 hours. The six untreated birds showed full recovery 12 hours after they were injected with B-vitamins.

An attempt to recreate the disease in the laboratory using suspect feed from Farm #1 was unsuccessful. None of the birds showed gross or microscopic lesions after 14 days on test.

*(product used on Farm #1 had B-vitamins in it, this one did not)

Discussion:

Today's poultry feeds are formulated to contain more than adequate amounts of vitamins needed for the normal growth and development of the broiler chicken. The feed formulas usually have a margin of safety for the major nutrients to compensate for any losses that may occur in processing, transporting, storage and/or adverse environmental conditions. When vitamin deficiencies do occur, it is usually due to the manufacturer accidently leaving out the nutrient or the destruction of the vitamin during storage or processing.

Riboflavin is a required nutrient in poultry diets. Riboflavin deficient chicks generally have a good appetite, but grow very slowly. They become emaciated, weak, and don't move except when forced to do so. The chicks walk on their hocks with toes curled inward. Curled toes are typical at rest as well as when they are walking. Leg muscles are usually atrophied and flabby, and in advanced stages they lie prostrated with their legs extended. There are usually no marked abnormalities of the internal organs, but there may be gross enlargement of the sciatic and brachial nerve sheaths. Microscopically, nerves show definite degenerative changes (1,2). Farm #1 and Farm #2 had these classical clinical signs, showed positive response to oral and/or injectable vitamin supplementation, and had typical neuropathological signs characteristic of a Riboflavin Deficiency Disease.

The feed company tested their vitamin pre-mix and discovered that it was not supplying the riboflavin needed in the feed.

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OSTEOPATHY AND BONE GROWTH IN BROILER CHICKENS

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Introduction

Leg weakness represents a substantial loss to the poultry broiler industry. There are at least two etiologies involved. One, which is associated with cartilage retention in the growth plates of long bones, is usually bilateral and has been shown to be due to several nutritional imbalances. The other is termed osteodystrophy because it is a bone disorder which is usually unilateral and does not involve cartilage plug retention in the growth plates (Riddell, 1981).

This study is concerned with the pathogenesis of osteodystrophy. We measured the serum levels of $1,25(OH)_2D$ and calcium, and removed mid-diaphyseal sections for histomorphometry of undecalcified bone at euthanasia of 7-week-old male Hubbard broilers. Serum levels of $1,25(OH)_2$ were determined using a calf thymus receptor according to the method of Reinhardt et al. (1984). A commercial kit introduced by ImmunoNuclear Corp. (Stillwater, MN) was employed. Values given in Tables 1 and 2 are means $\pm$ SEM.

Table 1

Group (n)	$1,25(OH)_2D$	Ca(mg/dl)	B.W. (g)
Control (6)	116 $\pm$ 8.0	9.0 $\pm$ 0.5	2.2 $\pm$ 0.13
Affected (14)	79 $\pm$ 6.0*	8.7 $\pm$ 0.6	2.4 $\pm$ 0.12

*$p < 0.05$ significantly different from control

Measurements of bone parameters were done on undecalcified sections utilizing a BIOQUANT computerized image analysis system and a microscope fitted with a camera lucida. Osteoid is expressed as the percentage of total measured endosteal surface that consists of unmineralized matrix. Osteoclasts are listed as the number of cells on total measured endosteal surface multiplied by 100.

Table 2

Groups (n)	Osteoid	Osteoclasts
Control limbs (4)	17.6 $\pm$ 3.8	.52 $\pm$ 0.17
Affected limbs (8)	20.3 $\pm$ 4.3	.67 $\pm$ 0.09*

*$p < 0.05$ significantly different from control

Our studies show $1,25(OH)_2D$ serum levels to be reduced and the number of osteoclasts on the endosteum to be increased compared to normal (control) broilers. Vitamin D reduction may necessitate activation of osteoclasts in the bone in an attempt to keep serum calcium levels at normal. This is apparently not a rachitic disorder that involves increased osteoid. There may be a genetic deficiency in the kidney hydroxylation of 25-(OH)D and/or a bone turnover disorder that weakens the diaphysis and predisposes the affected broiler to mechanically induced twisting and lameness.

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LUNG GROWTH OF TWO LINES OF TURKEYS

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Introduction

As the turkey industry has selected for a larger and larger turkey with large pectoral musculature, has the lung responded to the increase in muscle mass with a concomittant increase in volume especially within the exchange compartment?

A morphologic and morphometric study was done to describe turkey lung growth then to compare the lung growth of two lines of turkey, a purposefully unselected and a highly selected line.

There were three phases of turkey lung growth in this study: 1. Tissue Proliferation; 2. Equilibrated Growth; and 3. Regulated Growth. During tissue proliferation, 22 days incubation to hatch day (28 days incubation), most lung compartments grew more rapidly in volume than the lung. The exchange compartment grew explosively, with air and blood capillaries increasing seven- and eleven-fold respectively, in volume while lung volume increased two-fold. Interparabronchial connective tissue and interstitial tissue of the exchange compartment decreased. In equilibrated growth, 0 days to 28 days of age, volume of most lung compartments grew evenly with lung volume. During regulated growth, 28 days to adult, volume of most compartments grew less than lung volume, though the exchange compartment and atria grew evenly with lung volume. (Figure 1).

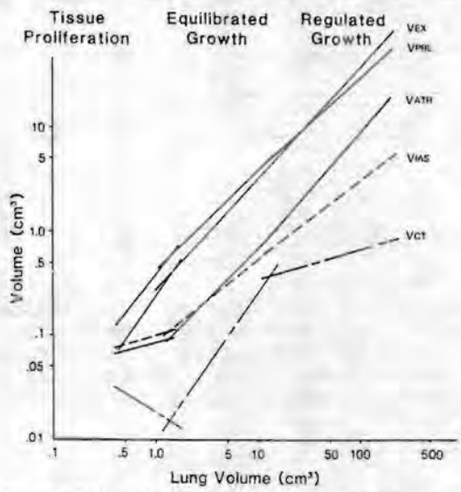

Fig. 1 Three phases of turkey lung growth

Allometric equations generated by log-log regression analysis of volumes and surfaces of compartments of the lung relative to lung volume and body weight were used to compare the lung growth of the two lines of turkey. Slopes and intercepts of the regression lines were compared to evaluate similarities and differences in growth of the lung of the two lines of turkey.

Materials and Methods

Male turkeys of a purposefully unselected line and a highly selected line were used in this study. Birds were sacrificed at 22 days of incubation, 25 days of incubation, hatch day, day 1, 4, 7, 10, 14, 21, 28, 112, and 420. Birds were weighed (W), euthanitized and phosphate-buffered formalin infused intratracheally at 20 cm H_2O pressure above the heart base. Lungs were removed, and lung volumes (VL) determined by weight displacement. A cascade sampling system was used to quantitate the compartments of the lung. Level one was counted on transversely cut lung slabs. Stratified random methods were used to select samples to process for microscopy. Level two and level three counts were done using the light microscope. The compartments studied were nonparenchyma (NP), which includes the large vessels (LVS) [greater than 100 u diameter] and the primary bronchus (PB), and parenchyma (P), which includes parabronchial (PRL) and atrial (ATR) lumen, interatrial septum (IAS), connective tissue (CT), small vessels (SVS), and the exchange compartment (EX). The exchange compartment is composed of the air capillaries (AC), blood capillaries (BC), and interstitium (INT). Volume densities of lung compartments and surface areas per reference volume were determined by point and intersection counting respectively. Absolute values were calculated and log-log regressions were done for allometric equations to determine stages of lung growth and differences or similarities between the two lines of turkey.

Conclusions

As the turkey industry has selected for a larger and larger turkey, with large pectoral musculature, it does not appear that the lung of the turkey has responded to the increase in muscle mass with a concomittant increase in lung parenchyma, especially the exchange compartment. The selected turkeys grow more rapidly than the unselected turkeys. (TimmWood, Julian)

Lung volume and body weight of both lines are similar at hatch but during the period of equilibrated lung growth the lung of the selected line of turkey grows more slowly relative to body weight than that of the unselected line. During the period of regulated growth, the lungs of the two lines appear to grow similarly relative to body weight (slope of the regression lines is similar) but the selected line has less lung volume relative to body weight than the unselected line. (Figure 2) Only the large vessels of the nonparenchyma grow more rapidly relative to the lung volume in the selected compared to the unselected line and grow the same relative to body weight in the two lines.

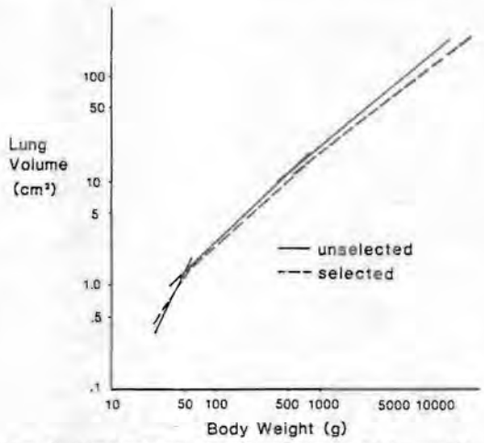

Fig. 2 Lung volume growth relative to body weight

The volume of exchange compartment grows similarly relative to lung volume in the two lines of turkey. But, growth of exchange compartment relative to body weight during equilibrated growth is less in the selected than unselected line of turkey; and during regulated growth, the exchange compartment grows similarly to body weight in the two lines but there is less exchange compartment for an equivalent weight turkey of the selected line than the unselected line. (Figure 3,4). Air capillary and blood capillary volumes and surfaces follow a similar pattern.

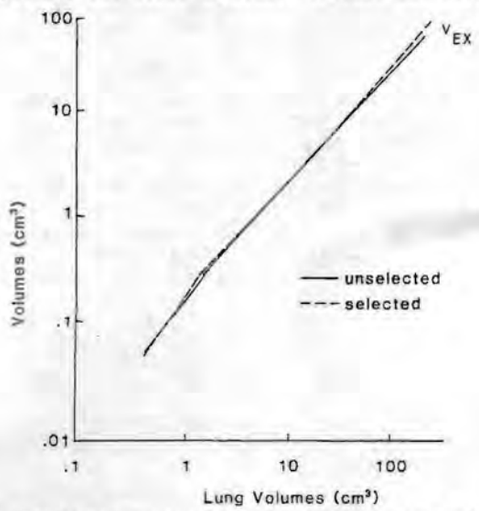

Fig. 3 Exchange compartment growth relative to lung volume

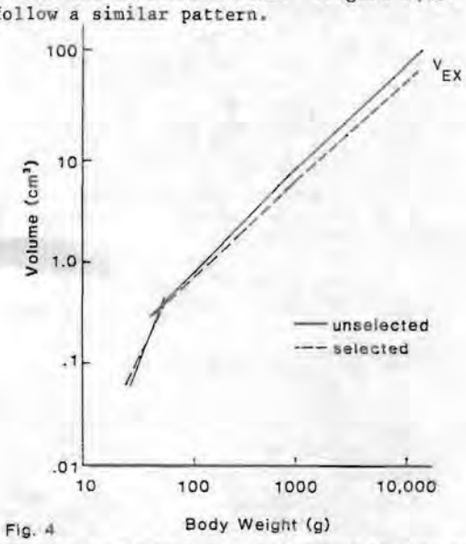

Fig. 4 Exchange compartment growth relative to body weight

In the adult turkeys, body weight is significantly greater in the selected birds while lung volume/100g body weight (specific lung volume), specific parenchymal volume, specific atrial volume, and specific volume of the exchange compartment are significantly lower (T-test p 0.05) in the selected line than the unselected line of turkey. (Table 1)

Table 1: Body Weight and Specific Volumes of the Lung and Some Compartments of the Unselected and Selected Lines of Turkey

Weight	Unselected Turkeys	Selected Turkeys
Lung volume/100 g W	1.344 ± 0.118	1.000 ± 0.049
Parenchymal volume/100 g W	1.162 ± 0.117	0.830 ± 0.034
Atrial volume/100 g W	0.110 ± 0.016	0.066 ± 0.011
Exchange compartment volume/100g	0.664 ± 0.057	0.465 ± 0.028

Is the turkey lung capable of developing more exchange compartment to respond to the large increase in muscle mass of the selected turkey? Either it is not, or, with more time further evolution of the lung will occur. The large increase in muscle mass of the selected turkeys has occurred only in the last 15-20 years, a very short time period.

Reference

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Cardiomyopathy and Ascites in Broiler Chickens

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In a survey of broiler chickens found dead on arrival at a processing plant in Saskatchewan, a low but widespread incidence of birds with ascites was found. In the examination of all mortality from two flocks 0.3 percent of total birds had ascites. In the two flocks different strains of broilers were identified by toe clipping and grown intermingled. All strains were affected. Birds with ascites were found as early as one week of age and the incidence increased throughout the life of the flocks until marketing at seven weeks of age. Seventy-two percent of affected broiler chickens were males. Affected birds had distended abdomens full of fluid and with large gelatinous clots. Hearts suffered from variable degrees of dilation. The ventricles were sometimes normal but when dilated the right was more affected than the left. The right atria and associated large veins were always dilated. The livers often had a thickened gray capsule and in some instances a fluid filled sac on the dorsal surface of the liver. This sac appeared to represent distension of the capsule. Most livers on cutting were firm and pale brown to red in color with distended large blood vessels. On preliminary histology no obvious microscopic lesions were found in the hearts but the livers had sinusoids distended with eosinophilic material and in some cases red blood cells. Many of the livers had greatly thickened fibrous capsules while others had widespread patchy coagulation necrosis. A few livers had both thickened capsules and necrosis. It is postulated that the primary defect in affected birds is cardiac dilation followed by intrahepatic edema with liver necrosis due to anoxia in birds dying from acute heart failure and the thickened liver capsule in birds dying from more chronic heart failure.

THE SIGNIFICANCE OF CAMPYLOBACTERIOSIS IN THE POULTRY INDUSTRY

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Introduction

Campylobacter jejuni is recognized as a primary pathogen in man[2] and domestic animals[3]. The organism which is responsible for enterocolitis can be isolated with frequency from a wide range of food-producing animals[5] including commercial chickens[24], turkeys[20], and their products[8,25]. The presence of C. jejuni in the intestinal tract is responsible for contamination of both processing equipment[27] and poultry meat at the point of sale[3,19]. The serological relationship between C. jejuni strains isolated from commercial poultry and the pathogens responsible for human enterocolitis[21] indicates the potential for infection. Epidemiological studies have implicated poultry as a source of human campylobacteriosis[1] and processing plant workers are more frequently infected than laborers in other agricultural occupations[10].

Classic "avian vibrionic hepatitis", which was frequently diagnosed in the 60's[16], is a specific clinical entity affecting mature breeders and layers. C. jejuni can be isolated so frequently from the feces of healthy broilers, breeders and layers[17] that the organism can be regarded as a commensal in the intestinal tract. Field and laboratory studies have shown that C. jejuni may be introduced into flocks by contaminated litter[14], equipment or infected flies[20], and free-living birds. Intraflock transmission and subsequent contamination of carcasses during processing results in a high prevalence of the organism in poultry meat and giblets[15,22]. Current methods of broiler growing on recycled litter at relatively high stocking density and the use of immersion tanks during processing favor the persistence and transmission of C. jejuni.

Possible approaches for reducing the potential for meat-borne campylobacteriosis include the application of competitive inhibition and the use of multi-tier mechanized growing which obviates the use of litter. The most practical, economic, short-term approach to decontamination is chemical or physical treatment of poultry carcasses and portions before packing and distribution.

Procedures

The objectives of the investigations which have been undertaken include the development of a method for quantifying C. jejuni on poultry meat, and evaluating the efficiency of alternative methods of decontamination including chemical treatment and irradiation.

The organism was isolated by inoculation of 0.01 ml aliquots of peptone solution onto brucella agar containing Butzler's supplement and 5% bovine blood. Plates were incubated at 43°C in sealed bags under microaerobic conditions (85% nitrogen: 10% carbon dioxide: 5% oxygen) for 48 hours. C. jejuni was identified on the basis of colonial morphology; nalidixic acid sensitivity; hydrolysis of hippurate and demonstration of characteristic motility after culture for 24 hours in brucella broth.

Broiler drumsticks were selected as the test system as they are essentially uniform in conformation and the surface area can be calculated by measurement of the length and girth of specific segments. Individual drumsticks were contaminated by immersion in plastic bags containing assayed suspensions of C. jejuni in peptone broth. Surface contamination was measured by immersing an infected drumstick in a bag containing peptone water followed by assay of serial dilutions of the wash solution.

The relative efficiency of chemical disinfectants was evaluated using a sequence of contamination, treatment and assay.

RESULTS AND DISCUSSION

The reduction in C. jejuni level, compared to controls is indicated in Table 1. Physical problems associated with chemical sprays or immersion, together with regulatory and economic restraints have stimulated an evaluation of irradiation as a method of decontamination[13]. Studies have shown the susceptibility of C. jejuni in meat products subjected to gamma irradiation derived from a Co_{60} source[12]. Although numerous workers have demonstrated the effectiveness of irradiation in reducing Salmonella contamination[9,18], the U.S. poultry and food industries have not applied this technique under commercial conditions. Initial problems with the organoleptic quality of products[4,7] were due to incorrect pre-irradiation treatment and high dosage levels. Using current technology it is possible to radappertize (sterilize by irradiation) chicken portions without producing deleterious effects on quality[26]. Experiments in the current series conducted concurrently with chemical decontaminants showed a reduction rate of 96% over controls using a radiation dose of 16 Krad with a level of contamination of 2.4×10^3 C. jejuni CFU/cm^2.

Current federal regulations consider irradiation as an additive, requiring expensive and complicated procedures to demonstrate that the unique radiolytic compounds which may be produced at dosages in excess of 10 Mrad are neither toxic, teratogenic, or mutagenic. At low dosages of 0.1 to 1 Mrad, no significant organoleptic or biophysical changes are produced, although pathogens and spoilage

microflora are destroyed. The need to develop innovative methods of sterilization of food products and the potential carcinogenicity of grain fumigants and chlorine-based disinfectants has led to a reappraisal of chemical preservatives. It is anticipated that the FDA will permit low level irradiation as a "process", facilitating commercial application of new technology. Based on the operation of pilot plants and processing units in 18 nations, irradiation at levels of 0.1 to 1 Mrad will be achieved at a cost of 2-4¢/lb.

Campylobacteriosis, which is emerging as a public health problem, has the potential for damaging the image of the poultry industry as a provider of high quality and inexpensive food. The use of innovative technology, manipulation of intestinal flora, and the application of low-level irradiation will all be involved in reducing the impact of C. jejuni and other enteric pathogens which detract from quality and wholesomeness.

TABLE 1

Decontamination of C. jejuni on drumsticks

Inoculum Concentration	1.8×10^6 CFU/ml		
Post-Contamination count	4640	CFU/cm^2	
Control count	2350	CFU/cm^2	% reduction
Hypochlorite 200 ppm; 15 minutes	681	CFU/cm^2	71
Glutaraldehyde 0.125%; 15 minutes	391	CFU/cm^2	83
Succinic acid 3%; 15 minutes	902	CFU/cm^2	62
PHMB 15 ppm; 15 minutes	342	CFU/cm^2	85
Irradiation 16 Krads	105	CFU/cm^2	96

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THE DEVELOPMENT OF PHARMACOKINETIC MODELS FOR POULTRY THERAPEUTICS

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Although the emphasis in poultry medicine is on prevention, the economic impact due to disease remains high. And though various primary insults including viruses may be the initiators of a disease problem, bacterial organisms and their close relatives often are responsible for the high economic loss associated with many disease syndromes in poultry. The use of various antimicrobial drugs to treat these problems is widespread; yet when one attempts to follow rational therapeutic strategy, a great deal of information seems to be lacking.

Rational antimicrobial therapy requires the following information be known to the clinician:

1. The antimicrobial sensitivity pattern of the disease-causing organism.
2. Knowledge of the pharmacokinetics of the various antimicrobial drugs which are available for the animal species in question.
3. Proper dosage of the antimicrobial drug of choice in order to achieve the necessary serum and tissue levels to successfully treat the disease.
4. Withdrawal period necessary to avoid product residues.

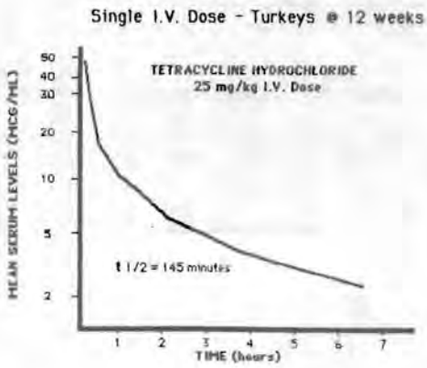
The antimicrobial sensitivity pattern of a disease-causing organism (minimum inhibitory concentration values) should allow the clinician to focus on the most promising drugs for the particular disease problem. The pharmacokinetic parameters of drug absorption, distribution, metabolism, and excretion (in the target animal species) provide required information to evaluate the practical application of a particular drug therapy strategy. The essence of this information will allow the clinician to arrive at the proper route and dosage of the most efficacious antimicrobial drug. It should be emphasized that there are probably more treatment failures due to improper dosage than due to the use of the improper drug.

Since the majority of antimicrobial drugs available for use in the poultry industry today were licensed before the requirements for pharmacokinetic and efficacy studies were in place, much of this information is not obtainable. It has been the goal of our group to develop this information for the antimicrobial drugs currently in use.

Our approach has been to design a series of animal models to evaluate various antimicrobial products in poultry species. These models include a single intravenous dose study, a single oral dose study, and a continuous oral administration in drinking water. We also have developed models for evaluating subcutaneous as well as intramuscular injection studies.

We recently completed some work evaluating the pharmacokinetics of tetracycline hydrochloride in turkeys. The examples below illustrate the type of information which is generated from these study models.

FIGURE 1



The data shown in Fig. 1 were generated using five birds. A single intravenous dose of 25 mg/kg was administered in the left wing vein. The birds were than bled at 5, 15, 30, 60, 90, 120, 180, 240, 360, and 480 minutes post injection. The samples were collected from the wing and jugular veins. We have recently revised this model to include ten birds with the early samples being taken at 10, 20, 35, and 60 minutes and the later samples going out to 12 hours. The curve derived from this model allows predictions on the distribution and elimination of the drug.

The data shown in Fig. 2 were generated with ten birds. A stomach tube is passed down to the region of the proventriculus where the calculated dose is delivered. The birds were then bled at 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, and 720 minutes post dosing. Using the stomach tube to bypass the crop results in more uniform delivery of the drug to the gastrointestinal tract. Analysis of these data along with the IV data allows predictions of drug absorption rates, percent absorption, and a modified drug "half life," (see Table 1).

FIGURE 2

Single Oral Dose - Turkeys @ 12 weeks

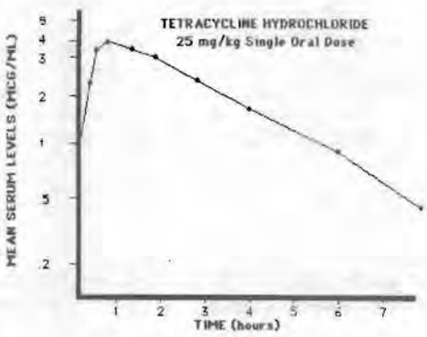


TABLE 1. Pharmacokinetics of tetracycline in turkeys (N=9) following oral administration of a single dose of tetracycline (HCl (25 mg/kg) by lavage tube.

Kinetic Parameter	Units	Experiment II
$C_{o(hypothetical)}$	µg/ml	5.07 ± 1.4
k_a	h^{-1}	3.0963 ± 1.189
$t_{\frac{1}{2}(a)}$	h	0.265 ± 0.14
k_d	h^{-1}	0.3086 ± 0.14
$t_{\frac{1}{2}(apparent)}$	h	2.44 ± 0.77
$AUC_{(po)}$	µg·hr/ml	16.75
F	%	28

The data shown in Fig. 3 were generated by housing turkeys in separate pens with 20 birds per pen. Each pen has a five gallon graduated container which delivers the treated or untreated water to each waterer. In this study, tetracycline hydrochloride solutions were prepared daily at 6.4 gm/gal, 3.2 gm/gal, 1.6 gm/gal, and 0.0 gm/gal of distilled water. Water consumption was monitored to evaluate palatability. Table 2 shows the water consumption data.

FIGURE 3

Continuous Oral Administration in Drinking Water
Turkeys @ 12 weeks

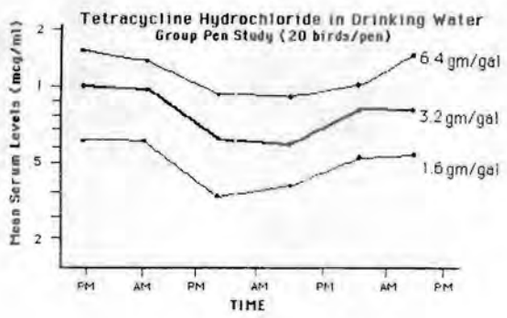


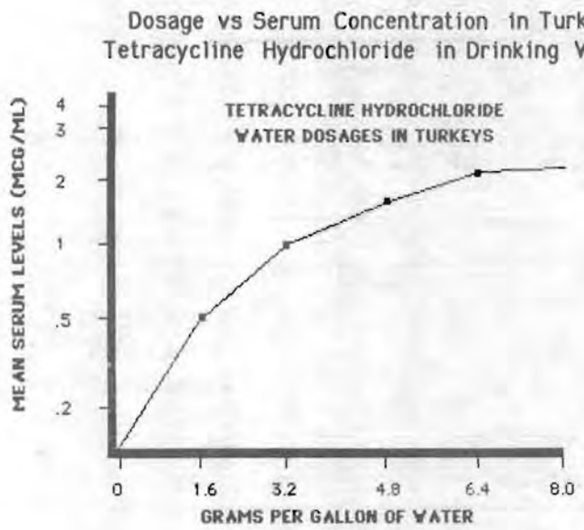
Table 2. Water Consumption in Turkeys with Various Doses of Tetracycline Hydrochloride in the Drinking Water.

Treatment	Water Consumption
Distilled Water Only	.22 gals/bird
1.6 gm/gal	.22 gals/bird
3.2 gm/gal	.22 gals/bird
6.4 gm/gal	.15 gals/bird

*Summer temperature average = 100F.

The information derived from these studies can be used to generate useful charts (e.g., Fig. 4) that can be an aid in planning clinical therapy. When you consider the economic costs which are at stake when a disease problem occurs, the best therapeutic course must be set. To successfully navigate this course requires accurate charts. These charts should allow us to follow rational, scientific, drug therapy so we may arrive at our destination of the most cost-beneficial outcome in the management of a particular disease problem.

FIGURE 4



THE EVALUATION OF FEED MEDICATIONS FOR TURKEY DISEASES: A COMPUTER SOFTWARE PROGRAM

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INTRODUCTION

The diagnosis of a serious disease in a flock of turkeys always leads to the difficult problem of the selection of the optimum medication. The selection process is complicated by the interaction of biological efficacy, cost, and drug withdrawal period. The biological efficacy of each product is reviewed by the veterinarian followed by the appropriate selection of the drug of choice. Often the cost of the medication is critical, especially in medication programs that require several weeks of continuous therapy. The drug withdrawal period is important if the flock requires medication within a few weeks of marketing to prevent tissue residues in the final meat product intended for human consumption.

The development of the computer has now made it possible to organize and greatly simplify the medication selection process for the veterinarian. The microcomputer, which now provides extensive computer capabilities at a nominal cost, is especially suited to aid the veterinarian in the diagnostic laboratory. With the necessary software, a microcomputer can become an important aid to the veterinarian to evaluate these complicated relationships.

The purpose of this paper is to describe the development of a unique software program that can be used as an aid by the veterinarian in the review of feed medications for turkey diseases. The software program "TURKEYMED" permits the veterinarian to efficiently review the total list of medications approved for the specific turkey disease including the specified levels of use and the required drug withdrawal periods. It automatically calculates the cost per bird of any of the selected medications making it possible for the veterinarian to use this additional data for the comparison of products with great speed and accuracy.

MATERIALS

A computer software program has been developed for the IBM-PC type computer using the common BASIC programming language. A total memory of 64 K is required for the computer to operate this program. BASIC was selected as the programming language because it is commonly available on the majority of all computers. The program is written in a manner that does not require any special knowledge of computer commands to operate the program, it is totally menu driven. Thus, anyone with access to an IBM-PC type or IBM compatible computer will be able to operate this evaluation program. All commands required to operate the program are given by the selection of a number from the screen. The program is initiated by turning the machine on, inserting the floppy disk into the machine and typing TURKEY. After a short interval required by the computer to load the program, the title will appear on the screen indicating that the program is ready for the first selection command. The exit from the program is automatically accomplished by answering a prompt on the screen.

OPERATION OF PROGRAM

The software program was developed by dividing the evaluation process into four different phases. The first phase is to list all the turkey diseases for which the U.S. Food and Drug Administration has approved the use of medicated products in the feed.

TABLE 1

```
                    ****TURKEYMED****
          PROGRAM TO EVALUATE FEED MEDICATIONS FOR TURKEY
*******************************************************************************
                          TURKEY DISEASES

 1.  BACTERIAL ENTERITIS              13.  MUCUS
 2.  BLUECOMB                         14.  MYCOSIS, CROP
 3.  BREAST BLISTERS                  15.  MYCOTIC DIARRHEA
 4.  CHRONIC RESP. DISEASE            16.  PARACOLON
 5.  COCCIDIOSIS                      17.  PARATYPHOID
 6.  FOWL CHOLERA                     18.  PULLORUM
 7.  FOWL TYPHOID                     19.  QUAIL DISEASE
 8.  HEXIMITIASIS                     20.  SECONDARY BACT. INVASIONS
 9.  HISTAMONIASIS                    21.  STRESS
10.  INFECTIOUS HEPATITIS             22.  SYNOVITIS
11.  INFECTIOUS SINUSITIS             23.  TRANSMISSABLE ENTERITIS
12.  LEUCOCYTOZOONOSIS                24.  WORMS
                          25.  EXIT PROGRAM
*******************************************************************************
     ENTER THE NUMBER OF YOUR SELECTION>
```

TABLE 1 lists the twenty-four different diseases of turkeys that comply with these standards. It is important to note that the list includes some of the disease related conditions such as STRESS that have approved feed medication regimens for turkeys. The veterinarian must select one of these diseases by typing the specific number followed by pressing the return key of the computer keyboard.

This command will cause the computer to react to display another table that will begin the next phase of the evaluation, a review of all of the approved feed medications for the selected disease. Each of the specific diseases listed have all the approved single entity drugs, the levels of approved use, and the withdrawal time, in days, that may be required for each medicated product. TABLE 2 is a typical example of these tables.

TABLE 2

```
**************************************************************************
HEXAMITIASIS

MEDICATION                          USE LEVEL          DAYS W/O REQ
   40.  BUTYNORATE                  40).0375%            40)  28
   41.  CHLORTETRACYCLINE           41) 50-200 G/TON     41)  0
   42.  FURAZOLIDONE                42) 100-200          42)  5
   43.  OXYTETRACYCLINE             43)  50-200          43)  3

 HISTAMONIASIS
   44.  CARABARSONE                 44).025-.0375%       44)  0
   45.  DIMETRIDAZOLE               45).015-.08%         45)  0
   46.  FURAZOLIDONE                46) 200 G/TON        46)  5
   47.  IPRONIDAZOLE                47).0625-.025%       47)  4
   48.  NITARSONE                   48).01875%           48)  5

 INFECTIOUS HEPATITIS
   49.  FURAZOLIDONE                49) 100 G/TON        49)  5

 LEUCOCYTOZOONOSIS
   50.  CLOPINDOL                   50).0125-.025%       50)   5

**************************************************************************
  ENTER THE NUMBER OF MEDICATION DESIRED>
```

TABLE 2 shows four diseases displayed in the same manner as they will appear on the computer screen. Note that there are five different feed medications approved for use against Histamoniasis. The display of all of the approved medicated products under the heading of the specific disease permits the veterinarian to review the total approved list. The range of the approved levels is listed but the veterinarian must use the appropriate level for prevention programs or therapeutic programs. The number of days required for drug withdrawal for each medicated product is also included in these tables. TABLE 2 shows the variation of 0 to 28 days being required for different products. Since the combinations of feed medications is an extremely long list, the combinations are not included in TABLE 2. In the interest of clarity, the combinations should be reviewed in the appropriate official documents if needed for a specific case. The veterinarian must select one of the medicated products by typing in the number of the selection which will automatically make the computer enter the next phase of the evaluation process.

The third phase of the program relates to the important question of the cost of the medication so that the veterinarian may compare any of the approved products on a cost per bird basis. This portion of the program will automatically calculate the cost of medicated feed per bird per day. The necessary information on the cost of the medication and the age of the birds must be entered in response to the specified questions.

TABLE 3 CALCULATION OF MEDICATION COST PER BIRD

```
**************************************************************************

    ENTER THE COST OF PER TON OF MEDICATED FEED
    NO DOLLAR SIGNS ($) PLEASE; EXAMPLE(186.47) > 186.55

    ENTER THE AGE OF THE BIRDS IN WEEKS
    WHOLE NUMBERS ONLY PLEASE (MAX=27)          >8
**************************************************************************

    FOR:        HISTAMONIASIS

    USING:      DIMETRIDAZOLE

    THE COST PER BIRD IS     > 2.6677 CENTS PER DAY
    (HEAVY BREEDS, TOMS)

    DO YOU WANT TO EVALUATE ANOTHER MEDICATION OR DISEASE? Y/N >
```

As shown in TABLE 3, the cost per ton of medicated feed must be entered in whole numbers. The program is constructed to do the calculations with either the cost of the medication per ton of feed or the cost of the entire ton of medicated feed. The second piece of data required is the age of the birds to the nearest week of age so that the feed consumption per day can be used in calculations of the cost per bird. The daily consumption of the birds at various ages has been calculated from the standard NRC tables (2). Upon entry of the age of the birds, the computer will automatically calculate the cost per bird per day for the selected medication.

An important part of the program, the fourth phase, is then initiated by the question "DO YOU WANT TO EVALUATE ANOTHER MEDICATION OR DISEASE?" which is displayed to invoke a yes or no response. A yes response will permit the veterinarian to select a second medication to compare costs. The comparison of medications can be repeated as many times as desired in order to complete the evaluation process. Due to the speed of the computer, a comparison can be made in a matter of seconds. A no, or N response automatically causes the computer to display the end of the program message and to prepare the computer for other assignments or a complete shutdown.

CONCLUSION

The diagnosis of a disease in a flock of turkeys must be followed by a complicated process regarding the evaluation and selection of the proper medication. A microcomputer with the software "TURKEYMED" can be used as an important aid to the veterinarian in this process. All the different medications that have been approved for use can be easily reviewed and the software will accurately calculate the cost per bird per day of any of the medications desired. Due to the tremendous speed of the computer, many different medications can be evaluated and compared with ease. "TURKEYMED" is an example of computer software that can be used by the veterinarian to improve the diagnostic procedures. There is a critical need for additional software programs to aid the veterinarian.

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A NEW TECHNOLOGY FOR SOLID PHASE ENZYME IMMUNOASSAY FOR DETECTING OF INFECTIOUS DISEASES

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In recent years enzyme-linked immunosorbent assay (ELISA) has gained widespread application for rapid viral diagnosis of both antigens and antibodies. The high sensitivity of the technique made this type of assay very attractive. Numerous enzyme-linked immunoassays for detecting avian pathogen antibodies in serum have been reported.

The present paper reports on the development of a new solid-phase enzyme immunoassay - ImmunoComb - that permits the visual detection of avian pathogen antibodies as well as the pathogens. The technique was developed on a new concept: operation of the kit in the field and immediate analysis of the results.

The ImmunoComb is a plastic device which has twelve projection teeth sensitized with an antigen. Ten of these teeth are for samples and two for internal positive and negative controls. On the upper part of the Comb a standard curve is developed simultaneously with the samples. The Comb is processed in a special plate equipped with compartments for each specimen, as well as for the immunological and enzymatic reactions. Most of the reagents are already in the suitable compartments. The last step of the test is formation of a colored insoluble product which is adsorbed to the plastic at the spots where the immunological reactions have taken place. The intensity of the color varies according to the amount of conjugate bound to the tooth and is proportional to the titer of antibodies in the sample tested. The use of the ImmunoComb makes the test versatile and its evaluation visually is accurate enough for practical purposes. Thus the test can be performed without a special laboratory. It can be easily used for photometric reading, however, by inserting the Comb into ELISA plate containing a substrate yielding a soluble colored product. Another advantage of ImmunoComb is the possibility to apply more than one antigen on the Comb. It is possible to detect in a single blood sample antibodies to four different pathogens. These properties endow the ImmunoComb Kit with field applicability. Therefore such a diagnostic kit could expedite the logistics of flock profiling in many ways.

A NOVEL RAPID TEST KIT FOR THE DETECTION OF NEWCASTLE DISEASE VIRUS ANTIBODIES

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A sensitive diagnostic kit for the rapid visual detection of Newcastle disease virus (NDV) antibodies is described. The kit - ImmunoComb - is based on a modification of the enzyme-linked immunosorbent assay and was found to be highly sensitive in detecting local immunity as well as marginal levels of humoral immunity. Therefore it provides the determination of state and efficiency of vaccination. The assay is simple and does not require the use of special equipment nor laboratory facilities. This endows the new kit with field applicability. The effectiveness and reliability of the kit were proven by a series of field experiments. Tracheal swabs and blood samples were taken from 20 flocks (chickens and turkeys) and were tested by the HI test and by the new kit. A comparative analysis showed a high correlation between the methods where the level of antibodies to NDV in the flocks ranged between negative and very high (Table 1).

Table 1. Summary of results obtained with ImmunoComb on blood and tracheal mucus from different flocks and agreement with serum HI titer

Bird Type	No. of flocks	Age	Number of flocks showing agreement[a] between ImmunoComb and HI	
			Blood	Tracheal mucus
Chicken				
Layer	7	12-17 months	6/7	6/6
Breeder	2	8-10 months	2/2	1/1
Broiler	4	5-6 weeks	4/4	1/4
Replacement	2	3-4 months	2/2	2/2
Turkey	5	9-10 weeks	5/5	5/5
TOTAL	20		19/20	15/18

[a]Significant difference level $p > 0.05$.

USE ON FIELD OF CONCENTRATED (HIGH ANTIGEN LEVEL) OIL EMULSION NEWCASTLE DISEASE VACCINES IN BROILERS.

Lozano D. Bernardo (A) Tinoco G. Hector (A)
Morfin Rogelio (B) Lozano D. Jorge (C)

INTRODUCTION:

Viscerotropic velogenic Newcastle disease (VVND) has been reported in México since 1946. Although many efforts has been carried out to prevent and control this disease, VVND is still enzootic in our country. Besides good husbandry and the implementation of sanitation measures, vaccination of our flocks, with live virus and inactivated virus oil emulsion (OE) vaccines is a common practice.

The purpose of emulsified vaccines (water in oil) is to elevate antibodies titer means on the HI test, in such a way that they can prevent mortality, (5,6) meanwhile with the use of a live virus one expectes to produce local immunity, that should help to prevent infection (1). More recently, research shows that very high HI titers, besides giving protection reducing mortality, will also avoid isolation of the virus after a challenge (7). European OE ND vaccines (2) (5) have shown to estimulate high HI titres in broilers, obtaining the benefits mentioned above. OE vaccines were launched commercially in México since 1969 and have been used massively since 1973. Some research (4) and unpublished data (3) show big differences between the immunological response produced by the six OE commercial ND vaccines available in México, confirming that just three of them produce acceptable levels of antibodies wich will decrease mortality rate yet they are not capable of preventing isolation of a challenged virus.

The purpose of this paper is, first of all, to report the results with an OE ND vaccine with high levels of antigen that could confere not only a good protection against mortality, but better epizootiological control of the disease reducing the time of isolation and shedding of the challenged virus.

A.- Pfizer Laboratories - Mexico C.- Anáhuac University - México
B.- Vineland Laboratories - Mexico

MATERIAL AND METHODS.

Chickens.

One hundred and ten commercial broilers of one day old, were housed in a commercial farm, which was kept empty 2 months prior to cleaning and desinfection in such a way that no evidence of NDV was founded by collected swabs and tested by CE inoculation. They were identified and divided into 3 groups, two groups of 50 chickens and one group of 10 chickens were kept as unvaccinated controls. They were fed commercial feed with a starter (1 Kg.) and finisher (4 Kg.) program.

Antigen and Experimental Vaccine preparation.

Antigen consists in strain La Sota of ND. It was propagated in embroyonated chicken eggs 9 days old. The allantoic fluid (AF) was harvested, clarified by centrifugation with a sharples centrifuged (4000 rpm) and inactivated with 0.5% formalin (V/V) and the absence of residual infectivity was confirmed by embryo inoculation. Thimerosal was added at 0.01% (V/V). The HA titer of the AF was 1280 U and the infectivity titer was 9.1 ELD 50% per 1.0 ml. OE experimental vaccine was made following commercial procedures, on a 50 liter batch, adding tween 80 as an aqueous phase emulsifier and span 80 (similar to Arlacel) as an oilphase emulsifier. Each dose of 0.5 ml contained 0.18 ml of AF.Mineral oil (Drakeol) was used as oil phase. The Aqueous - Oil ratio was 45:55. The viscosity was 259.86 centistocks (cts) and the stability was < 32 weeks at room temperature.

Experimental design.

Group A, consisting of 50 animals, received 0.5 ml subcutaneously (via neck route) of an OE ND commercial vaccine and one drop ocular route of commercial live virus vaccine (La Sota strain) at 10 days of age. At the same time 50 chicks of group B, received 0.5 ml subcutaneously of an OE experimental vaccine and live virus (same strain and route than A.) Group C with 10 birds were kept as unvaccinated controls on another premises. A ratio of 15 birds per group were bled on a weekly basis, and sera obtained was tested with HI microtest recording the titers on log. base 2. At 6 weeks of age, 10 birds of group A,B and C were challenged each with 10^6 CELD 50% / chicken via ocular route with a VVNDV, Chimalhuacán strain (native strain) and 5 birds of each group were bled and slaughter in order to achieve serological or histopatological changes for detecting IBD.

Vaccine Efficacy:

Vaccine efficacy was evaluated by two different means. The first consisted of a microtiter HI test and the other was the response to the challenge with virulent virus. Mortality was recorded during 14 days, post inoculation.

RESULTS.

No evidence of IBD was found in the histological and serological examinations. The unvaccinated controls were negative to the HI test until they were challenged. All of them died 5 days post-challenge.

The mean titers, expressed in $\log_2$, are summarized on table 1, and as it was expected, the experimental vaccine showed better responses on the HI test and similar results on protection versus the normal one (see table 2). The titers of the two vaccines showed significant differences ($P < 0.05$). The chicken showed very low maternal antibodies when vaccinated. (GMT = 0.75 $\log_2$).

Table 1

Mean titers of antibodies detected by the HI a) test.

AGE (days)	20	29	36	42	49	56
Commercial	.10 b	4.5 b	6.17 b	4.75 b	4.17 b	5.09 b
Experimental	.33 b	5.6 c	7.45 c	8.33 c	6.67 c	6.93 c

a) Expressed in $\log_2$.
b,c) Numbers with different letter differes statistically ($P < 0.05$)

Table 2

Results after challenge with virulent ND virus.

	Individual titers [a]	GMT	CH/D [c]	%Mortality
Commercial	3(1), 4(4), 5(4), 6(3) [b]	4.85	10/0	0
Experimental	6(2), 7(1), 8(5)	8.5	10/0	0
Unvaccinated	0(8), 1(2)	0.2	10/10	100

a) Expressed in $\log_2$.
b) Expresses the number of sera with the same titer.
c) Number of birds challenged / number of deaths.

DISCUSSION.

The OE experimental vaccine was produced following commercial procedures. The antigen was not concentrated by ultracentrifugation or PEG precipitation, but 3 times fold AF was added, compared with commercial vaccine. Indeed, there are some scientific evidence of using 100 times the antigen prepared by ultracentrifugation not obtaining good results (5). Another interesting point was the use of commercial chickens on field conditions. No matter the use of live ND vaccine was used, this result agree with other experiments in the sense that the potency of the vaccine was not increased by this, getting similar results in the sense of mortality. The fact of getting higher HI titers when more antigen was used is quite promising. We believe that these results and what others have reported, remarks that this type of OE vaccine can play a very important role in the disease control not only because the potency of the HI titers to prevent deaths, but also to reduce morbidity and virus multiplication on target systems of the body and its excretion.Actually another test is running in order to prove that this high antigen level OE vaccine, can be used in a reduced doses in one day old chicks, (2) in order to obtain high HI titers trying to correlate it with the time of excretion of challenge virus, and its persistance in the premises.

Finally, we want to emphasis the importance of good sanitation and husbandry for the prevention and control of the disease and not relying only on vaccination.

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DIAGNOSTIC OBSERVATIONS AT THE TURLOCK POULTRY PATHOLOGY LABORATORY

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This presentation will encompass two aspects of diagnostic activities at our laboratory - first a summary of the most common diagnoses during the past year (1983-84), and, secondly, some brief commentary on some recently observed disease problems. It seems appropriate to preface this paper with a brief summary of the poultry population we serve. The Turlock Laboratory provides diagnostic service to poultry populations in San Joaquin, Stanislaus, Merced and some surrounding counties. This geographic area encompasses production enterprises with about 11 million laying hens, annual broiler output of about 150 million birds and annual production of about 14 million turkeys. It is probably also appropriate to note that this area is served by private disease diagnostic laboratories and several veterinarians employed by producers, breeders or biologics manufacturers. The diagnostic summary presented here is based solely on necropsy accessions processed through the California Veterinary Laboratory Services facility at Turlock.

Summary of Most Frequently Diagnosed Diseases

Table 1 presents data on the 15 most frequently diagnosed diseases seen and types of poultry affected. You will note that, with the possible exception of Alcaligenes-induced respiratory disease, there's nothing very new or exotic on our "hit parade". The "big four" at the top of our list are coccidiosis, E. coli infections, Marek's disease and staphylococcal infection. It is somewhat surprising with all of our technology, that these diseases are seen with great frequency. However it is important to make a distinction between frequency of diagnosis and economic impact. One would hope at least that once diagnosed, these diseases can be more effectively treated or controlled in affected flocks than in the past. Similarly, neither morbidity nor mortality should be as great with Marek's disease breaks as would have been the case in pre-Marek's vaccine days. Disease frequency varies widely by poultry category - i.e. Marek's disease was the most common diagnosis in Leghorn chickens while E. coli infections topped the list for broiler chickens and turkeys. A sad and frightening statistic is the frequency of Mycoplasma gallisepticum infections in Northern California egg-type chickens and turkeys. M. gallisepticum infections accounted for 29 of 815 diagnoses rendered in 1983-84. The balance of information in the diagnostic frequency table will be left to speak for itself.

Table 1

MOST COMMON POULTRY DISEASE DIAGNOSES - TURLOCK LAB

	Chickens		Turkeys	Total Diagnoses
	Egg	Meat		
Accrued Diagnoses 7/1/83 - 6/30/84	408	51	356	815
1. Intestinal coccidiosis	42	5	30	77
2. E. coli related diseases	12	12	50	74
3. Marek's Disease	69	1	0	70
4. Staphylococcus related diseases	41	1	19	61
5. Enteritis/unidentified etiology	3	1	41	45
6. Enteritis - ulcerative/necrotic	27	0	18	45
7. Aspergillosis	3	0	38	41
8. Candidiasis	0	0	38	38
9. Mycoplasma gallisepticum (includes pos. serology)	16	0	13	29
10. Omphthalitis	22	4	0	26
11. Rickets	11	4	6	21
12. Pasteurellosis	8	0	13	21
13. Alcaligenes faecalis	0	10	10	20
14. Salmonella isolates	2	4	13	19
15. Round heart	0	0	14	14
	256	42	303	601

Specific Diseases or Syndromes of Interest

Three Diagnostic Challenges - Cranial Cholera, Bronchial Aspergillosis and Encephalomalacia in Turkey Poults. Frequently, in the diagnostic laboratory, a case is received which shows very little gross pathology on initial post-mortem, and a closer and more meticulous examination is required to find the lesions. Three such cases are discussed here.

The first case involves a flock of 14 week old meat turkeys with infectious sinusitis that were exhibiting torticollis, loss of balance, and extreme depression. The initial post-mortem exam was unremarkable, and it was not until the cranium was opened that a yellowish caseous exudate was seen filling the air spaces of the cranial bones, and the middle ear canal. Cultures from the exudate, and in some cases from cerebellar and cerebral tissues as well, yielded Pasteurella multocida. Histopathology revealed a fibrino-purulent process in the air spaces and meninges, with the infiltration of heterophils, production of fibrin and formation of giant cells around the caseous masses of exudate. This condition fits well with the description of cranial cholera, a chronic form of fowl cholera, and although this flock had experienced an outbreak of acute fowl cholera some three weeks earlier, no evidence of acute cholera was found in any of these birds.

A second case involves a group of young seven day old broiler chicks from a flock that was experiencing increased mortality and showing some respiratory distress. The birds were depressed, and some were gasping for air, but no rattles, gurgles, or other respiratory noises were audible. Initial examination showed little in the way of lesions, until a closer look was given to the bronchial tubes, from which mucus plugs could be expressed. A wet mount of this mucus material, stained with methylene blue, demonstrated the presence of fungal hyphae subsequently identified as Aspergillus fumigatus.

A third example of cases seen in the diagnostic laboratory, which show little in the way of pathology on initial post-mortem examination is encephalomalacia in turkey poults. Typically, these birds are male, approximately three weeks of age, and are often among the largest,healthiest appearing birds in the flock. They are often described by the grower as being "down in the legs", and they do appear ataxic. They may lay on their sides, and flap the wings in an attempt to stand. Once again, the birds appear remarkably free of lesions, until the cranium is opened to reveal a cerebellum which may be edematous and red to brownish in color. More often though, the cerebellum will look normal grossly, or at most contain small yellowish necrotic foci, and it is not until the tissue sections are examined histologically that the characteristic lesions of encephalomalacia are found.

Amyloidosis in Layer Ducks. A commercial balut (embryonated duck egg) producer with 6000 laying ducks complained of low egg production in the second cycle. The ducks laid about 80 to 90 percent the first cycle but obtained only a 50 percent egg production for the second lay cycle. The producer had primarily White Pekin, Rouen, and Khaki Campbell breeds. He produced approximately 150, 150, and 200 eggs/year from those three breeds, respectively. Samples consisting of eggs (day 14 of incubation), ducklings (1 day old), and laying hens (second lay cycle) were submitted to the lab for diagnostic workup.

Salmonella enteritidis and Escherichia coli were isolated from the eggs. Omphalitis caused by E. coli was diagnosed in the ducklings.

Necropsy of the three laying ducks revealed hepatomegaly and splenomegaly in all three ducks. Peritonitis and ascites were observed in two ducks. Pasteurella multocida was isolated from the liver, spleen and peritoneum. Gastrointestinal and respiratory systems appeared normal. All three were inactive layers.

The liver showed the most remarkable pathology. Grossly, it appeared three to five times normal size and occupied almost the entire abdominal cavity. It was white to yellow-orange and firm with very little areas of normal tissue present. On cut surface, the liver had a waxy appearance and lard-like consistancy. Histological sections stained with H & E revealed a dense and nearly homologous substance that replaced and obliterated preexisting hepatocytes. Fibrosis and clusters of bacteria were also observed. The section was stained with Congo Red. The homologous substance was stained orange while the normal hepatocytes were stained blue. Using polarized light, the substance had an apple green birefringence. A diagnosis of amyloidosis was made.

Amyloidosis in ducks is considered a metabolic problem and occurs often in the second lay cycle of ducks (Haider. Personal communication). In this case it appears to be secondary amyloidosis because of the isolation of P. multocida from the liver and the possible chronic antigenic stimulation. It appears that a substantial decrease in liver function occurs because of the lack of normal hepatocytes. The decrease in egg production could be caused by the pasteurellosis and the amyloidosis.

Fowl Cholera in Broilers. Within the past few months, for the first time in our collective experience, we have seen outbreaks of acute fowl cholera in 5-6 week old birds from two different broiler operations. The disease was characterized by a sudden onset of mortality which increased day by day. Total mortality prior to marketing approached 30% in one flock. Necropsy findings closely resembled those seen in turkeys - i.e. swollen livers and spleens with disseminated focal necrosis, consolidated gangrenous appearing lungs, serosal hemorrhages, fibrinous airsacculitis and pericarditis, fibrinopurulent exudate in hock joints, etc. Unique environmental circumstances accounting for these outbreaks were not readily apparent although, in one operation, the most severely affected house was accessible to predators (skunks and raccoons). Pasteurella multocida serotypes isolated form these two cases were serotyped as type 1 and type 1 crossed with 4.

Cellulitis in Broilers. We have seen several cases of this interesting condition affecting birds at around market age from two different broiler operations. Most frequently the condition is not recognized in live birds - the diagnosis being made at slaughter. Gross lesions include variable yellowing and dimpling of the skin ventral to the vent and over the ventro-caudal aspect of the breast. On incising the skin a leathery greyish-yellow membrane of inspissated exudate is noted in the subcutis. Frequently this film of exudate can be removed through the incision and forms a fairly extensive sheet sometimes extending laterally over the thighs. Histologically there is extensive deep dermatitis in the affected areas involving the dermis and subcutis. The inflammatory reaction includes edema and heterophil infiltration in more active areas and accumulation of a walled-off caseative sheet of exudate surrounded by a zone of giant cells in the more chronic areas of involvement. Coccobacillary bacteria can be seen in microcolonies within the exudate but we have not consistently recovered specific bacteria in our culture attempts. This condition may affect up to 5% of entire flocks at slaughter resulting in extensive trim-outs, downgrading and whole-carcass condemnation. There appears to be a correlation of this condition with the presence of coliform airsacculitis in affected flocks.

Staphylococcal Dermatitis in Chickens. This disease entity has been seen in two cases involving White Leghorn Chickens. In one accession 12-week-old chickens had uniformly severe atrophy of the bursa of Fabricius while the other case affected 18-week-old pullets with sulfaquinoxaline toxicity. The classical external feature in both cases was the presence of large areas of slimy gangrenous skin with underlying serosanguinous fluid over the breast and occasionally extending over the thighs. In the internal organs evidence of septicemia included swelling of livers and spleens and patchy to complete consolidation of lungs. Multifocal areas of necrosis were occasionally noted in livers and spleens. In severe cases lungs and spleens were almost totally liquefied - giving the appearance of fluid-filled sacs. Histologically, gangrenous dermatitis accompanied by subcuticular sheets of bacteria was a consistent finding. Necrosis in liver, spleen and lungs was also frequently accompanied by microcolonies or masses of cocci which seemed especially numerous around interstitial blood vessels. In both cases there was significant mortality (up to 40%) and the disease occurred with sudden onset and a very brief course(12-24 hours) in individual birds.

Bursal Cryptosporidiosis in Broilers. There is little to say about this protozoan infection except that we have observed Cryptosporidia frequently in the lining epithelium of the bursa of Fabricius from broilers in numerous flocks representing 3 different integrators. Generally, but not always, there is widespread follicular atrophy (presumed to represent a sequel to infectious bursal disease) in the affected bursas. The infected epithelium is usually hyperplastic with a foamy appearance of surface cells and there is variable infiltration of heterophils within the epithelium. Frequently strands or masses of fibrinopurulent exudate are seen in the bursal lumina. While frequently observed in obviously diseased bursas, the significance of Cryptosporidia is still uncertain.

Cage Layer Fatigue (Adult Rickets). We've seen several cases of this skeletal disorder in Leghorn chickens at or just past peak egg production. The classical features include deformities of the sternum, unilateral or bilateral fractures and collapse of the rib cage and extreme fragility of long bones. Frequently the hens are still in active egg production. This condition is known to be a metabolic problem involving deficiencies or imbalances of vitamin D_3, calcium or phosphorus. The frequent occurrence of the condition reflects a need for nutritional analysis and counseling in affected operations.

OUTBREAKS OF AVIAN ADENOVIRUS IN WASHINGTON FRYERS DURING 1984

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Avian adenovirus have been known to produce hepatitis in young and adult chickens. Evidence is mounting that adenoviruses can cause a variety of clinical and pathological conditions, e.g. temporary drops in egg production, hemorrhages, aplastic anemia, nervous signs and respiratory disease. Adenovirus isolations from tracheas have been made from commercial chickens under various circumstances.

Objectives

The objectives of this study include determination of etiological agents involved in respiratory problems in fryers and to evaluate the pathology induced on experimental inoculation

Materials and Methods

Necropsy examination: The fryers submitted to the Poultry Diagnostic Laboratory of Western Washington Research and Extension Center, Puyallup, Washington, with clinical history of respiratory involvement were necropsied. Selective tissues were collected for isolation of causative virus and/or bacteria and for microscopic examination.

Gross pathologic tracheal alterations include catarrhal tracheitis with presence of excessive mucoid exudate in the tracheas. In an occasional bird the lungs contained frothy material. Mild to moderate airsacculitis were other lesions present. Fibrinous perihepatitis and pericarditis lesions were observed in some groups; an E. coli was isolated in moderate to high numbers.

Microscopic alterations were of moderate to severe tracheitis and occasionally multifocal pneumonia. The liver or pancreas lesions were not seen in birds received with clinical history of respiratory problems.

Virus isolation: Tracheal and lung specimens collected from fryers with respiratory problems were inoculated via yolk sac in 6 to 7 day old SPF embryos. Two to three egg passages were made. The yolk material harvested from dead embryos was inoculated in chicken kidney cells to evaluate cytopathological effects. Electron microscopic evaluations were made on individual samples to identify the etiologic agent. An avian adeno virus was isolated and identified from about 18 field outbreaks of respiratory problem in fryers.

Virus Propagation: Six of these field isolants of avian adenovirus were selected and propagated in chicken-embryo kidney cells. The tissue culture fluid was harvested, titrated and maintained at -65° C.

Bird inoculation: One hundred forty straight run fryers were reared in groups of 20, on wood shaving litter in individual 3.6 x 5 M colony houses located 10 meters apart. The birds were wing banded. Food and water was provided ad libitum. Five birds, when 4 weeks old, were given an avian adenovirus 0.2 ml 10^4 TCID 50 intratracheally. Another 5 chicks received 0.05 ml of 2 x 10^9 CFU/ml of an E. coli intranasally in addition to the adenovirus. Five birds were administered only E. coli. Remaining 5 birds were treated as uninoculated contact controls. A group of 20 birds were kept in a colony house as uninoculated controls. The birds in each group were weighed at 4, 5, 6 and 7 weeks of age.

Results of Experimental Inoculation

Respiratory rales were observed in an occasional bird. All six adenovirus isolates produced moderate tracheitis in birds inoculated with adenovirus, adenovirus/E. coli combination, E. coli and uninoculated contact controls. Multifocal pneumonia was observed in an occasional bird. Adenovirus inoculated birds failed to attain weights compared to uninoculated controls. No microscopic tracheal or lung alterations were present in uninoculated controls.

MODEL STATE DISEASE PREVENTION PROGRAM

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The National Poultry Improvement Plan (NPIP) was requested to develop recommendations for a program to prevent poultry diseases. These recommendations could then serve as a model for individual States in developing a prevention program applicable to their State.

A special industry subcommittee of the General Conference Committee of the NPIP suggested three objectives for such a program. The first objective was to provide recommendations for preventing the initial introduction or occurrence of poultry diseases. The second objective was to provide guidelines and recommendations on how to respond to an outbreak of certain diseases. The third objective was to provide guidelines and recommendations under which the poultry industry could continue to operate and carry out its necessary functions under various disease situations.

The intent of the model program would be to prevent the introduction and spread of disruptive diseases of poultry at minimal cost with as few restrictions as possible and as uniformly as possible among States.

One of the first requirements of such a program is the designation of an Action Agency in each State to respond to an outbreak of a specific disease, to communicate disease control information, and to determine policy and make decisions concerning the prevention program.

Another requirement of an effective program is to ensure availability of authority, funding, facilities, and personnel to carry out a monitoring and surveillance program for specified diseases and to carry out the necessary control or eradication efforts.

Each State should determine the diseases that are of importance to its industry and the action which should be taken in each case. A proposed reporting scheme for the various types of diseases (i.e., emergency diseases, action diseases, and reportable diseases) will be presented. Some diseases that are being considered for a listing of reportable poultry diseases are: avian influenza, clinical Newcastle disease, pullorum and fowl typhoid, infectious laryngotracheitis, chlamydiosis in poultry, avian adenovirus 127 infection, infectious coryza in commercial chickens, mycoplasmosis in commercial poultry, avian cholera, and duck virus enteritis.

In order that various poultry products may move across State lines with as few restrictions as possible and minimal effort, a system for classifying States is being considered. The proposed classification would be based on disease control capabilities, disease control measures being taken, and the existing poultry disease situation within the particular State. Poultry health officials of importing States would be aware of poultry products entering their State either on an annual, periodic, or shipment basis depending upon a State's classification. It is expected that a proposed model program will evolve and can be presented at the next Biennial Plan Conference scheduled for June 24-26, 1986, in San Francisco, California.

PROFITABLE HEALTH AND MANAGEMENT SURVEILLANCE PROGRAMS

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Introduction

Profitable health and management surveillance programs are based first and foremost on knowing the immuno-capabilities of the individual chicken, and thereupon, the flock(s). A simple assessment to determine this competence is by two week and four week necropsy inspection of the Thymus, the Bursa, and the Bone Marrow. Relating this information then to serology and production efficiency parameters gives one a clear view of the past, the present, and the future probabilities inherent to flock performance. " LET THE CHICKEN(S) TELL YOU "

Thymus, Bursa, and Bone Marrow Necropsy Method

A flock sample consisting of 10 chicks selected as 5 small size, but active, and 5 average size (no culls) is collected. Samples are taken from the flock at two specific ages - 14 days (12 to 16 range) and 28 days (26 to 30 range).

The chickens are sacrificed by neck disarticulation and inspected according to the method depicted in figure 1.

Recording the score is done by comparing the Thymus in relationship to neck size, the Bursa in relationship to body size, and the Bone Marrow is a color comparison.

Score numbers are: 1 = severe damage
2 = moderate damage
3 = no damage

Damage assessed at two weeks has occurred during the time frame when active maturation and peripheralization of the T & B cells normally occurs. Significant and permanent generalized immunosuppression is the consequence of this damage.

FIGURE 1.

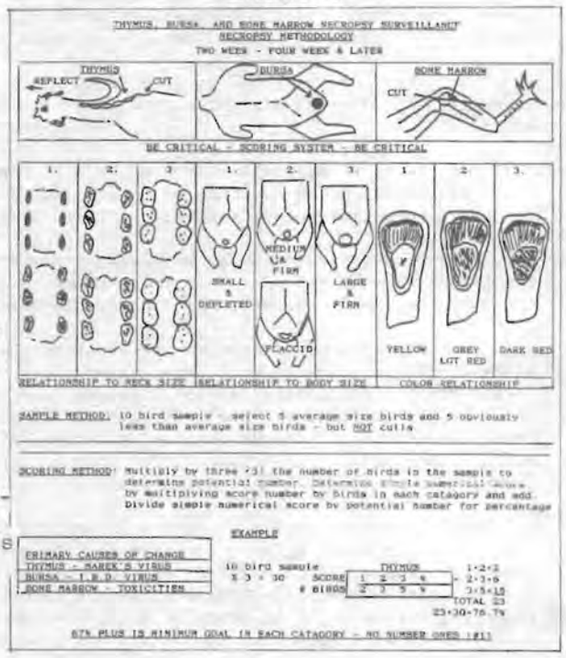


FIGURE 2.

NECROPSY SURVEILLANCE: INDIVIDUAL FLOCK IMMUNOCOMPETENCE. COMPANY: _____
FLOCK IDENT: _____ PARENT(S) IDENT: _____ IBD SERO 1DA-GMT: _____ %-STD: ___

	ORGAN	THYMUS			BURSA			BONE MAR.			SEX	ADDITIONAL NECROPSY OBSERVATIONS
	SCORE	1	2	3	1	2	3	1	2	3	♂ ♀	SMALL:
DATE	SML. 5											
12 DAYS 16	AVE. 5											AVERAGE:
ACTUAL												
TOTAL NUMBER												COMMENTS:
TOTAL SCORE												
SCORE / % / #1's												BY:

	ORGAN	THYMUS			BURSA			BONE MAR.			SEX	ADDITIONAL NECROPSY OBSERVATIONS
	SCORE	1	2	3	1	2	3	1	2	3	♂ ♀	SMALL:
DATE	SML. 5											
28 DAYS 30	AVE. 5											AVERAGE:
ACTUAL												
TOTAL NUMBER												COMMENTS:
TOTAL SCORE												
SCORE / % / #1's												BY:

	ORGAN	THYMUS	BURSA	BONE MAR.	SEX	ADDITIONAL NECROPSY OBSERVATIONS
DATE	SCORE				♂ ♀	
	SML. 5					
AGE	AVE. 5					COMMENTS:
TOTAL NUMBER						
TOTAL SCORE						
SCORE / % / #1's						BY:

Scoring the Necropsy Surveillance

After recording the determined numbers on a form as shown in Figure 2., the score is calculated as follows:

The number of birds in the sample is multiplied by 3, thus giving the highest numerical score possible.

The actual numerical value is determined as in Figure 2 then divided by the highest possible value to give a percentage (%) value.

The flock immunocompetency is reported as score % and % number of 1's.

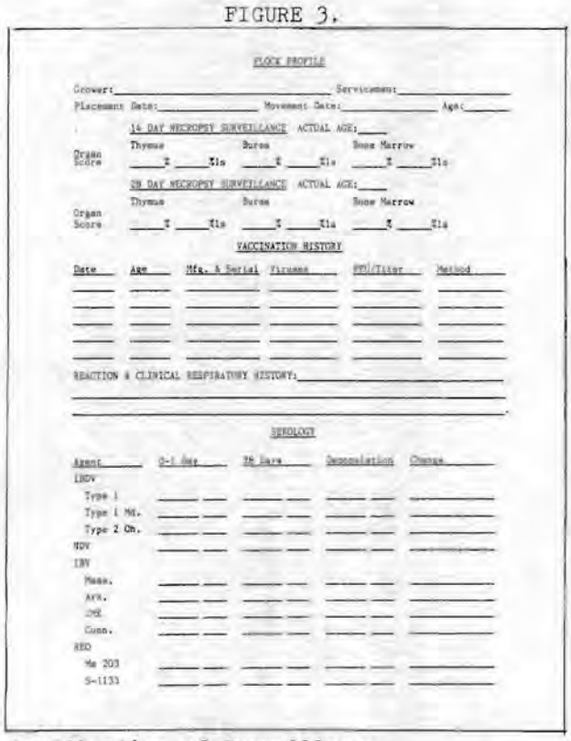
A low flock % indicates deficient flock immunocompetency. % 1's reveals individual host problems in competence, ie: uniformity.

Flock Serology Profiling

To be effective and of value, many factors must be included in a serological surveillance effort and coordinated, such as immunocompetence, vaccination history, clinical history, and paired serology. Figure 3 shows a simple control document designed to coordinate many of these factors in a broiler setting. There may be some slight variations of need from geographical area to area, but the requirement for continuity and coordinated information is ubiquitous.

Another valued form of surveillance is applying actual occurances against a probability model for the specific time frame of influence. Such a model is demonstrated in Figure 4.

FIGURE 3.



FLOCK PROFILE

Growers:_____ Servicemen:_____

Placement Date:_____ Movement Date:_____ Age:_____

14 DAY NECROPSY SURVEILLANCE ACTUAL AGE:____

	Thymus	Bursa	Bone Marrow
Organ Score	____% ____Tis	____% ____Tis	____% ____Tis

28 DAY NECROPSY SURVEILLANCE ACTUAL AGE:____

	Thymus	Bursa	Bone Marrow
Organ Score	____% ____Tis	____% ____Tis	____% ____Tis

VACCINATION HISTORY

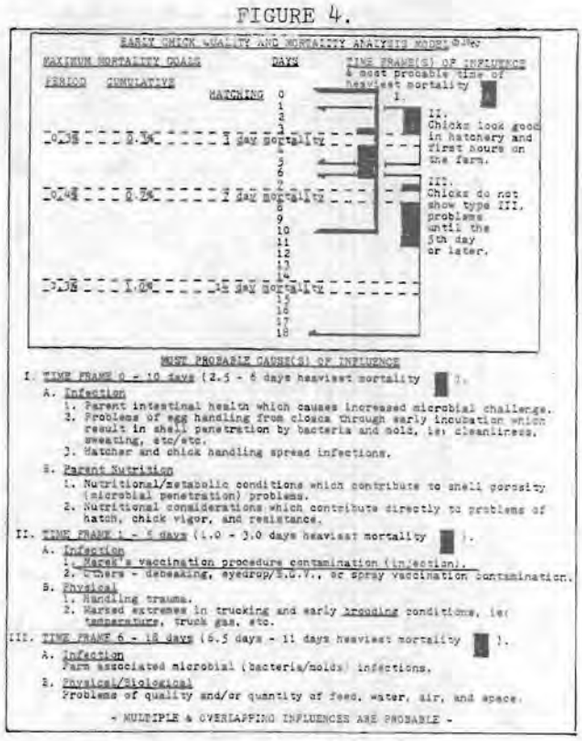
Date	Age	Mfg. & Serial	Viruses	PVO/Titer	Method

REACTION & CLINICAL RESPIRATORY HISTORY:_____

SEROLOGY

Agent	0-1 Day	28 Days	Seroconversion	Change
IBDV				
Type I				
Type I Md.				
Type 2 Oh.				
NDV				
IBV				
Mass.				
Ark.				
JMK				
Conn.				
REO				
Ma 203				
S-1133				

FIGURE 4.



EARLY CHICK QUALITY AND MORTALITY ANALYSIS MODEL ©Jaap

MAXIMUM MORTALITY CHART — DAYS — TIME FRAME(S) OF INFLUENCE & most probable time of heaviest mortality

PERIOD CUMULATIVE

HATCHING 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

I. 0.5% 0.5% 1 Day mortality

II. 0.5% 0.5% 1 Day mortality — Chicks look good in hatchery and first hours on the farm.

III. 0.5% 0.5% 1 Day mortality — Chicks do not show type III. problems until the 5th day or later.

MOST PROBABLE CAUSE(S) OF INFLUENCE

I. TIME FRAME 0 - 10 days (2.5 - 6 days heaviest mortality).

A. Infection
1. Parent intestinal health which causes increased microbial challenge.
2. Problems of egg handling from cloaca through early incubation which result in shell penetration by bacteria and mold, ie: cleanliness, sweating, etc/etc.
3. Hatcher and chick handling spread infections.

B. Parent Nutrition
1. Nutritional/metabolic conditions which contribute to shell porosity (microbial penetration) problems.
2. Nutritional considerations which contribute directly to problems of hatch, chick vigor, and resistance.

II. TIME FRAME 1 - 5 days (1.0 - 3.0 days heaviest mortality).

A. Infection
1. Marek's vaccination procedure contamination (in/action).
2. Others - debeaking, eyedrop/I.C.V., or spray vaccination contamination.

B. Physical
1. Handling trauma.
2. Warped extremes in trucking and early brooding conditions, ie: temperature, truck gas, etc.

III. TIME FRAME 6 - 18 days (8.5 days - 11 days heaviest mortality).

A. Infection
Farm associated microbial (bacteria/molds) infections.

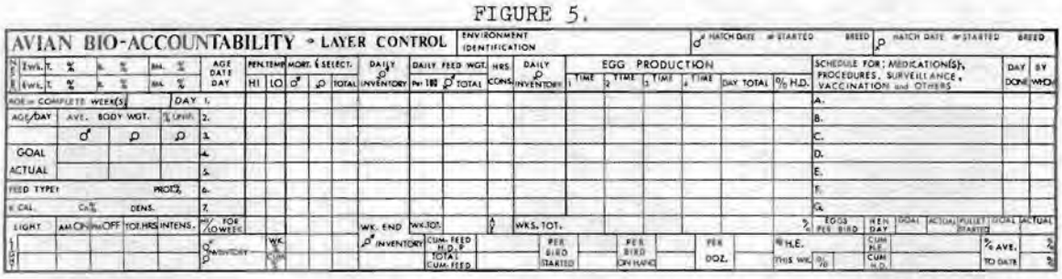
B. Physical/Biological
Problems of quality and/or quantity of feed, water, air, and space.

- MULTIPLE & OVERLAPPING INFLUENCES ARE PROBABLE -

Coordination of Surveillance

Problems determined by surveillance mean many different things to different individuals, depending on their particular interest(s). To the pathologist they mean serology, histopathology, and nutrition. To the nutritionist they mean protein, vitamins, and diseases. To the management specialist they mean lighting, feeding programs, nutrition, and disease. To the poultryman, be he an integrator or backyard putterer, problems determined by surveillance mean all of the above and correctly so.

FIGURE 5.



AVIAN BIO-ACCOUNTABILITY - LAYER CONTROL — ENVIRONMENT IDENTIFICATION — ♂ HATCH DATE # STARTED BREED — ♀ HATCH DATE # STARTED BREED

N / R — Wk. T. % h. % bn. % — AGE DATE DAY — PEN TEMP HI LO — MORT. & SELECT. ♂ ♀ TOTAL — DAILY ♀ INVENTORY — DAILY FEED WGT. Per 100 ♀ TOTAL — HRS CONS. — DAILY ♂ INVENTORY — EGG PRODUCTION TIME 1 TIME 2 TIME 3 TIME 4 TIME DAY TOTAL % H.D. — SCHEDULE FOR: MEDICATION(S), PROCEDURES, SURVEILLANCE, VACCINATION and OTHERS — DAY DONE — BY WHOM

ROE in COMPLETE WEEK(S) DAY 1.

AGE/DAY | AVE. BODY WGT. | % UNIF. | ♂ | ♀ | ♀ | 2. | 3. | 4. | 5. | 6. | 7.

GOAL | ACTUAL

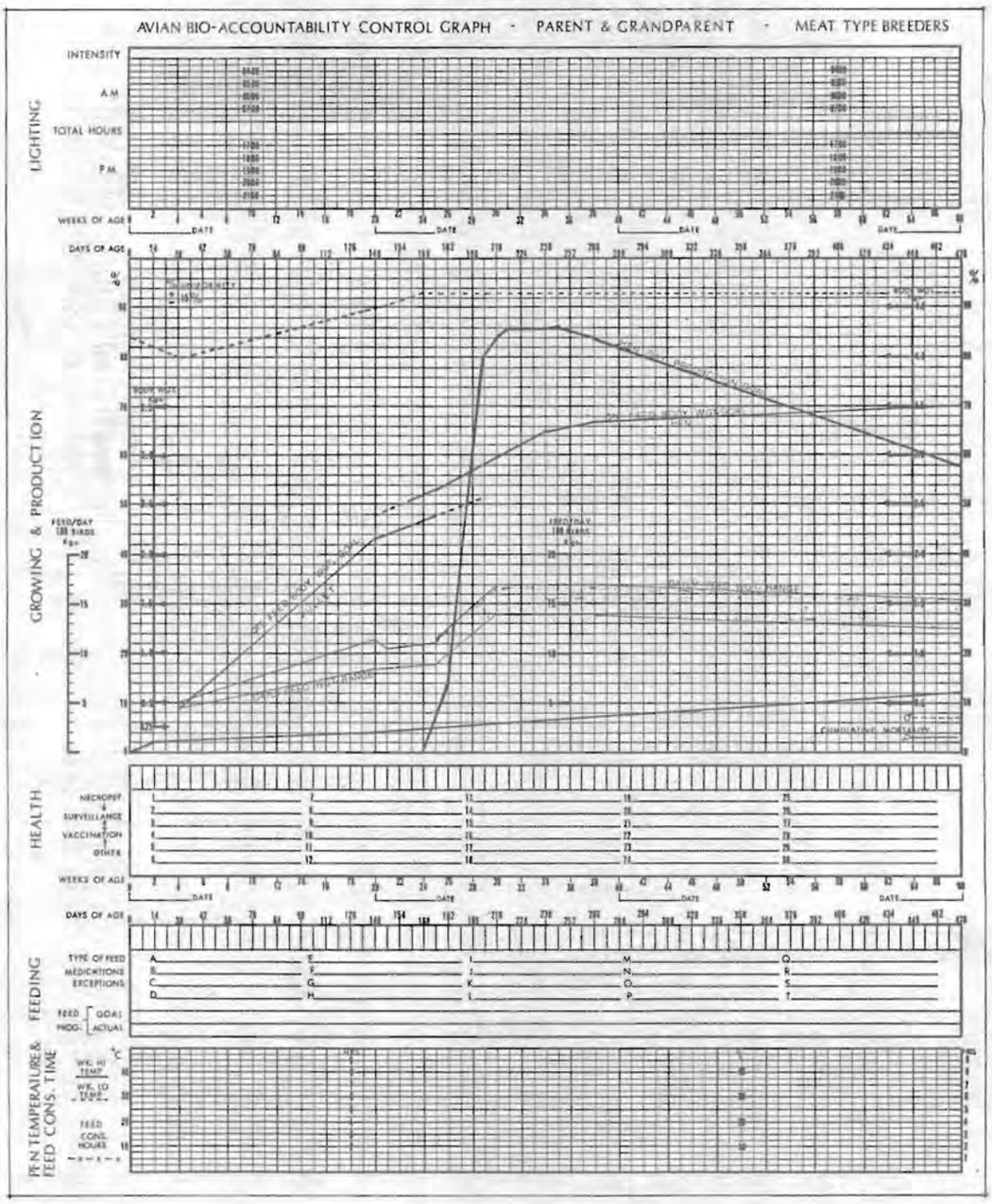
FEED TYPE: PROT% | # CAL. | Ca% | DENS.

LIGHT | AM ON | PM OFF | TOT. HRS | INTENS. | % FOR LOWEST | WK. END ♂ INVENTORY | WK. TOT. ♀ | WKS. TOT.

1 / 2 / 3 — ♂ INVENTORY | WK. CUM. | CUM. FEED M.D.P. TOTAL CUM. FEED | PER BIRD STARTED | PER BIRD ON HAND | PER DOZ. | % H.E. This Wk. % | CUM. DOZ. CUM. H.D. | % EGGS PER BIRD | HEN DAY GOAL | HEN DAY ACTUAL | PULLET STARTER GOAL | PULLET STARTER ACTUAL | % AVE. TO DATE

Surveillance must be coordinated, analyzed, and used to be profitable. Figure 5 shows a control document which has been very helpful in this regard. It takes into consideration all the major factors relative to chicken productivity (this example happens to be for heavy breed layers). Continue the numbers accumulated by Figure 5 and submit them to graphic analysis as shown by Figure 6., and really most of the obscure problems become, if not absolutely clear, then certainly more likely solved.

FIGURE 6.



Summary

Know the immunocompetence of the flock(s) as represented by individuals and as determined first by two and four week necropsy procedures. Track, follow, and know the other major indicators of health and management. Collate, coordinate, and analyze the information in a single format.

Use the information to circumvent and prevent repetition of problems in the future. Health and management surveillance is primarily effective for future planning, not fire fighting.

AND REMEMBER

The essential bio-physical and social needs of the individual chicken must be met if a flock, company, or integration is to achieve health and productivity goals.

! LET THE CHICKEN(S) TELL YOU !

Profiles Of Chickens. ROY E. LUGINBUHL, Ph.D., SPAFAS, INC.

The poultry industry has entered a new era in health monitoring. Techniques are now available utilizing automatic readers and computers. The enzyme-linked immunosorbent assay (ELISA) for antigen-antibody reactions allows the laboratory to obtain a titration on a single dilution of serum. This value is automatically printed on paper or in a computer. The computer can be instructed to print a histogram with bar values for relative titers. The histogram allows an immediate evaluation of the serological status of the flock.

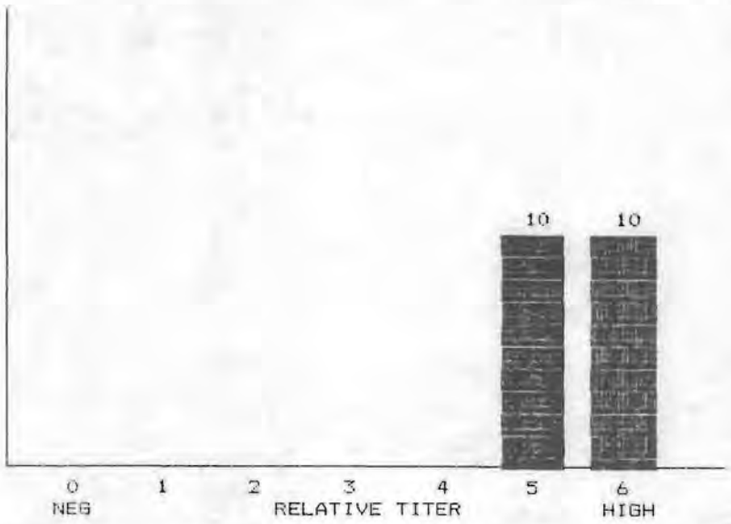
Serum profiles can now be performed for the following avian diseases:

- Newcastle disease
- Infectious bronchitis
- Avian encephalomyelitis
- Reovirus
- Infectious bursal disease
- Mycoplasma gallisepticum
- Mycoplasma synoviae

Profiles on baby chicks monitor maternal antibodies and thereby warn the poultryman of the need for a booster vaccination in the breeder flock. Profiles on growing birds evaluate the efficacy of the immunization program. Profiles on mature birds provide a warning system for revaccination.

FARM: Joe Smith
FLOCK: B, 1 day-old

INFECTIOUS BURSAL DISEASE
DATE: 6-1-84

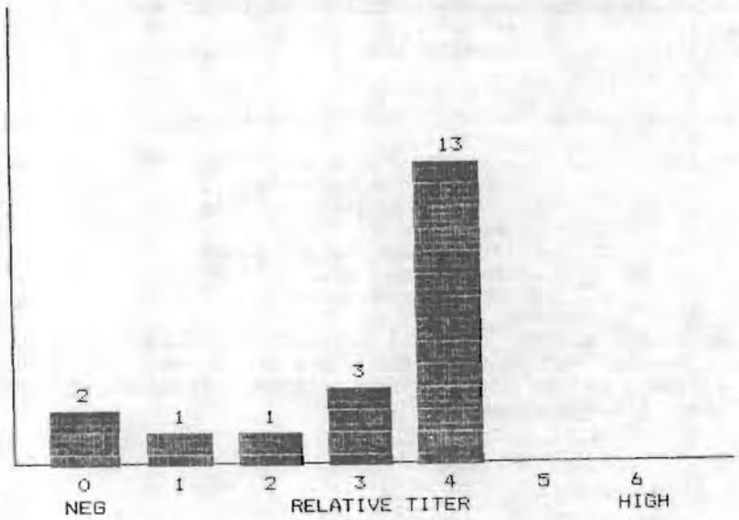


NO. ABOVE COLUMN(S) = NO. OF SAMPLES

THE ANTIBODY LEVEL IN THE PARENTS OF THESE BIRDS IS EXCELLENT. NO VACCINATION IS NEEDED.

FARM: Joe Smith
FLOCK: B, 1 day-old

INFECTIOUS BURSAL DISEASE
DATE: 6-1-84



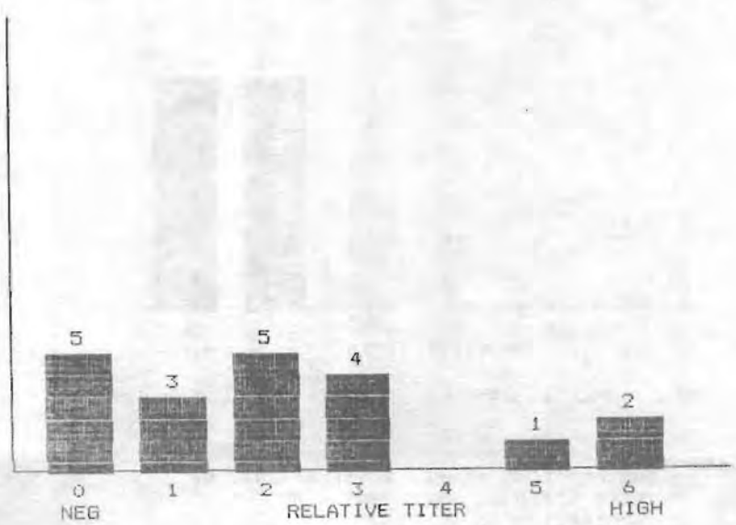
NO. ABOVE COLUMN(S) = NO. OF SAMPLES

THE ANTIBODY LEVEL IN THE PARENTS OF THESE BIRDS IS MARGINAL.

ANOTHER PROFILE SHOULD BE OBTAINED IN ABOUT ONE MONTH.

FARM: Joe Smith
FLOCK: B, 1 day-old

INFECTIOUS BURSAL DISEASE
DATE: 6-1-84



NO. ABOVE COLUMN(S) = NO. OF SAMPLES

THE ANTIBODY LEVEL IN THE PARENTS OF THESE BIRDS IS POOR. THE PARENT FLOCK NEEDS A VACCINATION.

MONOCLONAL ANTIBODY TO DIFFERENTIATE EXOGENOUS FROM ENDOGENOUS AVIAN LEUKOSIS VIRUSES

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Avian retroviruses are congenitally transmitted by exogenously infected hens. Consequently, tolerant progeny recycle avian leukosis viruses (ALVs) of subgroup A, B, C and D to succeeding generations (6,9). Subgroups A and B viruses are the commonly occurring field strains and typically cause bursal-dependent lymphoid tumors in chickens. Subgroups C and D viruses have not yet been detected in the field in the United States. Moreover, genetically related but nononcogenic, endogenous retroviruses which are classified in subgroup E are transmitted through the germline and are inherited as Mendelian genes (3).

An ALV group-specific antigen (p27) is detected at high titers in egg albumens from infected breeding hens. The presence of this antigen is correlated with congenital transmission of exogenous ALV (3,4,6,9). In ALV reduction or eradication programs, p27-negative dams are selected by commercial breeders for reproduction. Antiserum to p27 is used for selection in the field, but it cannot distinguish between endogenous and exogenous virus infections. The enhanced sensitivity of enzyme-linked immunosorbent assays (ELISA) tends to confuse classification of the status of ALV infection in field flocks because some normal chickens with ev genes may express p27. On the other hand, hybridomas that secrete monoclonal antibodies (MCA) specific only for exogenous ALV antigens may overcome ambiguities in interpretation of the field status of flocks. We report the development of a MCA, 6AL42, which can be used either in an indirect ELISA or a double-antibody sandwich ELISA with rabbit antiserum to p27 to differentiate between endogenous viruses from exogenous ALVs.

Chicken embryo fibroblasts (CEF) from 11-day-old SPAFAS White Leghorn embryos (C/O) were cultured in medium 199 and medium F10 supplemented with 4% calf serum. Avian leukosis viruses used in this study included subgroup A viruses (two field isolates, RPL-40 and RPL-42); subgroups A and B viruses (avian myeloblastosis viruses); a subgroup B virus (the Rous-associated virus, type 2, RAV-2); a subgroup C virus (the Praque C strain of Rous sarcoma virus, PrC-RSV); a subgroup D virus (the Carr-Zilber strain of RSV, CZ-RSV); and a subgroup E virus (Rous-associated virus, type 0, RAV-0) (3). Another retrovirus, reticuloendotheliosis virus (strain T, REV-T) was included for testing the specificity of MCAs. Albumen samples were obtained from chickens infected with RPL-40 virus and chickens that spontaneously produced endogenous RAV-0 (3).

Hybridomas were produced by fusing spleen cells, obtained from Balb/c female mice immunized with Schmidt-Ruppin strain of Rous sarcoma virus (RSV) transformed Balb/c mouse cells, with NS-1 myeloma cells in the presence of 35% polyethylene glycol as previously reported (5). Hybridoma supernatants (50 ul) were added to wells of microtiter plates previously coated with 250 ng of purified RPL-40 virus and allowed to react for 60 min at 37 C. Unbound antibodies were removed by washing three times with phosphate buffer saline (PBS) with 1% Tween 80. Goat anti-mouse IgG (100 ul) conjugated to horseradish peroxidase was added to the wells and reacted for 60 min at 37 C. Finally, 100 ul aminosalicylic acid (1 mg/ml) in H_2O_2 was added and absorbancies were measured after 1 hr in an ELISA minireader. For double antibody ELISA, the procedure was essentially the same except that the wells were coated with the first antibody and antigens or albumen samples were reacted for 60 min followed by adding either the homologous or heterologous second conjugated antibody. For the determination of the titer of the ascitic antibody, the endpoint dilution of the antibody, which gave an absorbance of 0.25 at 490 nm, was considered as the endpoint titer.

Twelve separate fusions produced only one MCA, 6AL42, specific for p27. The reactivity of this MCA against several viruses is summarized in Table 1. It reacted in an indirect ELISA with exogenous subgroup A, B, C and D viruses (RPL-40, AMV, RAV-2, PrC-RSV and CZ-RSV) to a titer of 10^5-10^7, but only to a titer of 10^4 with endogenous RAV-0 virus. MCA 6AL42 is highly specific for ALV; no reactivity was observed with REV-T preparations or uninfected CEF extracts. The MCA 6AL42 was found to be of the IgG2a isotype by double diffusion agar gel using rabbit antisera to specific mouse immunoglobulin isotypes.

To further test the ability of 6AL42 to distinguish between infections by exogenous and endogenous viruses, CEF cells were separately infected with three exogenous ALVs (RPL-40, RPL-42 and RAV-1) and one endogenous ALV (RAV-0). At intervals between 4 and 21 days after infection, cells were trypsinized and seeded in micro-ELISA plates for assay of p27 using an indirect ELISA (2). The results of a representative test, at 9 days after infection, with anti-p27 antibody and MCA 6AL42 from ascites are summarized in Table 2. As shown, anti-p27 was positive for cells infected with RPL-40, RPL-42, RAV-1 and RAV-0, whereas 6AL42 reacted with all but RAV-0-infected cells. The finding further supports the conclusion that 6AL42 can differeniate RAV-0 from other viruses.

Antiserum to p27 has been employed in the field among commercial breeders. However, it cannot distinguish between endogenous and exogenous virus infections. MCA 6AL42 was developed for differential diagnosis of exogenous ALV infection, or, more specifically, infections by endogenous RAV-0

and exogenous field strains such as RPL-40. Preliminary data using 6AL42 to detect p27 in albumen indicated that it is useful in identifying hens shedding exogenous gs antigens. The reason for the discrimination is probably because of the preferential reactivities of 6AL42 which may reflect differences in the structure and configuration of p27 encoded by endogenous and exogenous ALV (1,7).

Table 1

Reactivity of MCA 6AL42 (IgG2a) Supernatant and Ascites Fluid Against ALVs in ELISAs

Virus	Hybridomas Supernatants[b] (O.D. 490)	Ascites Fluid[c] (O.D. 490)	Ascites Fluid[c] (Titer)
RPL-40	1.18	0.35	10^7
AMV	1.07	0.31	10^7
RAV-2	1.03	0.48	10^5
Pr-C	0.64	0.40	10^5
Carr-Zilber	0.90	0.30	10^6
RAV-0	0.35	0.28	10^4
REV-T	0.09	0.26	<10
CEF	0.12	0.25	<10

[a]Wells were coated with 100 ul (250 ng) of purified ALV. 100 ul of 6AL42 at various dilutions was reacted for 60 min followed by rabbit anti-mouse IgG conjugated to horseradish peroxidase. The reactivity was expressed as absorbance at 490 nm.

[b]Supernatant antibody without dilution.

[c]Endpoint titer was determined at the end-point dilution of the antibody which gave an absorbance at 490 nm of 0.25.

Table 2

6AL42 Ascites for p27 Detection in ALV-Infected Cells[a]

Virus-infected cells	Anti-p27	6AL42
RPL-40	>2.00[b]	1.70
RPL-42	>2.00	1.68
RAV-1	>2.00	1.63
RAV-0	>2.00	0.20
REV-T	0.15	0.09
15B-CEF	0.10	0.01
Line 0-CEF	0.09	0.02
SPAFAS-CEF	0.11	0.03

[a]5×10^4 infected CEF at 9 days after infection with ALVs. Infected cells were fixed with acetone. The dilutions of 6AL42 ascites used was 1-500.

[b]Absorbance at 490 nm.

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DIARRHEA, ENTERITIS, MALABSORPTION, MALDIGESTION AND VIRUSES

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The title of this article has a small sampling of some of the descriptions given to a condition recognized in broiler chicks and turkey poults appearing in the first two weeks of life. The problem has become of economic concern and extensive investigations are proceeding in many laboratories in an attempt to understand its pathogenesis and to define its etiology/ies. A variety of other terms has been used to describe the condition and these terms refer to clinical signs observed in affected birds. Many believe these signs are secondary nutritional deficiences that follow the original diarrhea/enteritis. In a brief review of the literature, diarrhea was the most consistent early clinical sign described in outbreaks of the disease followed by unevenness of the affected flocks. Excessive drinking and nervousness were noticed during the early stage of the disease with increased mortality. Abnormal feathering, skeletal abnormalities, and various signs that could be related to nutrient deficiences appear in the aftermath of the acute stage of the disease. An interesting facet about this condition is the similarities in signs and lesions observed in the broiler chick and the turkey poult. It should be mentioned that differences in clinical signs and lesions were observed in broilers in different parts of the world. That does not necessarily mean that we are dealing with more than one disease.

Observations on Possible Etiology/ies: The failure to incriminate recognized enteropathogens as causes for the condition led to extensive searches for viruses and other agents that might be associated with the condition. A variety of viruses were identified in gut contents of affected broilers. These included: different groups of rotaviruses, reoviruses, enteroviruses, adenoviruses, caliciviruses, coronavirus, parvoviruses and an unidentified small round RNA viruses. In turkey poults the following viruses were identified: two groups of rotaviruses, astroviruses, reoviruses, enteroviruses, adenoviruses, and parvoviruses. The similarities between the viral agents detected in poults and chicks are obvious.

Researchers in the US have been very interested in the role of reoviruses and it was possible to reproduce some of the lesions of malabsorption syndrome in broilers with some reovirus isolates, but the results were not always consistent. A cloned reovirus was reported to cause low absorption of D-Xylose in turkey poults in the US. In Ireland, broilers inoculated with a combination of an entero-like virus and a reovirus developed some signs of malabsorption.

At the present time the cause/s of the syndrome is not completely clear. The possibility exists that an agent or agents that have not been as yet associated with the disease may singly or in combination initiate the disease.

In the following part of this report we present information generated in our laboratory on the detection of viruses in the gut contents of diarrheic poults and on experimental studies in turkey poults.

Samples were collected or submitted to our laboratory from 47 flocks in the following states: California, Indiana, Iowa, Minnesota, Nebraska, North Carolina and Ohio. The flocks of origin varied from 10 to 21 days of age. The main clinical signs described for these flocks were diarrhea and variations in size accompanied by increase in morbidity and mortality. Electron microscopy, immune-electron microscopy and polyacrylamide gel electrophoresis were the techniques used for detection of viruses. Standard bacteriological techniques were used for bacterial isolation.

Viruses were detected in all but one of the above flocks. The following viruses were detected and are listed in order of prevelance: a rotavirus-like agent (RVLA), astroviruses, reoviruses, rotaviruses, enteroviruses and adenoviruses. The majority of the samples had more than one type of virus. Salmonella was isolated from one of the flocks examined. The rotavirus and RVLA were morphologically indistinguishable but were differentiated by immune-electron microscopy. The turkey rotavirus reacted with antisera to bovine and swine rotavirus, whereas the RVLA did not. The RNA electrophoretic migration pattern of the RVLA was different from that of conventional rotaviruses and reoviruses.

In earlier experiments in our laboratory, a combination of the RVLA and the astro-like virus inoculated into specific pathogen free (SPF) turkey poults resulted in diarrhea. The rotavirus alone was associated with very mild clinical signs and lesions. Later, Dr. Reynolds of our laboratory was able to separate the astro-like virus and proceeded to inoculate SPF poults. The birds developed profuse diarrhea similar to that observed in poults inoculated with the combination of RVLA and the astro-like virus. The intestinal tracts of these birds were distended, lost tone, were full of gas and fluid contents and the coeca had frothy contents. There were statistically significant differences in the weight and of D-Xylose absorption between the inoculated and control birds as seen in the table.

Comparison of Day-Old Poults Inoculated with Astro-like Virus to Uninoculated Control Poults.[A]

	Days Post Inoculation						
	1	3	5	7	9	11	13
Ave. Body Wgt. (Kg)							
Controls	0.044	0.078	0.084	0.118	0.148	0.170	0.200
Inoculated	0.046	0.062*	0.058*	0.086*	0.114*	0.142*	0.162*
D-Xylose absorption (mg/dl)[B]							
Controls	23.798	19.162	29.144	18.40	41.026	20.972	20.59
Inoculated	20.678	10.196*	14.14*	10.52	24.092*	16.634	16.162

*Statistically significant difference from controls.

[A] 35 control birds and 35 inoculated birds were housed in separate sterilized isolators, 5 birds/group were evaluated on days indicated.

[B] Average serum D-Xylose values at 90 minutes after oral administration.

These results have been repeatedly obtained in several experiments. Unfortunately, these experiments were of short term, hence, any possible long term nutritional deficiences were not observed. Yet, it was demonstrated that the astro-like virus does cause an enteric disease in turkey poults. Whether this virus plays a role in the malabsorption syndrome of broilers is not known. It should be mentioned that these astro-like viruses are not easy to identify, and only some ofthese viruses show the typical five or six point star morphology. They also fall in the same size range as enteroviruses.

We learned from the survey that in most diarrheal outbreaks of turkey poults, more than one viral agent was present. Information on the pathogenicity of these viruses is rather limited. With the exception of reoviruses and some rotaviruses, the other viruses reported are difficult to propagate in vitro. Reoviruses in particular are the easiest group to propagate in vitro and are rather widespread in turkey flocks (a similar widespread occurrence of reoviruses has been reported in broilers). Because of the widespread occurrence of a variety of viruses in commercial flocks, the choice of birds to be used for experimental studies becomes very important.

Currently, we are concentrating our efforts on defining the role of different viruses in enteric disease/malabsorption of turkey poults. Plans are also underway to conduct similar studies with broiler chicks. It is felt that defining the role of the different viruses singly and in combination could lead to a better understanding of the etiology/ies of enteritis/malabsorption syndrome of poultry.

MALABSORPTION - "DO WE SPEAK THE SAME LANGUAGE ON BOTH SIDES OF THE ATLANTIC ?"

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Introduction

During the 33rd WPDC congress there were a number of presentations on M.A.S. and in the discussions it appeared to the authors that apart of the ideas on the etiology of the disease, also differences in clinical symptoms, gross lesions, histo pathological findings, prophylaxus and economical importance were evident between the U.S. and Europe and even within the U.S. and between European countries. In order to check this observation the authors together with Dr. Ben Kouwenhoven of the Dutch Poultry Health Institute composed an inquiry consisting of 35 questions, which, to their opinion, seemed all relevant as far as MAS is concerned. These questionnaires were sent out to experts on M.A.S. via the outlets in, the US, Europe and the Far and Middle East. This inquiry was not ment to fulfil all demands of a statistically highly ranked investigation, but must be seen as an attempt to know where are we walking hand in hand and where do we split up. The presentation is decided in two parts. In the first part the preliminary outcome of the quiry is given divided in the headings 1. Clinical symptoms. 2. P.M.'s, 3. miscellaneous.
In the second part more details are given on the etiology of the disease, as it is seen in Europe to-day. Questionaires come back from Arkanses, Alabama, Georgia (3x), California, Minisota, Ohio, U.K. (3x), Northern Ireland, Holland, Germany, Italy, Denmark, Israel, Mexico, the Philippines(3x). Also 3 questionaires from the U.S. and 1 from the U.K. based on M.A.S. in turkeys were received.
In order to make this overview no too complicated and too extended, authors restrict themselves here only to the U.S. and Europe and uniquely to broilers.

Results outcome inquiry

1. Clinical symptoms of M.A.S. on which we mutually agree.
   - 1.1 The time of onset of runting/stunting
     Almost all inquiries mentioned the onset of first signs as early as 5-7 days of age.
   - 1.2 Depression of bodyweight as a result of M.A.S.
     We do agree, that M.A.S. in general has its effects on the bodyweight of the diseased bird only and not on that of their healthy littermates.
   - 1.3 Mortality
     Variations reported between 0-15%. But no differences between U.S.-Europe.
   - 1.4 Morbidity
     Also varying, from 0-40% but no difference between continents.
   - 1.5 Poor feathering in general ?
     Marked feature in N. Ireland and Holland, but was reported on all guestionaires that came back.
   - 1.6 Helicopter like feathers
     Seen on both sides of the ocean. But only to a very low percentage(<0.5%)
   - 1.7 Wet litter
     Seen both in U.S. as in Europe, but to a varying degree. In Holland however it is considered as a marked feature in the disease.

2. Clinical symptom reported as part of M.A.S. on which there is not mutual agreement.
   - 2.1 Abnormal shank colour ? (that means very pale shanks)
     In Europe 7 out of 9 questionnaires reported "This is not a feature of M.A.S. to our opinion". Only Holland and Denmark reported with yes. In the U.S. however, 6 out of 8 answered positively. Probably the cereals used are of influence. For instance in Europe wheat is the main base of the ration (Exception is Holland where corn is widely in U.S. as it is, in the U.S. So the malabsorbed carotene from the corn may be at the base of the difference of opinion.
   - 2.2 Mucoid diarrhea (orange droppings)
     In Europe an occasional yes, but in U.S. a definitve yes. Even 5-10% of total birds in affected flocks. Seen from 7 days of age onwards.
   - 2.3 Undigested food in litter
     Europe in general a varying symptom. Yes reported by Holland and Italy. Not considered as important by N. Ireland, Denmark.
     U.S. in majority yes.
   - 2.4 Evidence of Encephalomalacia ? (not A.E.)
     Europe: 5 out 9 questionnaires reported no, but yes by Holland, Germany, Denmark and Israel. U.S. 5 out 7 reported "yes this is important".

3. Postmortem findings on which we mutually agree in M.A.S.
   - 3.1 Abnormalities of proventiculitis
     Europe, only Italy and Israel reported it as a marked feature.
     U.S. in general no, only the lists from Arkansas and Georgia mentioned yes.
   - 3.2 Swollen intestine
     Europe yes, marked feature in Holland, N. Ireland.
     U.S. common picture, only Minesota and Ohio reported negatively.

3.3 Gaseous Ceaca
Europe not important.
U.S. not important.

3.4 Size bursa F
Both U.S. and Europe: smaller than normal
some reported diseased birds are also small so importance in M.A.S. is questionned.

3.5 Thymus
Not considered as an important feature for the disease.

3.6 Any Rib caartilage abnormalities ?
Europe: only Holland, Germany and Israel were positive.
U.S.: only Alabama and Georgia positive.

3.7 Any soft Rubber like bones
Europe : in general occasional low %.
U.S. : occasionally yes but low % of birds affected.

4. PM findings we do not or only partly agree on.

4.1 Undigested gut content
In Europe this is not considered as an important feature, but in the U.S. 6/6 reported yes.

4.2 Softening or snap-off cartilage of long bones (tybia, femur)
Europe varying feature in general but a very prominent one in Holland, Germany, Italy, Israel. We call this "osteoperosis".
U.S. All lists that cameback reported "yes we do see that".

4.3 Any enlarged bone growth plates ?
Europe: very important feature, starting 2 - 3 weeks onwards.
U.S. : only Alabama and Ohio reacted positively.

4.4 Any serum colour abnormality ?
In general in Europe: No. Only in Holland considered as part of the symptoms.
U.S.: 4/6 reported yes - 2 did not look at it at all.

5."Miscellaneous questions"

We further asked for histo pathological changes of bones, intestines,pancreas etc. If they looked at it at all, the U.S. and in Europe reported lesions of bones, intestines,of which the pancreas was the organ most frequently mentioned. Only Israel reported also liver and kidney lesions as part of the syndrom.
When feedstuffs were controlled for mycotoxines, only a very few questionnaires those from Arkansol, Ohio and Israel mentioned occasional contaminations. Vomitoxines were checked in U.K. + 30% of rations from 1 integration proved positive. Important ?
Europe and the U.S. both agreed in majority on a Parenstock-offspring relation.
- especially in young flocks in the beginning of their egg production.
- No differences in economical importance of the disease could be notified between the U.S. and Europe. However many variations exist between the European countries and within individual states.
The actual situation has been described as: "a serious problem together with coccodiosis and Gumboro as problem nr. 1 in Holland and Denmark, to a rather unimportant problem nowadays in N. Ireland.

In U.S. M.A.S. still is very important in Georgia and Arkansas but a smaller problem in Alabama and Minesota as was the situation early 1984.
Last winter period M.A.S. was again a very important problem in Holland. Control measures seem in Europe only concentrated on strict cleaning, good desinfection and management measures, whereas vaccination of broiler breeders with Reo vaccines is considered in the U.S. also aspart of the control measures.

To summarize these preliminary outcomes of the inquiry we may conclude:
1- that in general, the differences between clinical symptoms, postmortems, histopathological and biochemical findings reported either from Europe or the U.S., are not significant, so we do speak the samelanguage as far as M.A.S. is concerned.
2- certainly differences exist, but variations within the U.S.-states and within European countries are sometimes bigger than those between continents.
3- The impression exists that when problems with M.A.S. were reported, the severity and the influence of it on mortality and morbidity was more pronounced in the U.S. than in Europe. But here differences in feedingredients, management and climatological conditions have to be taken into account as well.

Second part of the presentation
Most differences we mentioned sofar were of minor importance. But an important difference remains an that is our ideas on the aetiology of the syndrom. To the question: "Is there any virus involvement ?" 3 out of 5 states reported the isolation of a Reo virus and no single other virus. In Europe Reo virus involvement in the disease however is more subject for discussion than it seems in the W.S. Reo's were also isolated in the U.K,Holland, Italy and Denmark, but their role in M.A.S. is denied or considered as of minor importance.

1- Other viruses isolated or demonstrated in connection with M.A.S. in Europe are for instance when Peter Wyeth demonstrated the presence of a calicivirus. But its role in Infectious stunting however is questionable.

2- Mc.Nulty and Mc.Ferran in Northern Ireland and Sparkman c.s. in England demonstrated or isolated small enteroviruses which were 24-30 mm. in diameter. Both groups were able to reproduce M.A.S. like symptoms in day old broilers with a crude inoculum prepared out of gut material from diseased birds, but with purified virus particles however they were unable to repeat this symptom sufficiently in day-old chicks.

3- Dr. Farmer at the Houghton Poultry Research station works on a 3 years grant of the British Poultry Industry on comparable small viruses which he calls SRV(small round) viruses.

4- Kisary c.s. Hungary publishe in Avian Pathology (1984) the isolation of small viral particles of 19 to 24 mm. in diameter, which are suggested to belong to the Parvoviridae. Purified virus particles derived from whole intestines of orally infected SPF chickens were able to introduce M.A.S. like symptoms in day-old broilers. He reported: growth retardation (Runting), bad feathering, soft long bones.
Kisary's theory about Parvo in itself is interesting. Parvo infections he says have a natural affinity to fast growing cells. As you know in dogs parvo affected primarily the embryo and the young pup, in pigs parvo is the problem of embryonatal death (all fast growing cells). In chicken M.A.S. is by far a bigger problem in broilers than it is in layer type birds and because broilers grow very fast Parvo might be important.
Kisary is working now on a 2 years grant of Solvay's Animal Health division to which Duphar and Salsbury belong as you may know.

5- Very recently Mrs. Farmer published in the Vet. Record the isolation of a new virus, which she named F.E.W. virus. It is a RNA virus and capable of causing mortality varying from 20-60% after inoculation if day-old SPF chickens. But its importance in M.A.S. is still unknown.

6- It's not our intension to summarize all the work in process in M.A.S. in Europe but one last development you may not miss. Already for a longtime researchers at the Poultry Health Institute in Doorn, Holland, don't believe in virus involvement in M.A.S.. So far, however they failed to demonstrate the presence of other contageous agent(s) in the aetiology. Speculations were made of bacteria involvement or their toxines. Very recently Dr. Davelaar of the same Institute isolated from a flock of laying hens with diarrhoea, drop off egg production, spirochetes which fluoreced by the fluorescent antibody test (F.A.T.) with an antiserum to Treponema hyodysenteriae. Day-old broilers infected with a crude homogenate containing treponema's developed wonderful malabsorption like symptoms like: diarrhoea, growth retardation, osteoperosis, undigested food in intestine, a high alkaline fosphate activity and a low serum carotene content. He checked 150 broilerflocks in the field, 50 of them proved to be positive (F.A.T.) for spirochetes. Interesting finding ? More details of this study will be published in Avaian Pathology and presented at the world congress in Jerusalem next August.
The idea that we probably frustrated your knowledge of the aetiology of M.A.S..
M.A.S. even more than it already was,makes us feel quilty. So I like to end my presentation with something more positive. not going to reveal to you the name of the virus of bacteria causing M.A.S., nor the pesentation of a vaccine based on this antigen. No,we like to present you the results of a first experiment with a product that is probably well known to you, 3-Nitro. Clients in the U.K. who used this product for many years, reported favourable effects of it in reducing M.A.S. in their broiler blocks. Davelaar and Kouwenhoven of the Dutch P.H.I.. in Doorn, both known experts on M.A.S. were interested to test this product in their M.A.S. set up;

At the institute 2 groups of each 15 commercial day-old broiler chicks were placed in 2 isolators. Both groups were fed a diet with ± 66% corn in it. The metabolizable energy of this diet was appr. 3000 K cal. The controlgroup received the diet as such, the 3-Nitro group received 50 ppm 3-Nitro via de food.
Both groups were inoculated at day-1 with the standard M.A.S. inoculum of the Institute.
From 3 days of life onwards the controlgroup developed the typical M.A.S. symptoms, as they normally see them with their inoculum.
- depressed chicken
- yellow slimy faeces.
- Our testgroup also, but from 5 days onwards the colour of the faeces improved already. At day-7 the general appearance of the test chicks was less "depressed" than that of the controls.
- From 10 days on there was a definite breakthrough in the development of the chciks in favour of the 3-Nitro group.
- P.M.'s at day 13 revealed no pale intestines and livers in the test group, contrary to those found in the controlgroup.

Table - Next Page

Average bodyweight

1- at 12 days of age. The 3-Nitro group had a 27.8% higher bodyweight than the controls.
- at 20 days the difference was decreased to 12.2% in favour of the 3-Nitro.
Differences.These may have been more in favour of the 3-Nitro group when sexes were more equally divied within the control group. only 4 ♀ and 7 ♂♂.

2- No difference in the bone deformation index could be demonstrated.

3- The Institute considers the Alkaline fosfatase activity as a parameter for M.A.S.
You see confusing figures at 12 days and 20 days. high - low ??

4. Serum carotene content is also considered as an important parameter. You may observe at 12 and 20 days significant higher serum carotene contents in the 3-Nitro group. This indicates a better resorption of carotene from the feed into the intestine.

SALSBURY 3-NITRO AND MALABSORPTION

group	inoculated with -	treated with 3-Nitro (50 ppm)
1) weight day 12 ♀ and ♂	158± 42	202± 51 +27.8%
	3 and 12	9 and 6
weight day 20 ♀ and ♂	336± 68	377± 86 +12.2%
	4 and 7	6 and 7
2) bone deformation		
day 12	4	3
day 20	1.3	1.2
3) ALP		
day 12	4.295± 1.912	7.046± 4.516
day 20	4.144± 2.409	1.748± 1.035
4) carotene		
day 12	0.084± 0.045	0.219± 0.068
day 20	0.240± 0.097	0.484± 0.037

Summarizing the results we may state that treatment with 3-Nitro had obvious a favourable effect on preventing some important clinical symptoms of M.A.S. A second test now with a higher dosis of 3-Nitro (75 ppm) is planned.

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STUDIES OF 078 COLISEPTICEMIA IN CHICKENS

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Colisepticemia in poultry is caused by a variety of E. coli strains. We studied the virulence of several strains in day-old chicks and found that 078 and 02 were the most virulent.

E. coli 078 is pathogenic to humans also and has been reported to be responsible for diarrhea and sepsis, both in infants and in adults. We could demonstrate that:

1. Chicks (LD50 = 10^2 - 10^4) were more sensitive than mice (LD50 = 10^7) to all the E. coli 078 isolates tested (19).

2. 078 from human enteritis (5 isolates) show low virulence to mice, and are more virulent to chicks.

3. 078 from severe epidemic of hospital neonatal sepsis in Hungary (3 isolates) were virulent in mice, but more in chicks.

4. 078 from humans (8 isolates) was less virulent to chicks than 078 from poultry specimens (11 isolates).

The results raise the following questions:

1. Do human and avian isolates of 078 share common antigenic determinants other than the O-antigen?

2. Are enteritis-related strains carried in poultry?

3. Was the severe hospital epidemic of 078 (Hungary) caused by an avian strain?

4. Can avian colisepticemia be caused by human strains?

SOME NEW CONCEPTS OF MAREK'S DISEASE PATHOGENESIS

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The salient features of MD pathogenesis are well known (1,6). For instance, it has long been known that lymphoid organs are the primary early target for the virus and that an acute lymphoreticulitis is the consequence. Virus replication in lymphocytes results in cell death followed by inflammation, and if the damage is sufficient, atrophy of the bursa and thymus. Relatively new information about this very important phase of MD pathogenesis came from studies by Shek et al (8), in our laboratory, who found that the major target lymphocyte at this time is the B-cell, no matter which organ is involved. Relatively few T-cells are cytolytically infected (4) even though it is the T-cell that ultimately is involved in tumors.

After 5-7 days, the infection type switches from cytolytic to latent, with viremia becoming evident. Frequently, there is splenic enlargement. The latent infection involves mostly T-cells plus a few B-cells (4,8). The T-cells are activated based on the presence of Ia antigen on their surface (4). At this same time, perhaps not coincidentally, the first immune responses are evident, even though there is a transient loss of mitogen responsiveness. Cells bearing the putative tumor-associated antigen, MATSA, first appear during this period. Up until this time, neither qualitative nor quantitative differences in the infection pattern can be associated with the genetic strain (with the possible rare exception of the resistant line 6), age, or virus strain virulence among the oncogenic serotype MDVs. None of the various features of early pathogenesis can be used to predict whether or not tumors will eventually develop.

During the next few days, infection spreads to other organs, probably by way of infected PBL. Infection is essentially focal in nature, perhaps only involving a small cluster of cells in a kidney tubule, for example. Focal infections are necrotizing and they do incite an inflammatory reaction.

The second major phase of MD begins coincident with the development of permanent immunosuppression about 14-21 days postinfection. There is a reappearance of cytolytic infection in the lymphoid organs, and nerve lesions, both inflammatory and proliferative, develop. Unlike the situation during the early cytolytic phase of infection, when genotype, age, virus strain, etc. are not influential, major differences are seen during the second phase. Moderate to high levels of infection, pathology, immunosuppression, etc. correlate with genetic susceptibility, virulent virus etc.

The ultimate response to MDV infection is, of course, cellular alteration. The most obvious type is neoplastic transformation which leads to the lymphomas we associate as the final lesion in MD. In chickens, this has invariably involved activated T-cells, which are the fundamental element in a lymphoma although the actual proportion of a tumor made up of transformed T-cells may vary from less than 5 to more than 50%. Gross tumors can be found as early as about 2 weeks after infection, but the incubation period is quite variable and depends on a number of factors including genetic constitution and virus strain. Atherosclerosis can result from another type of cellular alteration (5), but will not be considered here.

These, then, constitute the essential features of infection of chickens with oncogenic (serotype 1) MDV. There is something to be learned from a brief look at what happens in infections with other related but nononcogenic viruses like serotype 2 MDVs (example, SB-1), serotype 3 HVT, or attenuated serotype 1 MDVs (see 1,3,6). The most striking difference is that there is minimal or no early cytolytic infection associated with any of these viruses. HVT and SB-1 apparently do not infect B-cells easily (or at all?), and attenuated MDV may fail to infect lymphocytes of any type. On the other hand, MATSA-bearing cells can be found, there is an early period of splenic enlargement and a transient loss of mitogen responsiveness, and latent infections in the spleen and PBL persist with some or all of the nononcogenic viruses. There may even be transient proliferative lesions, for example in the nerves of HVT-infected birds. Permanent immunosuppression does not occur, and no tumors develop.

The questions that arise are numerous when we attempt to determine which features are requisite to tumor development. For instance, is it necessary to have the early B-cell infection and the consequent lymphoreticulitis? Or, how important is the latent phase of infection? Can the differences in oncogenic potential of serotype 1 vs. 2 or 3 viruses be related to immunosuppressive potential, or predilection for specific target subpopulations, or transforming potential? Is the oncogenicity or nononcogenicity of the various related herpesviruses due strictly to their ability to infect certain target cells, or rather to inherent differences in the viral genome (e.g. presence or number of "oncogenes")? What are the genotypically determined differences which affect pathogenesis, and how do they operate? These are examples; there are many others.

In an attempt to address these questions in a systematic fashion, we have developed a hypothesis related to the basis of the sequential changes that collectively constitute the pathogenesis of infection with an oncogenic strain of MDV in a genetically susceptible chicken (3,7).

1. B-cells but not T-cells (resting) are susceptible.

2. Cytolytic B-cell infection leads to "reticulitis" with infiltration of inflammatory cells.

3. T-cells become activated as part of the response.

4. Activated T-cells are susceptible to infection (likely because of the presence of Ia antigen). The number that become infected may be genetically controlled through differences in susceptibility to infection (direct effect) or through differences in the rate of activation (indirect effect). The number of infected T-cells may be the single most significant event in pathogenesis!

5. The outcome of infection in T-cells is subject to host influences through lymphokines, immune mechanisms or other mediators. Supportive evidence includes: (i) The switch from cytolytic to latent infection occurs simultaneously in B- and T-cells and coincides with the first evidence of immune responses. (ii) The second phase of cytolytic infection coincides with permanent immunosuppression. (iii) Explantation from host to cell culture activates latent infection.

6. Transformation is subject to virus-cell interactions and may require integration of viral DNA. Latent infections differ from transformation in that they are easily "turned on" whereas in the latter, virus "turn on" is less frequent and more variable. Furthermore, transformation is associated with a population of proliferating T-lymphoblasts which makes DNA integration possible.

7. Transformed cells must escape immunosurveillance. This can result from immunosuppression. Also, tumor cells may have variable levels of genome expression related to antigens necessary for recognition.

Following this hypothesis, we can identify several possible reasons for the failure of infection to induce tumors. First, failure to infect B-cells, as apparently occurs with the nononcogenic viruses like SB-1 and HVT obviates not only humoral immunosuppression, but also the reticulitis. Without the inflammatory reaction, there would be little T-cell activation and hence few T-cells would become infected. The fewer T-cells infected, the less chance for either suppression of CMI or transformation.

A second possible reason for nononcogenicity would be that the virus might not infect T-cells, activated or not. Yet another possibility is that the virus is quite able to infect T-cells, but does not kill them and hence does not cause immunosuppression. Alternatively, infected T-cells which are targets for transformation may always enter cytolytic infection, or express potent immunogens to cause rejection. Finally, there is the obvious possibility that a given virus might have few or no oncogenes required for transformation or that there is a failure of viral genes to integrate in the host cell genome.

The actual importance of T-cell activation can only be speculated at this time. However, it could be all-important as the common denominator of many of the observations already made regarding pathogenesis and the influence of various factors such as genetic constitution (see 2). For instance, it appears that there may be a correlation between genetic susceptibility and cell-mediated immune responses like mitogen response, GVH, etc.

What needs to be done? First, we need to learn what factors dictate the outcome of the virus-cell encounter and how these differ among chickens or viruses to affect pathogenesis. Second, the basis for immunosuppression needs clarification. Is it due only to cytolytic infection of effector cells, or is there proliferation and enhanced activity of suppressor cells? Also, what is the role of modifying factors such as lymphokines, macrophages, etc.? Finally, the factors which affect the frequency of tumors in various tissue locations need definition.

From all of this, it is clear that we have come a long way toward understanding MD pathogenesis, but the job is not yet finished.

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BIRDS AND THE 1984 ST. LOUIS ENCEPHALITIS EPIDEMIC IN SOUTHERN CALIFORNIA

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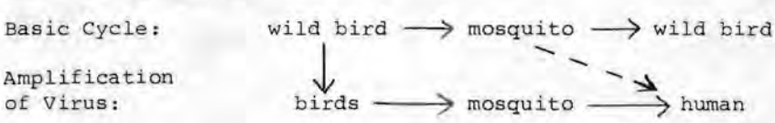
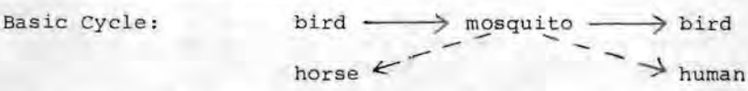
There was a major combined outbreak of Western Equine Encephalitis (WEE) and St. Louis Encephalitis (StLE) in California during the 1950's in the Sacramento and San Joaquin Valley. The two diseases seemingly remained quiescent in California until 1983 when extensive flooding of the lower Colorado River basin created conditions suitable for viral/avian/mosquito amplification. Nine cases of human StLE were reported in California in 1983. Most were associated with residence near, or travel to, the lower Colorado River basin. Two of the nine human cases of StLE recorded in Southern California were not associated with travel to the lower Colorado River basin. In 1984, two sentinel chicken flocks were placed; one in Long Beach and one in Irvine. On August 2, 1984 a human case was reported in central Los Angeles. The sentinel chickens in Long Beach and Irvine seroconverted, on August 30, 1984, to StLE and WEE. Encephalitis virus was found in mosquitos collected on September 13, 1984. By the end of September, there were seven confirmed human cases of StLE in Los Angeles County; by the end of October there were 16 cases. In 1984, twenty-six human cases of StLE, including three deaths occurred in Southern California. It is estimated that one confirmed clinical case represents about 200 subclinical cases. It is evident that the sentinel chicken flocks did not seroconvert to StLE and WEE early enough to alert the medical community that an epidemic was eminent. The 1984 human index case, rather than the chickens, was the sentinel. Counting the incubation period, the bird/mosquito amplification of the virus must have been occurring in July of 1984.

Cycle

St. Louis Encephalitis



Western Equine Encephalitis



Comparative Medical and Veterinary Services, Los Angeles County Department of Health Services, and the UCLA School of Public Health began to search the bird population for a better sentinel. Wild ducks and American coots were found to have titers to StLE but it could not be determined which birds were resident or which were migratory. It has been proposed that migratory birds carry encephalitis into a susceptible area and that domestic ducks are better sentinels. Local domestic ducks were found with serotiters to StLE. Feral pigeons (Rock Doves, Columbia livia) are ubiquitous in both urban and rural areas and do not migrate. Feral pigeons were found antibody positive for both StLE and WEE. Wild Passarine birds, especially sparrows, play an important

role in amplification of the virus in an urban setting. It is difficult to obtain enough serum for testing from the small species, some of which are migratory. Pheasants are susceptible to both infection and develop clinical signs of encephalitis to StLE and WEE. The few pheasants tested were negative to StLE and WEE. Western Equine Encephalitis outbreaks are often associated with StLE epidemics. As of April 1, 1985, there has not been a confirmed case of WEE in Southern California.

PREVALENCE OF HI ANTIBODIES TO ST. LOUIS ENCEPHALITIS VIRUS
IN BIRDS COLLECTED IN LOS ANGELES COUNTY, CALIFORNIA 1984

AREA	DUCKS	GEESE	COOTS	PIGEONS	OTHER
Lancaster	NS	NS	NS	NS	1/1*
Castaic	NS	NS	NS	NS	1/1
San Fernando	NS	NS	NS	NS	1/5
Granada Hills	NS	NS	NS	2/5	3/10
Torrance	1/1	NS	NS	NS	NS
Whittier	7/13	NS	NS	NS	NS
Marina Del Rey	0/11	1/6	NS	1/6	NS
Los Angeles	NS	NS	NS	1/1	NS
Long Beach	NS	NS	NS	6/7	NS
Carson	2/16	0/2	NS	NS	0/1
Downey	0/1	0/1	NS	NS	0/2
Santa Fe Dam	NS	NS	9/22	NS	NS
TOTAL	10/42 (24%)	1/9 (11%)	9/22 (41%)	10/19 (52%)	6/20 (30%)

Collections made from October 1984 through January 1985

HI: Hemagglutination-inhibition
NS: Not sampled

*Number with Positive HI titer of 1:20 and over/number collected

Adopted from Work, et. al. January 1985

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RECENT INVESTIGATIONS ON ASCITES IN MEXICO

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The Ascitic Syndrome (AS) is one of the most important problems that have affected the poultry industry in the recent years. In one study of 166 flocks, with a total population of 5,165,000 broilers within the Mexico City Valley, over the period from 1981 to 1984, the incidence of AS was 37.7% of total mortality wich for this group was 13.79% (1).

Several factors have been mentioned as important in this syndrome: genetics, nutrition , instalation, management and environment.

Much work has been done during the last three years. A summary of this work is presented here.

Under field conditions, several "feeding-treatments" have come into use against AS, the most common of these are feeding corn alone for several days, taking the animals off-feed for a short time, a reduction in the nutritive value of the feed and feed restriction. In broiler breeds, the incidence of AS is low and the severity of the problem decreases with the iniciation of feed restriction.

An experimental model was devised such that 294 broilers were divided into 3 groups with different feed and illumination programs. The first received light during 24 hours and feed ad libitum, the second received light during 24 hours with total food restriction during 7 hours in this same period and the third received 10 hours of illumination and feed ad libitum. The feed employed in this model had "produced" (bben associated with the presentation of) AS in a previous work.

Effects of feed and lighting programs used against AS in broilers

PARAMETER	GROUP 1	2	3
Mortality for AS (%)*	11.2	3.0	10.2
Feed consumption (g)*	3680	3413	3898
Feed/bird/day (g)*	146	124.6	156.5
Body weight (g)*	2022	1785	2096
Feed conversion *	2.12	2.29	1.83
% heart weight+	1.4	1.9	1.2
% liver weight+	3.8	4.2	4.6

* at 7th week; + related to body weight

In 3 birds with AS there was a considerable amount of fluid in the abdominal cavity without the presentation of hydropericardium. No pathogenic bacterial could be isolated from samples of heart, liver, spleen, yolk sac, gall blader, ascitic fluid

It would appear that the mortality due to AS could be reduced through a feed-restriction program (5).

Another study considered the repercussions of the advocated feed-restriction programs in the production. This model was done in the Mexico City Valley (2,300 m. altitude) in the period february thru april 1984 using 240 chicks distributed in 6 treatment groups with 4 repetitions; the feed employed in this model was obtained from a farm where the mortality due to AS was high..

The treatments used were:

1. Control (water and feed ad libitum)
2. Total feed restriction during 5 hours per 24 hours cycle on the 10^{th}, 21^{th}, 35^{th} and 42^{nd} days and during 24 hours on the 30^{th} and 45^{th} days.
3. 20% less than the registered feed consumption of group 1
4. Corn alone as feed for 2 periods of 3 days each during the 3^{rd} and 5^{th} week.
5. Administration of layer ration (16% crude protein) during 2 periods of 3 days each during the 3^{rd} and 5^{th} weeks.
6. Feed restriction 1 day a week during the entire experimental period.

Table 2

Effects of different feeding programs in broilers on production parameters

Parameter	Feed Consum.	Weight gain	Feed Conver.	Mort AS	1/	2/
1. Control	5683 de	2497 e	2.27 a	3/40	-	100
2. 5 and 24 hours	5732 e	2350 de	2.44 ab	2/40	2	107.8
3. 20% less	4456 a	1928 a	2.31 ab	0/40	9	105.5
4. Corn	5364 bc	2165 bc	2.47 ab	1/40	5	106
5. Laying feed	5421 bcd	2331 cd	2.32 ab	2/40	3	100.6* subsidized feed
6. 24 hours/week	5182 b	2087 ab	2.49 b	2/40	7	111

Different letter in the same column are different statistically (p 0.05)
1/ Extra days to get same weight than control
2/ Increase in cost of feed to get same weight

The treatment that showed the best results with respect to the production parameters, was group 1 this is so even though this same group had the highest incidence of AS. Here is the importance of an adequate evaluation of the magnitude of the problem before using any feed program that can affect the productivity (5).

The following experiment evaluated the effect of low-energy diet (2850, 2900 and 2950 Kcal/kg), low levels of vegetable oil (30,7 and 18 Kg/ton), high levels of methionine (0.5,0.48 and 0.44%), and the addition of vitamine E (30,000 IU/ton , vitamin C (300 g/ton , vitamin B1 3 g/ton, vitamin B/ 6 g/ton and sodium selenite 1ppm) together with good management and an emphasis on adequate temperature control to avoid diurnal and nocturnal fluctuations through the use of properly functioning heaters and drinkers and the correct management of litter and curtains. Eight houses of 15,000 birds each were used in Guanajuato, Mex. from april through may 1984, the experimental design was as follows:

1. Normal management and normal feed
2. Normal management and special feed

3. Special managemnet and normal feed
4. Special management and special feed.

Table 3

Effects of different feed and management programs against AS

Experimental Group	1	2	3	4
Feed consumption (g)	3960	3855	3749	3687
Weight gain (g)	1731	1811	1786	1800
Feed conversion	2.25	2.12	2.09	2.04
Total mortality	5.98	6.16	5.87	6.03
Mortality for AS	1.21	0.85	0.73	0.83

Statistically no significant differences.

The ranch and period of the year choosen for the trials have been associated with a high incidence of AS in the past, the low prevalence of the AS during this particular year is apparently inexplicable (4).

In another study, evaluation of data from laboratory of normal birds and those affected with AS raised at different altitudes were made. One hundred twelve broilers of different ages were studied (50% had AS).

Table 4

Data from laboratory analysis of normal birds and with AS raised at different altitudes

Alt.	Hemat (%)	hemog (g/dl)	plas. prot. (g/dl)	TGO (URF/L	FAS (UB)	Na	K
			Broilers without AS				
0*	33.1	10.4	3.9	58.9	55.8	133.3	4.7
2200*	33.5	9.8	4.1	60.6	74.4	131.8	4.3
2800+	37.1	10.7	3.3	60.6	66.7	131.5	2.6
			Broilers with AS				
0*	45.5	13.2	3.5	59	23.9	133	6.0
2200*	43.5	12.3	3.2	48	45	134	6.0
2800+	32.7	8.4	3.9	58.5	41.8	132.5	3.5

* 10^{th} week of age; + 7^{th} week of age.

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PRIMING AGENTS FOR BROILER BREEDER VACCINATION AGAINST INFECTIOUS BURSAL DISEASE (IBD). *

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Summary

Three different Infectious Bursal Disease virus (IBDV) strains were used to water vaccinate broiler breeders at 12 weeks of age, followed by an oil emulsified vaccine at 18 weeks of age. For evaluation, sera from breeders was collected before and after vaccinations and every four weeks from 30 to 50 weeks of age for titration against IBDV. At the same time eggs were saved (using trap nests), incubated and hatched; the progeny was wing banded and bled for titration against IBDV at 1, 7, 14 and 21 days. The results suggested that the mesogenic 2512 IBDV strain gave a more uniform response regardless if the birds were exposed or not to field IBDV during the growing period (1 day to 12 weeks). That the breeders raised under field conditions became susceptible to the field IBDV when their titers were between 160 and 320.

Introduction

Soon after the use of inactivated oil emulsified vaccine against IBD started, the poultry operators empirically realized that a combination of a live vaccine given at 10-12 weeks of age, followed by the inactivated vaccine, given at 18-20 weeks of age; gave higher titers and longer lasting immunity in the broiler breeders against IBDV, and that the progeny was also better protected against IBDV at an early age.

This study was conducted to determine the immunogenicity of three different IBDV strains used as priming agents with and without the influence of the environment, in broiler breeders and in their progeny.

Experimental Design

The breeders were individually identified at one day of age and separated into two groups: one group was raised under simulated field conditions, the other group raised in isolation. At twelve weeks of age each group was divided into three sub-groups; each one received a different strain of IBDV (Lukert, experimental 2512 and Bursa Vac), one recomended dose in the water per chicken and were placed in six different isolators. At 18 weeks of age every chicken was injected with one dose of inactivated emulsified IBDV vaccine and remain in the same isolator to eliminate the influence of the environment. At 24 weeks all the birds were moved into three laying houses with trap nests (one house per priming agent) and kept up to 50 weeks of age.

Blood from the breeders was taken at one day of age, at two weeks, at 4, 12, 18 and 24 weeks of age, and every four weeks from 30 to 50 weeks of age to measure the circulating antibodies against IBDV. Eggs from individual hens were collected and identified every four weeks from 30 to 50 weeks of age and incubated. When the chickens hatched they were wing banded.Virus neutralizing antibody titers against IBDV in the progeny were determined at one, 7, 14 and 21 days of age.

Laboratory Procedures

To titrate the anti-IBDV circulating antibodies, the beta method was used (constant virus-diluting serum). Two fold dilutions of the sera were done in 50 μl of 199 medium, when completed; 50 μl of an IBDV suspension was added to each titration well and to the virus control wells (Bursa Vac M strain was used, containing 2000 PFU per 50 μl) finally, a chicken-embryo fibroblast suspension (1 ml of packed

* Based on the MS thesis of the author conducted at the University of Delaware, Dept. of Animal Science. Newark, Delaware.

cells per 700 ml of growth medium) was added to every well (including the cell control wells) 50 µl per well. Positive anti-IBDV chicken-serum and negative chicken-serum was routinely included in all tests.

The micro-titer plates were placed in a 5% CO_2 incubator at 37°C saturated with water vapor. The plates were examined daily under an inverted microscope, by the third day the monolayer was usually confluent in the cell control wells and showed severe cytopathic effect in the virus control wells. At this stage the plates were fixed and stained in one step using a buffered 10% formalin-0.5% gentian violet solution. The plates were thoroughly washed under tap water and macroscopically read.

Results

Table 1

Infectious Bursal Disease Virus Neutralizing Antibody Titers in Breeders.

12 weeks IBDV Vaccine	Breeders Environment	Geometric Mean Titers					
		1 day	2 weeks	4 weeks	12 weeks[A]	18 weeks[B]	24 weeks
Lukert	Isolation	1741.81	207.49	31.74	<10	27.21	1612.69
Lukert	Field	2560.00	176.65	215.34	1810.19	1974.02	44667.19
2512	Isolation	1522.18	113.13	13.19	<10	6088.74	10240.00
2512	Field	2079.36	219.25	269.08	1015.93	2873.50	20480.00
Bursa Vac	Isolation	1560.33	195.04	26.91	<10	22611.75	37098.44
Bursa Vac	Field	1974.02	160.00	262.50	1940.11	8778.17	31041.87

12 weeks IBDV Vaccine	Breeders Environment	Geometric Mean Titers					
		30 weeks	34 weeks	38 weeks	42 weeks	46 weeks	50 weeks
Lukert	Isolation	452.54	1114.30	2152.69	1688.97	1280.00	452.54
Lukert	Field	6755.88	47050.68	23525.34	11762.67	5120.00	5120.00
2512	Isolation	2031.87	5120.00	6450.79	16254.98	8127.49	5120.00
2512	Field	7608.29	22988.02	7760.46	13782.01	7608.29	4561.40
Bursa Vac	Isolation	10240.00	14481.54	3044.37	5120.00	4063.74	3620.38
Bursa Vac	Field	4695.06	24354.96	5120.00	6241.34	4063.74	3620.38

[A] Priming vaccine given in the water

[B] Emulsified vaccine injected

Table 2

Infectious Bursal Disease Virus Neutralizing Antibody Titers in 34 Weeks Old Breeders and their Progeny.

Breeders		Geometric Mean Titers				
Priming Agent	Environment	Breeders	Progeny			
		34 weeks	1 day	7 days	14 days	21 days
Lukert	Isolation	1114.30	74(9)	62(8)	18(7)	11(5)
Lukert	Field	47050.68	761(8)	431(7)	113(6)	24(4)
2512	Isolation	5120.00	1280(5)	381(4)	127(3)	40(2)
2512	Field	22988.02	2301(13)	823(11)	288(13)	80(7)
Bursa Vac	Isolation	14481.96	NP	NP	NP	NP
Bursa Vac	Field	24354.96	780(7)	508(6)	160(5)	40(4)

( ) = number of chickens
NP = no progeny

Table 3

Infectious Bursal Disease Virus Neutralizing Antibody Titers in 42 Weeks Old Breeders and their Progeny

Breeders		Geometric Mean Titers				
Priming Agent	Environment	Breeders 42 weeks	Progeny 1 day	Progeny 7 days	Progeny 14 days	Progeny 21 days
Lukert	Isolation	1688.97	958(12)	359(12)	75(11)	17(12)
Lukert	Field	11762.67	7525(9)	2560(9)	587(8)	59(9)
2512	Isolation	16254.98	6451(9)	2792(8)	123(8)	26(8)
2512	Field	13782.01	7760(10)	2560(9)	274(9)	40(9)
Bursa Vac	Isolation	5120.00	8127(3)	3225(3)	202(3)	40(3)
Bursa Vac	Field	6241.34	NP	NP	NP	NP

Table 4

Infectious Bursal Disease Virus Neutralizing Antibody Titers in 50 Weeks Old Breeders and their Progeny

Breeders		Geometric Mean Titers				
Priming Agent	Environment	Breeders 50 weeks	Progeny 1 day	Progeny 7 days	Progeny 14 days	Progeny 21 days
Lukert	Isolation	452.54	32(3)	13(3)	<10(3)	<10(3)
Lukert	Field	5120.00	7241(2)	1810(2)	160(2)	10(2)
2512	Isolation	5120.00	2560(3)	403(3)	50(3)	8(3)
2512	Field	4561.40	3225(3)	640(3)	127(3)	13(3)
Bursa Vac	Isolation	3620.38	NP	NP	NP	NP
Bursa Vac	Field	3620.38	NP	NP	NP	NP

( ) = number of chickens
NP = no progeny

Conclusions

The results suggests that:

1. The strain of choice to be used as priming agent for broiler breeder vaccination against IBD should be a mesogenic strain of IBDV capable to override the field IBDV exposure but not too pathogenic to damage the bursa of Fabricius in susceptible chickens. In both cases such strain should be able to generate or increase the antibody titers against IBDV and the number of memory cells that will produce the plasma cells (manufactures of the antibodies), when the inactivated emulsified vaccine is administer. The IBDV, low-intermediate egg passage (p50-p55) 2512 strain meets this characteristics.
2. The 2512 IBDV strain seems to give a more uniform response regardless if the birds were exposed or not to the field IBDV during the growing period.
3. That the response to the different priming IBDV strains followed by the same inactivated emulsified vaccine will remain throughout the life of the breeder and also will be seen in their progeny.
4. It appears that the response to the Lukert and to the Bursa Vac IBDV strain will greatly depend on the exposure to field IBDV at an early age.

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LABORATORY TESTING OF A NEW KILLED E.COLI VACCINE FOR CHICKENS AND TURKEYS

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Introduction

Colibacillosis is considered to be a multimillion dollar problem in both turkeys and chickens. The use of day-of-age injectable antibiotics such as gentamicin has been effective in reducing early chick and poult mortality due to E.coli. Unfortunately, the commonly used antibiotics which are used to treat colibacillosis later in the bird's life are becoming less and less effective due to drug resistance. At present, in many cases the grower may find that none of the drugs that he has available to him will stop the problem.

Therefore, a logical approach to the control of colibaccillosis is the development of an E.coli bacterin. Fortunately for the industry, there are three major serotypes (01, 02, and 078). Other serotypes obviously do exist and are isolated at a less frequent rate.

The goal of our research effort was to develop an oil-emulsion bacterin that contained the three major serotypes and would be efficacious when administered at one day of age. In addition, we would want the same product to be effective as a breeder vaccine that would offer significant protection to recently hatched poults and chicks against E.coli induced mortality.

Today I would like to discuss the first aspect of our research program, namely the development of a day-of age bacterin.

Materials and Methods

Briefly, the bacterins were produced by growing the E.coli strains under conditions that would enable them to express pili. Standard methods were then used to prepare the oil-emulsion bacterins.

Broiler chicks and poults at various ages were vaccinated with the experimental bacterins and challenged primarily by the intramuscular route with homologous challenge cultures. The birds were observed for 8-10 days for mortality. At the end of the observation period, the birds were sacrificed, a post mortem examination performed on each bird, and gross lesions scored. The scoring system was as follows:

0 = normal, no lesions

1 = slight cloudiness of the pericardium and/or cloudy air sacs

2 = moderate thickening and cloudiness of the pericardium and/or moderate cloudiness of the air sacs

3 = severe pericarditis or thickening of the air sacs with fibrinous deposits or perihepatitis

Results:

Table 1

Evaluation of a Single Dose of an E.coli Bacterin Containing 01, 02 and 078 Strains in Turkeys Vaccinated Subcutaneously at 2 Weeks of Age and Challenged* at 4 Weeks of Age

Challenge Group	# Birds	Dose (ml)	# Birds Died (%)	Mean Lesion Score	Protection Index (%)
		EXPERIMENT 1			
01 Strain Vaccinates	10	0.5	0	0.1	100
Controls	10	-	6(60)	1.8	-
02 Strain Vaccinates	10	0.5	0	0	100
Controls	10	-	4(40)	2.6	-
078 Strain Vaccinates	10	0.5	0	0	100
Controls	10	-	9(90)	3.0	-

*IM Challenge

Turkeys vaccinated at 2 weeks of age and challenged 2 weeks later were protected against challenge. In a similar experiment, not shown, turkeys were vaccinated at 1 day of age and challenged at 4 weeks of age with similar type results.

Table 2

Serum Agglutination Titer to 01, 02 and 078 Strains in Turkeys Vaccinated at 2 Weeks of Age and Bled at 4 Weeks of Age

01 Strain		02 Strain		078 Strain	
Cont.	Vacc.	Cont.	Vacc.	Cont.	Vacc.
		EXPERIMENT 1			
16	4096	4	2048	64	4096
32	256	8	512	32	512
8	256	32	2048	32	128
8	128	16	256	64	512
8	256	2	512	64	256
16	512	8	1024	64	256
8	512	16	128	32	512
8	256	8	256	32	128
Mean 13	784	12	848	48	800

Eight turkeys, at random, from experiment 1 (Table 1) were bled at 4 weeks of age and a serum agglutination test performed on each serum. The results indicate excellent serological response to all three serotypes of E.coli present in the vaccine.

Table 3

Evaluation of a Single Dose of an E.coli Bacterin Containing 01, 02 and 078 Strains in Chicks Vaccinated Subcutaneously at Day of Age and Challenged at 4 Weeks of Age

Challenged Group	# Birds	Dose (ml)	Route of Challenge	# Birds Died	% Birds Died
		EXPERIMENT 2			
01 Strain Vaccinates	20	0.1	IV	2	10
Controls	20	-	IV	12	60
02 Strain Vaccinates	23	0.1	IM	13	57
Controls	23	-	IM	23	100
078 Strain Vaccinates	20	0.1	IV	0	0
Controls	17	-	IV	11	65

Chickens vaccinated at one day of age and challenged 4 weeks later showed very good levels of protection, as compared to the nonvaccinated challenged controls.

Table 4

Serum Agglutination Titers to 01, 02 and 078 Antigens in Chicks Vaccinated at Day-Of-Age and 3 Weeks of Age with Blood Samples Collected at 4 Weeks of Age

01 Strain		02 Strain		078 Strain	
Cont.	Vacc.	Cont.	Vacc.	Cont.	Vacc.
		EXPERIMENT 2			
64	512	8	256	16	128
16	2048	4	512	32	128
32	1024	2	256	16	64
32	256	4	256	8	16
32	64	8	32	32	32
64	4096	4	32	8	256
32	256	8	2048	8	128
16	512	2	128	32	256
Mean 36	1096	5	440	19	126

Eight chickens, at random, that were vaccinated in experiment 2 (Table 3) were bled at 4 weeks of age and a serum agglutination test performed on each sample. Most of the chickens in each group showed at least a 4 fold increase in titer, compared to the nonvaccinated controls.

Conclusion:

Chicks and poults vaccinated at day-of age or at 2 weeks of age demonstrated significant protection against challenge with 01, 02, and 078 strains. Similarly, serum from vaccinated birds showed significant increases in agglutination titers to all three serotypes.

FIELD TRIALS WITH INACTIVATED E. COLI VACCINE

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Extensive losses to the poultry and turkey industries due to E. coli infections are well recognized (4,5). In spite of the occurrence of a large number of serogroups associated with E. coli in poultry, only organisms belonging to a few serogroups, particularly those of 01, 02 and 078 have been found to be pathogenic. Although efforts were made in the past to develop E. coli vaccines, unfortunately, due to one or the other reason, no vaccines were licensed for commercial production (1,2,3). In view of the increased drug resistance of these organisms further efforts were made to develop E. coli vaccine for poultry. One such vaccine produced by Vineland Laboratories for use in chickens and turkeys was recently licensed by the USDA. The present report describes the use of Vineland's vaccine under field conditions.

The results of testing of the licensed vaccines (S# 6608) under laboratory conditions in chickens and turkeys are given in Tables I and II and Figures 1 and 2.

Table I

Results Of Vaccination Of Chickens With
E. coli Vaccine And Subsequent Challenge

Group	No. of Birds	No. Died of Colisepticemia	% Protection
Vaccinated	20	7	65
Control	20	19	5

Chickens were vaccinated at 3 and 6 weeks of age. They were challenged at 7 weeks intravenously using 0.5 ml of a 10^{-1} dilution of culture containing 3×10^9 ml organisms (078).

FIGURE 1

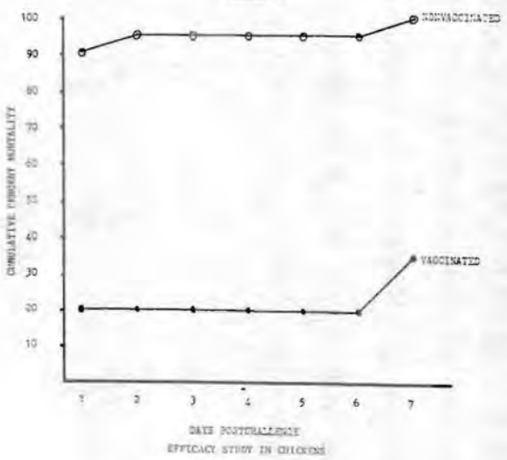

FIGURE 2

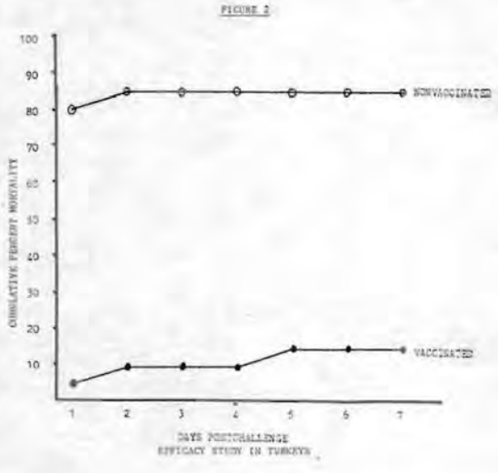

Table II

Results Of Vaccination Of Turkeys With
E. coli Vaccine And Subsequent Challenge

Group	No. of Birds	No. Died of Colisepticemia	% Protection
Vaccinated	20	3	85
Control	19	17	10

Turkeys were vaccinated at 2 and 5 weeks of age. They were challenged at 6 weeks of age intravenously using 0.5 ml of a 10^{-1} dilution of culture containing 2.4×10^9/ml organisms (078).

Not more than 20 % of the vaccinated chickens and turkeys had a pea sized granulomatous lump after first vaccination. This lump could not be seen at the time of second vaccination. Other than this, no other untoward post vaccination reactions were seen. It is evident that in spite of a very heavy challenge given intravenously, there was good protection of the vaccinated chickens and turkeys.

The field trials with this vaccine were conducted in broilers and in broiler-breeders. For broiler vaccination, four farms were selected with no specific E. coli problem. At all farms, vaccination was done using automatic vaccinating equipment. However, the farm managers used only one dose of vaccine. At Farm A, the vaccine was used imm-diately after the Marek's vaccine, while at the other farms it was used after an interval of at least 8 hours. The results are shown in Table III.

Table III

Results Of Field Trials In Broilers

Group	Treatment	No. of Birds Used	% Condemnation Air Sac/Sep Tox	% Condemnation Total
A	Control	93,500	.93	1.79
	Vaccinated	95,600	.70	1.65
B	Control	22,700	1.6	1.62
	Vaccinated	22,700	0.81	.82
C	Control	13,700	0.41	0.48
	Vaccinated	13,700	0.20	0.81
D	Control	10,000	0.55	0.56
	Vaccinated	10,000	0.32	0.33

In Group A when both E. coli and Marek's vaccinations were done one after another, there was marginal increase in leucosis condemnations, but there was reduction in condemnations due to air sac sep. toxemia and in total condemnations. In the rest of the groups there was almost 50 % reduction in condemnations due to air sacculitis and septicemia toxemia.

The results of vaccination with E. coli vaccine in a problem farm are given in Table IV.

Table IV

Results Of Field Trials In E. coli Problem Broiler Farm

Group	Treatment	No. of Birds Used	% Condemnation Air Sac/Sep Tox	% Condemnation Total
A	Control	10,400	1.89	4.06
	Vaccinated	10,800	.79	1.84

Vaccination at this farm was done at 10 days of age. Against the recommended two vaccinations, only one vaccination was done. It is very evident that again the losses due to condemnation were significantly reduced.

Vaccinations were also attempted in broiler breeders. In the field trials over 250,000 breeders have been vaccinated. There was no deleterious effect after first or second vaccination. The first progeny broilers are now ready to go for processing. In the meantime, progeny from the vaccinated breeders have been brought into the laboratory and tested. The results of challenge of progeny are presented in Table V.

Table V

Results Of Challenge Of 1-Day-Old Broiler Chickens Via The Intratracheal Route With E. coli Serotypes 02: and 078:

Breeder Flock Identity	Breeder Vaccination[A]	Age of Breeder	Progeny[B] Challenge Results (% Protect.)
1	E. coli Bacterin 2X	36 wks.	69 %
2	E. coli Bacterin 2X	32 wks.	81 %
3	E. coli Bacterin 2X	32 wks.	81 %
4	E. coli Bacterin 2X	30 wks.	57 %
5	None	28 wks.	0 %

A Vaccinations were done at 20 and 25 weeks of age.

B Progeny were challenged at 1 day of age via the tracheal route with 10^8 CFU of E. coli/bird. Average of results with 02 and 078 challenge.

It is quite evident that the progeny was protected against a very heavy challenge.

The results of duration of maternal immunity are given in Table VI.

Table VI

Duration Of Passive Immunity In Progeny From Nonvaccinated Breeders And Breeders Vaccinated With An E. coli Bacterin

Age at E. coli Challenge(Route)[A]	E. coli Vaccination[B] Nonvaccinated % Affected	Vaccinated 1X % Affected	Vaccinated 2X % Affected
1 Day (IT)	88	20	6
3 Days (IT)	81	38	7
7 Days (IT)	56	0	6
14 Days (IT)	13	7	0
21 Days (IV)	69	--	25

[A] Birds were challenged with 10^5 through 10^8 CFUs of E. coli (02A:K1:NM) Intratracheally (IT) or Intravenously (IV).

[B] Hens were vaccinated once at 20 wks. of age or twice at 20 and 25 wks.

It is very evident that the protection was seen up to 21 days, the longest period for which the broiler progeny was tested.

The results of effect of maternal immunity on progeny vaccination are presented in Table VII.

Table VII

Effect Of Maternal Antibody On Broiler Vaccination Of Day Old Chickens With An E. coli Bacterin

Breeder Treatment[A]	Broiler Treatment[B]	% Affected[C]
Vaccinated	Vaccinated	48
Nonvaccinated	Nonvaccinated	79
Vaccinated	Nonvaccinated	29

[A] Breeders were vaccinated at 20 and 25 weeks of age with 0.5 ml subcutaneously

[B] Broilers were vaccinated at one day of age with 0.25 ml subcutaneously

[C] Percentage of birds dead or with moderate to severe lesions following challenge (IV) with 02A:K1:NM at 2 1/2 weeks of age.

It is apparent from the table that progeny from vaccinated breeders were more resistant to challenge than the progeny of nonvaccinated breeders. However, if progeny from vaccinated breeders were revaccinated at one day of age after 2 1/2 weeks, they were found to have less resistance to infection than those which came from vaccinated breeders.

In order to ascertain if the resistance to challenge in the progeny from the vaccinated breeders was limited to only the serotype strain contained in the vaccine, the progeny was challenged with six Group 02 virulent field isolates. It was found that the progeny from the vaccinated breeders was resistant to all six field isolates while the progeny of nonvaccinated breeders was susceptible.

In conclusion, it may be said that the Vineland E. coli vaccine was found to be safe and efficacious both for broiler vaccination and for broiler breeder vaccination.

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Note: Part of this work was done in the Department of Pathology at Iowa State University, Ames, Iowa, which is gratefully acknowledged.

VACCINATION AGAINST COLISEPTICEMIA IN CHICKENS

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Colisepticemia is caused by the invasion of primarily stressed tissues with pathogenic strains of Escherichia coli - E. coli. The antigenic structures of the organism are identified by O - somatic antigen, K - capsular antigen, H - flagellar antigen and P - pili. There are recognized 164 O-antigen, 100 K-antigen, 49 H-antigen serotypes, as well as an unknown number of morphologically and antigenically different pili. Disease outbreaks have been shown to be caused by 79 different O-antigen subgroups of which O1, O2 and O78 were the dominant serotypes, causing about 50% of the outbreaks.

The two main difficulties in containing the disease are:

1. The poor response to treatment due to the rapid development of resistance to the various available antibacterial agents. This resistance is usually genetically transferable from one strain to another, making the problem more severe. The development of new drugs for poultry is inhibited by the high cost of reseach and regulatory problems.
2. Vaccination is not very effective due to the large number of pathogenic E. coli strains which are immunologically different. Even so, the use is limited to inactivated (killed) bacterins containing several pathogenic strains and the only means of applying such a vaccine is by individual injection. This method is unapplicable for mass treatment and it is impossible to cover all the E. coli strains which are potential pathogens.

We have developed a mutant of E. coli K-12, a labortory strain, which has been chemically mutagenized. It is defective in the biosynthesis of the species specific O-antigen and therefore stimulates the production of antibodies against these part of the lipopolylyposacharide (LPS) which are common to all the enteric bacteria. In addition, it activates macrophages and phagocytes, thus elevating non-specific immunity. This strain - LR-2 - has the following properties:

1. Safety - Our mutant is completely avirulent - no damage was detected in mice or chicks repeatedly injected with more than 10^8 bacteria per animal. In addition, the rate of reversion of LR-2 is lower than $1:10^9$.

2. Multivalent vaccine - antibodies obtained in immunized rabbits cross reacted with chemically purified LPS from a wide variety of enteric bacteria including, in addition to E. coli - Klebsiella, Enterobacter, Serratia and Proteus.

3. Effective protection - a small number of vaccinations was shown to be sufficient for protection.

These characteristics of LR-2 enable its use as a live polyvalent vaccine. Thus, it can be used for mass vaccination by means of aerosolization, nebutalization or in the drinking water.

In laboratory experiments we demonstrated the protective effect of LR-2 on subsequent intratracheal challenge with virulent strains of E. coli, O2 and O78. Several parameters were examined: weight gain, mortality and pathological examinations. The results indicate that:

a. Protection against several virulent strains of E. coli can be obtained after one vaccination. Measurements of weight gain, which is an objective and economically important parameter, indicated that vaccinated chicks increased their weight after a challenge with pathogenic E. coli by 45% in three weeks, in contrast to unvaccinated challenged controls which only increased weight by 6%.
b. Vaccination can be given effectively at age 5 - 35 days.
c. Protection after one vaccination lasts for a minimal period of 20 days.
d. Vaccinated chicks are more resistant to repeated infections with virulent bacteria than previously unvaccinated chicks.

INFECTIOUS BURSAL DISEASE VIRUS VACCINE STUDIES

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Introduction

Vaccination of breeder replacements with sequentially administered live and inactivated infectious bursal disease virus (IBDV) is a common practice. This is done to obtain immune progeny chicks that can resist early IBDV exposure. Generally, this vaccination program is successful in controlling early IBDV infection and its immunosuppressive aftermath. However, immune chicks usually are not protected against clinical IBD as maternal antibody declines steadily following hatch and is essentially gone by 3 to 4 weeks of age. Therefore, chicks placed on farms already seeded with virulent field strains of IBDV should be vaccinated early in life; often in the face of maternal antibody. The purpose of such a practice is to induce an active immune response that will protect chicks against clinical IBD.

The objectives of the research to be described were three-fold:

1. To evaluate the efficiency of IBDV maternal antibody transfer from breeder hen to progeny chickens.

2. To study maternal antibody decay in immune chicks.

3. To study the immune response of susceptible and immune chicks to commercial vaccines.

Materials and Methods

Susceptible and immune heavy breed chickens used in this study were derived from SPF White Plymouth Rocks and commercial broiler breeders, respectively. The latter averaged 56-weeks-of-age, were trapnested, and had been vaccinated with live and inactivated IBDV. Both sources of chickens (25-35/vaccine) were held in isolation cabinets and vaccinated (1 dose, subcutaneously or intraocularly) at 2, 6, 10, 14 and 21 days of age with 3 or 4 different IBD vaccines. At 4 days post-vaccination, bursae and spleens were collected (5 chicks/vaccine) for virus replication assays by direct immunofluorescence (FA) and histopathology. At 14 days post-IBD vaccination 10-20 chicks per vaccine type were challenged (0.2 ml per os) with 100 EID_{50} of the IM strain of IBDV. Fourteen days post-challenge, IBD immunity was evaluated by calculating bursa:body weight ratios. IBD vaccines were also examined for immunosuppressive potential by vaccinating 5-10 chicks per treatment with Newcastle disease virus (NDV; B_1 strain; 1 dose ocularly) at 20 days post-IBD vaccination. Newcastle disease immunity was evaluated 20 days post-ND vaccination by challenge (Texas GB strain NDV; 10,000 ELD_{50} per 0.2 ml, intramuscularly; record mortality) and hemagglutination inhibition (HI) testing. An appropriate number of unvaccinated-unchallenged, unvaccinated-IBDV challenged and unvaccinated-NDV challenged controls were also included in these studies.

Egg yolks and/or sera from commercial breeders and progeny chicks were examined for IBDV and NDV antibody by agar-gel precipitin (AGP), microtiter virus neutralization (VN) and HI test procedures.

Results

An examination of sera and egg yolks from nearly 400 trapnested broiler breeders revealed IBDV antibody levels ranging from 1:2,000 - 128,000. A majority (77%; both sera and yolk) of sample titers were about mid-range. Serum/yolk antibodies of individual breeders rarely exceeded a 4-fold titer difference. As a result of commonly high levels of breeder serum/yolk antibody, high levels of maternal antibody (62% tested; 1:512 - 4,096) were usually transferred to progeny chicks. Maternal antibody was found to persist in unvaccinated immune chicks for at least 21 days. Maternal antibody levels of vaccinated passively immune chicks were not found to be appreciably different than their unvaccinated counterparts.

Results of IBD vaccination in this laboratory trial revealed that the vaccine lots tested couldn't stimulate an active immune response in chicks with maternal antibody levels greater than 1:64. Fully susceptible chicks responded well, immunologically, to the commercial vaccines tested. Immunofluorescent and histopathologic examination of bursae and spleens added support to the serologic findings. FA reactions of bursae and spleens in susceptible chicks were abundant, whereas in immune chicks reactions were very limited, most of them occurring in 10 and 14 day old chicks. Bursal and splenic changes consistent with invasiveness were found on examination of H & E stained sections. These changes were more pronounced in susceptible than immune chicks. Chicks experiencing an active immune response as a result of IBD vaccination were usually found to resist an IBD challenge. Additionally, the immune system of IBD vaccinated chicks was not compromised as revealed by NDV hemagglutination inhibition and immunity challenge tests.

Discussion

This study has demonstrated that inactivated oil emulsion vaccine is highly immunogenic, as has been noted by several other researchers (1,3,4). Maternal antibody was shown to be efficiently

transferred from breeder hen to progeny chicks and to persist for at least 21 days. Such data strongly support the concept of breeder vaccination to protect progeny against early infection. Generally, early vaccination did not adversely affect passive antibody titers or their decay rate. This study interestingly revealed that maternal antibody levels must be relatively low (1:4-64) before IBD vaccines (of varying invasiveness) can stimulate an active antibody response. A similar finding has been reported by Naqi et al. of Texas A&M University (2). In practical terms, these data suggest that chicken flocks with relatively uniform and high levels of maternal antibody can't be effectively vaccinated against IBD until they have reached 2-3 weeks of age and more importantly that producers of commercial breeders or broilers know the maternal antibody level of placed chicks so as to determine proper vaccination timing.

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STRUCTURAL ANALYSIS OF INFECTIOUS BURSAL DISEASE VIRUS

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Infectious bursal disease, or Gumboro disease, is an economically significant infection of chicken flocks. The characteristic pathological lesion is bursal necrosis, resulting in defects of humoral immunity. The responsible agent is a novel virus related to infectious pancreatic necrosis virus of salmonids, tellina virus of bivalve molluscs, and drosophila X virus. Infectious bursal disease virus (IBDV) is a naked icosahedron of about 60 nm with a buoyant density of 1.33 g/cc. Virus preparations are composed of two sedimenting species, the faster of which possesses virtually all of the specific infectivity. Interferon is elicited in CEF cultures; consequently, the virus is cultured in Vero cells where maximum titers of extracellular virus of about 5×10^7 pfu/ml are reached 48-120 h post-infection.

Immunoprecipitation with monospecific antisera and peptide mapping with V8 protease indicate that the viral structural proteins mature via post-translational cleavage of one or more polyproteins and that a number of minor proteins observed in SDS-PAGE are actually incomplete cleavage intermediates of the major polypeptides. Attempts to obtain the primary structure of two mature proteins, p40 and p31, by gas phase amino acid sequencing were not successful, presumably due to amino terminal acetylation. DEAE chromatography achieved purification of p40; hyperimmune rabbit anti-p40 serum exhibited strong virus neutralizing activity in plaque reduction assay.

The viral genome is composed of two apparently unrelated segments of double-stranded RNA, with molecular weights of approximately 2.5 and 2.2 million and a velocity of about 22 S. The genome is very resistant to denaturation, having a T_m near 95°C. Oligo d(T)-cellulose chromatography indicates the absence of polyadenylation. Poly(A) tails can be added with poly(A) polymerase; however, this results in apparent nicking of the RNA and a subsequent failure to obtain cDNA by oligo d(T)-priming of reverse transcription. Eventually, melted RNA was primed with calf thymus DNA and first strand cDNA synthesized with reverse transcriptase. Molecular sieve chromatography was used to select RNA-DNA hybrids containing cDNA of weight average equivalent to 600 bp. The heteroduplexes were C-tailed with terminal transferase and cloned into the G-tailed Pst I site of pBR322. The vector was transformed into HB-101 and a small number of transformants obtained by tetracycline selection and ampicillin screening. Southern blotting with nick-translated pBR322 DNA and viral cDNA probes were used to ascertain the specificity of the cloned inserts. The relationship of the clones is being assessed by matrix slot-blot hybridization and the genomic complexity by Northern blot analysis of the genome with selected clones as probe. The primary structure of the inserts in the size range of 1 Kbp is being obtained by M13 sequencing and additional clones are being prepared from cDNA synthesized by specific priming with mixtures of random-primed cloned DNA.

In summary, then, we have further characterized the biological activity of infectious bursal disease virus, examined the nature of viral protein synthesis and post-translational modification, and identified a viral polypeptide - p40 - that elicits antibody with in vitro viral neutralizing activity. Further, we have obtained recombinant DNA clones of the viral double-stranded, bisegmented RNA genome by the somewhat novel method of cloning an RNA-cDNA hybrid. We are sequencing these clones right now and also using them as specific primers for further cDNA cloning. Once sufficient sequence data is available and we have mapped the polypeptides within the genome, we will attempt to subclone protein-encoding regions, specifically the p40 gene, into suitable expression vectors for the production of candidate vaccine materials.

COMPARING IBD MICRO-VIRUS NEUTRALIZATION TITERS IN DAMS, EGG YOLKS AND NEONATAL CHICKS

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Joseph Schultz
Douglas Allen

Introduction

The individual chick titers of broiler breeder progeny has become a subject for considerable discussion. This is particularly true when the breeders are in the transition period between the use of a live IBD vaccination program to a combination live-inactivated program.

There are a lot of factors involved. There is considerable difference in the results between laboratories; the age of the breeder is an important factor, it is observed that some pure lines respond with different intensity to certain antigens. We felt it was worthwhile to look at the matter a little more comprehensively than we had in the past. We had two objectives in mind: the variation in the titers of individual chicks from the same dam,and comparison of antibody titers between yolk and chick serum.

Materials and Methods

Hens: A group of 92 Broiler Breeder type pure line hens were trap nested and the eggs identified for a period of 14 days. These hens had only live IBD vaccine used for immunization against IBD. They were also vaccinated for fowl pox, infectious bronchitis, Newcastle disease, Avian encephalomyelitis, and vital arthritis. The 50 that laid a minimum of 10 eggs were selected for the trial. The first and last eggs, as well as one that was laid in mid period, were selected for yolk assay. The rest of the eggs were incubated. One hen died during the trap nest period and two had no eggs that hatched, so these were eliminated, leaving 47 hens in the trial. The hens were bled on the 15th day to avoid handling and/or mortality during the trap nest period.

Yolk: The large end of the egg shell was removed and 1 ml. of yolk was removed and combined with 1 ml. of chloroform and 1 ml. of PBS, thoroughly mixed and allowed to set for one hour. It was then centrifuged for 15 minutes at 1500 rpm. The aqueous portion was saved for assay.

Chick: The chicks that hatched were held for 24 hours and blood taken and the chicks discarded. The blood was allowed to clot and the sera separated and saved for assay.

The sera and yolk extracts were coded, along with negative controls were sent to a private laboratory for virus micro-neutralization tests for IBD.

Results

The Geometric Mean Titer of the hens was 1:981, the yolk was 1:608 and the chicks was 1:576 (Table 1). The difference was for all practical purposes 1 dilution between the dams and the egg or the chick.

However, the results are not really that simple. We grouped the hens by individual hen titer and studied the frequency distribution of the titers of the progeny. The results are shown in the tables.

Twenty-nine of the 47 hens (61.7%) selected for the test had individual titers of 1:320 (17) or 1:1280 (12). These were selected for further consideration of the variation of titer of the progeny. The results are shown in Tables 2 and 3 and Figures 1 and 2.

Discussion

There is considerable variation in the neutralizing antibody extracted from the yolk and in the chick sera from individual hens with the same neutralizing titer. This is considered a pilot trial and it will be repeated using the ELISA method as well as Micro VN. If there is truly this much variation in the titers of individual dams, more thought is going to have to be given to the most effective method of vaccinating the progeny for protection against IBD.

TABLE 1

IBD IMMUNITY SURVEY, TRAP NESTED HENS

	Hens Number	Hens GMT	Yolk 3 Eggs/Hen GMT	Yolk Range Individual Titer	# Chicks	Chicks GMT	Chick Range Individual Titer
	2	1:160	1:587	1:160 1:2560	13	1:245	1:80 1:640
	17	1:320	1:256	1:40 1:1280	81	1:240	1:40 1:1280
	3	1:640	1:640	1:160 1:5120	13	1:218	1:80 1:640
	12	1:1280	1:874	1:160 1:2560	62	1:832	1:40 1:2560
	6	1:2560	1:1088	1:320 1:2560	25	1:704	1:80 1:2560
	5	1:5120	1:1621	1:160 1:5120	27	1:1920	1:640 1:5120
	2	1:10240	1:3413	1:1280 1:5120	9	1:3157	1:1280 1:5120
TOTAL	47	1:981 RANGE 1:40 1:10, 240	1:608	1:20 1:5120	230	1:576	1:40 1:5120

TABLE 2

1:320

HEN	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
A41			11		11			
A204							1	
A1176				1111	111	1		
A1257	11	11						
A1734	1	1	1111					
A2012			111	11				
A2780			11					
A2796			1		11	1		
A3287			1111	11				
A3374				1	111			
B233			11					
B418					111	111		
B536		1	1111	1				
B546				~~1111~~	11			
B1032	1		111					
B1042		11	~~1111~~	111				
B1190			1	1				
TOTAL 17	4	6	31	19	15	5	1	

TABLE 3

1:1280

HEN	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
A224					111	111	11	
A265			11	11				
A275					1			
A2067				11	111	1		
A2075						1	11	
A2090						1	11	1
A2706	1	1	1	11	1			
B96				1		1111	11	
B945				1	11	1111	11	
B963				111	1			
B1087				111		1		
A1781					111	11	1	
TOTAL 12	1	1	3	14	14	17	11	1

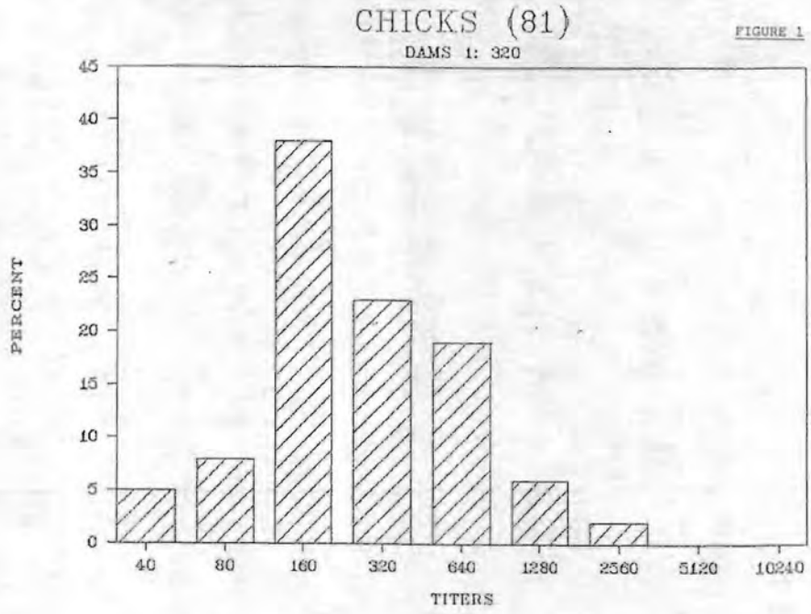

FIGURE 1

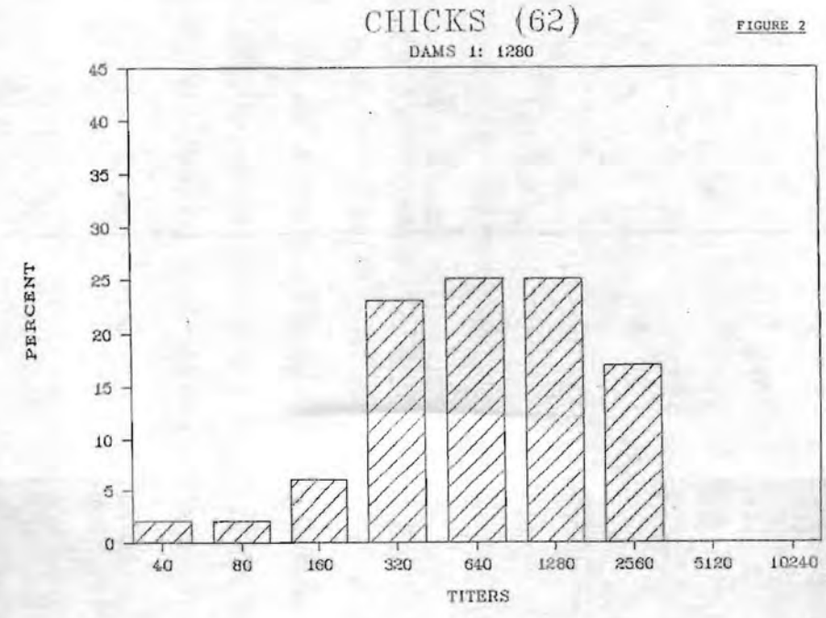

FIGURE 2

COMPARISON OF INFECTIOUS BURSAL DISEASE ANTIBODY LEVELS BY ENZYME LINKED IMMUNOSORBENT ASSAY AND VIRUS NEUTRALIZATION TESTING IN COMMERCIAL CHICKENS.

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Two flocks of commercial type White Leghorn chickens were tested sequentially for antibody levels against Infectious Bursal Disease (IBD) using the Enzyme Linked Immunosorbent Assay (ELISA) and the Micro Virus Neutralization (VN) test.

Serum samples were collected once from the parental breeder flocks and from progeny chickens in one flock at 9, 12, 20, 27, 35, 42 and 47 days of age. Progeny chickens of the 2nd. flock were tested at 7, 15, 20, 27, 43, 49, 57 and 75 days of age. The first progeny flock was vaccinated against IBD at 16 days of age, the second progeny flock at 10 and 21 days of age.

The ELISA testing was performed in 96-well polystyrene plates (Dynatech), which were precoated with IBDV antigen. The plates were washed 3 times in wash solution (H_2O - Tween). Then 50 ul of serum, diluted 1:50 in diluent (Tris-EDTA-saline with 0.1% BSA) was added to each well. Appropriate high and low titer and negative serum controls were added at the same time. The plates were incubated at 37° C. for 45 minutes after which the plates were washed 4 times with wash solution.

Then 50 ul of goat anti-chicken IgG-horseradish peroxidase conjugate (K&P) diluted 1:200 in PBS was added to each well and the plates incubated at room temperature for 30 minutes. Plates were then washed 4 times in wash solution, holding the last wash for 3 minutes before discarding. Then 100 ul of O-phenylenediamine (OPD) substrate was added to each well and the plates were incubated for 10-15 minutes at room temperature. The reaction was stopped by adding 50 ul of 2.0-2.5N H_2SO_4 to each well. The highly positive control should read .600-.700 at 490 nm. Histograms were prepared by grouping samples by their absorbance value into 7 groups: 0.000-0.099 = group 0, 0.100-0.199 = group 1, etc.

Micro Virus Neutralization (VN) tests were performed on the same serum samples, using the constant virus-serum dilution method with 1000 $TCID_{50}$ of IBD virus per well and serial two-fold dilution of serum.

The results indicated good correlation between VN and ELISA antibody titers. It was observed that the breeders had high levels of IBD antibody. IBD maternal antibody levels in the first progeny flock became negative at 27 days of age, and then remained negative until 49 days when an increase in antibody titers was noted. In the second flock (maternal) antibody could be detected at appreciable levels until 42 days of age, at which time no more IBD antibody levels could be found. At 47 days of age antibody titers were again observed. It is conceivable that immune suppression was a factor in this late development of IBD antibody, since bursal follicular depletion of lymphocytes was observed in several birds examined at 46 days of age. Gross lesions of bursal and splenic atrophy as well as coccidiosis and ulcerative enteritis were found in these birds. Such lesions are frequently found together, in our laboratory. It is thought that immune suppression is involved with the incidence of coccidiosis and ulcerative enteritis.

In conclusion, it appears that ELISA is a reliable testing method for IBD antibody and correlates well with conventional VN testing.

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A VIRAL DISEASE OF GROUSE

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Very little is known about diseases of upland game birds, except perhaps of the more common species of quail and pheasants.

The Oregon State University Veterinary Diagnostic Laboratory received three Franklin spruce grouse (Dendragapus canadenis var. Franklinii) raised by a quail breeders' society. These birds were approximately two years old and sexually mature. This species is extremely difficult to raise, and probably no more than 20-25 individuals exist in captivity in the United States.

The symptoms observed by the breeders were depression, fluffed-up appearance, and watery, foamy fecal droppings. The onset of symptoms was usually rapid, with mortality occurring within a few days. Some of the individuals displayed respiratory difficulty and gasping. They were raised together on wire-floor pens. The breeders also raised a variety of other grouse and quail; they appeared healthy.

On necropsy examination, the grouse were well-fleshed. The lungs were dark red and firm. The spleens were enlarged and showed randomly distributed, prominent, white foci. The intestines contained brown, fluid, fecal contents. Aerobic and anerobic routine bacterial cultures did not demonstrate significant pathogenic organisms.

Microscopically, lesions were confined to the lung and spleen. In the spleen, large, multifocal areas of fibrinoid necrosis with karryorhexis of lymphocyte nuclei and reticuloendothelial cell degeneration were seen. Amyloid was not demonstrated by histochemical means (Congo Red stains). Numerous intranuclear basophilic and eosinophilic inclusion bodies were seen within swollen reticuloendothelial cells. Interstitial pneumonitis, fibrinous pleuritis, and varying degrees of pulmonary edema and congestion were seen.

Virus isolation from lung, spleen, and liver was attempted by inoculating embryonated chicken eggs and chicken embryo kidney and liver cell cultures. Both were unsuccessful. Virus isolation and experimental poult inoculation were performed by Dr. Fadly, USDA Regional Poultry Research Laboratory veterinarian. These attempts were also unsuccessful.

Thin sections of formalin-fixed grouse spleen tissue were examined by transmission electron microscopy. Non-enveloped virus particles (100-110nm) were seen within the nucleus of affected cells. These findings are suggestive of herpes virus infection or a very large adenovirus.

The clinical findings, as well as the gross and microscopic lesions, are strikingly similar to Marble Spleen disease of pheasants. However, the disease could not be reproduced in experimental hosts, unlike Adenovirus Group II agents. The causative agent, therefore, remains obscure.

Unfortunately, the birds did not survive long enough to breed. Avicultural groups such as the Quail Breeder's Society struggle to raise potentially endangered species of upland game birds to aid in reintroduction of these animals into their increasingly depleted habitats. Breeder efforts are often thwarted by infectious diseases. Increased understanding and research are needed in this area.

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LYMPHOPLASMACYTIC ENCEPHALITIS, MYELITIS AND MENINGITIS IN A GROUP OF PIONUS SPP. PARROTS

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Introduction:

In the late fall of 1982 a shipment of South American psittacines was brought to a United States Department of Agriculture (USDA) approved private quarantine station in San Francisco, California. The shipment included blue-headed (Pionus mentruus) and dusky parrots (P. fuscus) and parrolets (Touit spp). The shipment had originated in Suranime, and had traveled through Amsterdam and Frankfurt before arriving in San Francisco.

There was about a 15 percent mortality among the Pionus parrots during the first 15 days in quarantine. The parrolets were less affected. The early mortality was attributed to Pseudomonas septicemias and a few cases of pox. Routine cloacal swabs from living birds and specimens taken from dead birds cultured at the USDA's Veterinary Services Laboratory in Ames Iowa yielded a non-Newcastle hemagglutinating virus. During the 30 day quarantine period many birds developed neurologic signs which persisted after the shipment was released from quarantine. Because these signs did not appear to be diminishing, 24 living and 2 recently dead Pionus spp. parrots were donated to the University of California at Davis for further investigations.

Clinical signs:

Twenty-one birds were given detailed clinical examination. Three died prior to evaluation. The signs exhibited included: tremors (9 birds) circling (9 birds), ataxia, falling or staggering (8 birds), torticollis (7 birds), weakness or paresis (5 birds), depression (1 bird) and unspecified neurologic signs in 2 birds. Several birds exhibited more than one sign. All birds could see and were capable of purposeful movement toward food or away from people. All could perch.

Postmortem findings:

Complete gross and histopathologic examinations were performed on 23 of the donated Pionus spp. parrots: 15 dusky parrots; 7 blue headed parrots; and one whose species was not identified. Fourteen were males, 9 were females. Two had died and 21 were euthanatized because of persistent neurologic disease. Five were sacrificed on receipt (about one and a half months after entry into quarantine); three were euthanized one month after that; 3 birds died and were accidently disposed of before necropsy; and the remaining 13 birds were euthanized after an additional 6 weeks (about 4 months after arrival in quarantine) because of persistent neurologic signs.

External examination revealed weight loss in 7 birds, and adequate or good body condition in 14. (In two, nutritional status was not recorded). In one bird which died there were extensive caseonecrotic plaques in the oval cavity, around the choanal slit and in the esophagus. These were pox complicated by candidiasis. Seven other birds had foci of scarring or depigmentation in the oral cavities suggestive of healed pox infections. Foci of pneumonia were present in the one bird with active pox and in an additional bird which had died at the quarantine station. Splenomegaly was present in 10 birds; hepatomegaly in only two. Additional lesions included a small or granular pancreas in 11 birds and pale renal nodules in one bird.

Brain lesions were difficult to appreciate grossly but in at least one bird the ventricles over the posterior cerebral cortices were distended by clear cerebral spinal fluid indicating hydrocephalus. Yellow periventricular discoloration was occasionally seen.

Histopathology

Lesions were present in the central nervous system of all 23 parrots examined. Encephalitis was present in 22; myelitis in 17; cerebral meningitis in 16; and meningitis of the cord in 11. Prominent lymphoplasmacytic perivascular cuffs were seen predominantly in periventricular (subependymal) areas of all levels of the brain and around the central canal sometimes accompanied by loosening (edema) of the neuropil. Meningeal and penetrating vessels were also invested by cuffs. Spherules representing neuronal necrosis and axonal degeneration were seen in only two birds sacrificed upon receipt from quarantine and were absent in ones sacrificed later in the disease. Cuffing was still florid in birds sacrificed nearly 3 months later. Some of these birds had obliteration of the central canal and one had multiple undefined foci of cerebral gliosis. Ependymal cells in the last birds sacrificed often had swollen clear nuclei. Equivocal eosinophilic Cowdry type A inclusions were noted in some ependymal cells and in rare subependymal glial cells or small neurons in the chronic cases.

In addition to lesions in the central nervous system, pancreatitis was present in 20 of the 23 birds. In the mild cases this consisted of interstitial fibrosis accompanied by moderate numbers of lymphocytes and plasma cells. In the severe cases ascinar tissue was obliterated by fibrosis and inflammation and only hyperplastic ductules remained. Large lymphoid follicles were occasionally present in the pancreas.

Lymphoid hyperplasia with marked plasmacytosis was noted in the spleens of 20 of the birds and prominent follicular development was noted in 13 of these. (As apposed to spleens of domestic fowl, lymphoid follicle development is relatively rare in psittacine spleens examined at the UCD pathology service). Subacute enteritis was present in 19 birds associated with prominent gut associated lymphoid tissue in 4 birds. Lymphoplasmacytic interstitial nephritis was present in eight birds. In two birds, large lymphoid patches had been visible grossly in intestine and kidney.

Other lesions included: caseonecrotic tracheobronchitis in one bird which had died in quarantine; mild acute necrotizing enteritis in one bird; focal fungal (Aspergillus) pneumonia in two; focal fungal encephalitis in one, cestodiasis in two and large intranuclear inclusion bodies (compatible with adenovirus inclusions) in the intestines of the last bird sacrificed.

Microbiology

Virus isolation was attempted from the brains of the first 9 birds sacrificed. No viruses were isolated after three passages in embryonated chick eggs. Bacterial cultures of brain were also negative. E. coli was isolated from intestines and other tissues of the bird with acute necrotizing enteritis and E. coli, Klebsiella and Pasturella were culture from the bird with caseonecrotic bronchitis which had died at the quarantine station.

Discussion

The presence of neurological signs such as tremors and torticollis in psittacine birds arouses suspicion of Newcastle Disease (ND) (1). Cultures in quarantine did not confirm a diagnosis of ND but did reveal the presence of a non-Newcastle hemagglutinating virus within the shipment. Other non-Newcastle panamyxoviruses (mainly PMV3) have been associated with neurologic signs in psittacine birds (2). The lesions reported in ND and these other entities are: neuronal degeneration and necrosis; multifocal gliosis; lymphocytic perivascular cuffing and hypertrophy and hyperplasia of endothelial cells. In experimental cases ND viruses may cause large foci of necrosis and gliosis. Visceral lesions reported in ND in psittacines are usually mild and primarily acute hemorrhage. Pancreatitis is not a reported feature. (3)

The lesions in the Pionus parrots differed appreciably from those described above. Neuronal necrosis was a rare event. Although necrosis may have been present earlier in the course of the disease, residual glial nodules or other indicators of past necrosis were not found. Gliosis was present in only one bird and was mild. The marked plasmacytic component and the orientation of the lesions around internal (periventricular) and external (meningeal) surfaces of the brain are not features described in other viral encephalitities in avian species. (4)

In reviewing the pathology files at UCD, lesions identical to those in the Pionus were found in a group of Neophema spp. parakeets. These birds had been donated by the same quarantine station two years previously because of persistent torticollis. "Star gazing" in Neophema has been associated, in Europe, with a paramyxovirus (5), however the lesions seen in the Neophema from California did not fit the description of that disease. Some members of the group of Neophema were also suffering from herpesvirus associated proliferative bronchitis (6). Although herpesvirus particles were readily detected in the lungs of these parakeets, no virions were found in CNS tissue examined by electron microscopy. In addition, the Neophema had pancreatitis and marked lymphoid hyperplasia and plasmacytosis in spleen and other tissues similar to the Pionus.

The lymphoplasmacytic nature of the CNS lesions and evidence of generalized lymphoid stimulation suggests a chronic antigenic stimulation which might occur in a persistent viral or bacterial infection. Mycoplasma has been associated with such stimulation in mammalian and avian species but avian mycoplasma is not usually neurotropic (7). Persistent neutrotrophic viral infections have been reported in mammalian species. In reviewing the literature on comparative neuropathology, the disease with morphology and persistence most similar to the entity seen in the Pionus is lymphocytic choriomeningitis (LCM) in rodents (caused by an arena virus) (8). Of the myxoviruses, measles (rubeola) virus is capable of causing a disease in man called subacute combined sclerosing panencephalitis (SSPE) (9). The lesions and distribution differ from those in the Pionus, however. In both LCM and SSPE the viruses and inflammation persist for a long time after initial infection. LCM is also associated with systemic lymphoid hyperplasia in some species (eg. hamsters).

It may be that the cause of the encephalomyelitis, pancreatitis and lymphoid stimulation in this group of Pionus parrots will remain a mystery, although the subependymal inclusion bodies are being persued by electron microscopy. The sterotypic nature of the lesions, their consistancy from bird to bird and the previous cases in Neophema make it likely that this is a descrete psittacine entity and one which will be seen again. Hopefully, awareness of this disease and use of psittacine derived tissue culture systems will allow identification of the causative agent (or agents) in future cases.

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Intestinal Cryptosporidiosis and Reovirus Isolation from Young Pen-Raised Bobwhite Quail with Severe Diarrhea and High Mortality

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Today I want to share with you some information that we have gathered in the course of investigating an outbreak of severe diarrhea, with high mortality, in commercial pen-raised Bobwhite quail. The problem first began in July of 1982 as a diarrheal disease in birds that were 4 to 5 weeks old. Mortality was higher than normal but many of the affected birds did recover. As the production season continued, a similar disease occurred in progressively younger birds, as young as 8 days of age, with increasingly higher mortality. The same pattern was repeated in the 1983 production season.

The first hatch of the 1984 production season was raised free of clinical disease. However, birds from subsequent hatches once again began showing signs of diarrhea and increased mortality, sometimes within 5 days of hatching. It was at this point, in July of 1984, that we were asked to investigate this problem. We began with a farm visit to obtain a more detailed history and some specimens for our diagnostic work-up.

Bobwhite quail have been raised commercially on this farm for twenty years. Approximately 500 thousand birds are marketed each year. This was essentially a closed operation until 1981 when two thousand males, originating from another state, were added to the breeding population without benefit of quarantine. As I mentioned earlier, there were no major problems at this farm until the summer of 1982, so its tempting to suggest that the newly introduced birds may have brought the problem with them.

The facilities, as you will see, were not what you would call modern, but they appeared to be adquate, and the farm was fairly well managed. Cleaning and disinfection practices appeared to be good. The grow-out buildings are divided into rooms and have automatic curtins on two sides. Each hatch is placed on new pine shavings. Each room has an outdoor flight pen; the birds are

allowed outside beginning at 3 weeks of age, but are always put in at night. On the same farm are some Southeastern wild turkeys, some Buborn red turkeys, and peafowl. None of these other birds have had any unusual problems.

The disease, as it occurred on the farm, was characterized by a profuse, whitish, watery diarrhea affecting birds as young as 5 days of age and up to approximately 5 weeks of age. Severe dehydration and death occurred within 1 to 3 days of onset of clinical signs. Younger birds were more severely affected than were older birds, and the breeders have not shown any signs of this disease. Recovered birds were stunted, and affected hatches were very uneven in size. Mortality could be very high. We were told that total mortality has ranged from 40 to 90%.

Figure 1 illustrates daily and cumulative mortality that occurred in a flock hatched June 29, 1984. A peak in mortality occurred very early, at 3 days post-hatch. A brooder malfunction at 14 days post hatch contributed to the second mortality peak. Cumulative mortality through 17 days was 44%. Figure 2 shows similar data for a flock hatched the following month. There is a very distinct peak in mortality occurring at 8-11 days post hatch. Cumulative mortality was 43%.

We returned to the veterinary school with several groups of quail of various ages. There are two of these groups that I want to focus on. We took 31 birds right from the hatcher, I'll refer to these as the younger birds; and 31 birds that were 14-days- old, from a pen that had typical dissease, I'll refer to these as the older birds. These birds were housed together in a single brooder cage. Within three days the younger group of birds began showing clinical signs that were consistent with the disease observed on the farm. They had severe, white, watery diarrhea, were dehydrated and died. The mortality data (Figure 3) tell the story very well. Mortality began 3 days post-hatch, peaked between 6 and 9 days, and all 31 birds were dead by 10 days post-hatch.

(Slide) Here are two examples of 4-day-old quail that died from this disease. They were very dehydrated, and the diarrhea they had was so fluid that that it did not paste around the vent, but you can see some caked to their feet. (Slide) This is one of the birds at necropsy. You can see that the intestine is thin walled and fluid filled. (Slide) Here is a closer view of the intestine. It is thin walled and fluid filled in its entire length. There are areas of gas accumulation and whitish casts within the intestinal lumen. This gross appreparance was consistent in virtually all of the affected birds that we examined.

The microscopic findings in the intestines of these birds was most interesting. (Slide) This is an H&E stained section of small intestine, I believe ileum, from a quail only 2 days old. At this magnfication you can see that there is villus atrophy and fusion, and some sloughing of cells at the tips of the villi. There is little or no inflammatory response. And if you look very carefully along the villus boarder, you see many small spherical organisms that we identified as cryptosporidia.

When we examined the 2-week-old birds we found much the same thing. (Slide) This section is jejunum, and shows villus atrophy, villus fusion, sloughing of cells at the tips of the villi, and there is some vacuolation of cells in the lamina propria and at the villi tips, and again there are some crypostsporidia along the villus boarder.

Let me just show you a few more examples to convience you that this was a consistent finding. (Slide) This section of jejunum, from a 2-week-old bird, shows the villus fusion and atrophy very well, and a large number of cryptosporidia along the villi. (Slide) Here is another example from the same bird at a higher power so you can more easily see the cryptosporidia.

Transmission electron microscopy gave us a very interesting look at these organisms and their association with the intestinal villi. (Slide) This is duodenum from one of the younger group of quail at 7 days of age. There are three different stages of the cryptosporidium life cycle represented here. From the top right to the lower left, there is a gamete, next is a schizont, and below that a trophozoite. (Slide) Here is another area in the duodenum of a 7-day-old quail showing a high density of these parasites, and their very intimate association with the villi. There has been some controversy as to whether these organsms are intra- or extracellular parasites. Most recently, it has been proposed that the so-called parasitophorous vacuole is of host origin. In that case, cryptosporidia would be classified as intracellular, extracytoplasmic parasites.

(Slide) This section of duodenum from a 7-day-old quail shows two gametes and a trophozoite at the top, and a merozoite just below them. Also, the trophozoite is sectioned so that you can see this interesting structure which is called the feeding organelle.

(Slide) This is a section of jejunum, also from a 7-day-old quail, that shows several cryptosporidia and a cell about to be sloughed from the villus tip. (Slide) Another section of jejunum, which shows a schizont in the upper left, below that are two trophozoites, and a gamete. Here again you can see the feeder organelles in close association with the host cell.

When we looked at the older group of quail, we found the same thing. (Slide) This is a section of jejunum from one of the 2-week-old birds. You can clearly see how the microvilli are disrupted where this trophozoite is attached to the host cell. The electron dense objects are bacteria in the intestinal lumen.

Based on these observations, we felt that the cryptosporidia were at least a possible cause of the enteritis. There has recently been an explosion of interest in cryptosporidiosis, because diarrhea caused by cryptosporidia is one of the leading causes of death in immune compromised individuals, particularly AIDS patients. And now that more people are looking for it, there is a growing list of diarrhea cases being attributed to crytosporidiosis.

So it appeared that we had some good circumstantial evidence for intestinal cryptosporidiosis resulting in severe diarrhea in very young quail. However, we had some additional laboratory findings that we need to consider. I should mention that aerobic and anaerobic bacterial cultures did not yield anything signifcant, and fecal floats did not reveal any parasites or ova other than cryptosporidia.

Droppings were submitted for negative stain electron microscopy and virus isolation. Reovirus particles were observed in the droppings of both the younger and older groups of quail. The virus was isoloated in chich embryo kidney cell culture and embryonating chicken eggs. It grows very well and forms syncitia in cell culture.

At this point, I believe we have identified two possible etiologies in this case of severe diarrhea and high mortality in Bobwhite quail. Our next step will be infectivity trials to see if either or both of these agents is capable of reproducing the disease that we observed on the farm.

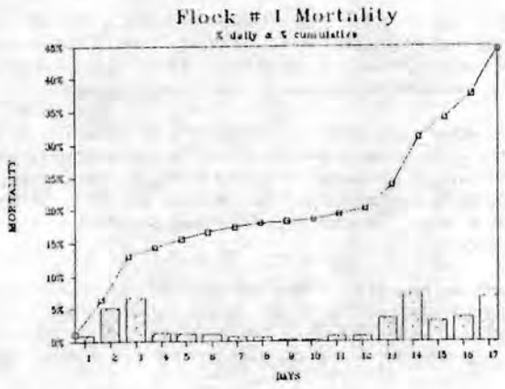

Fig. 1

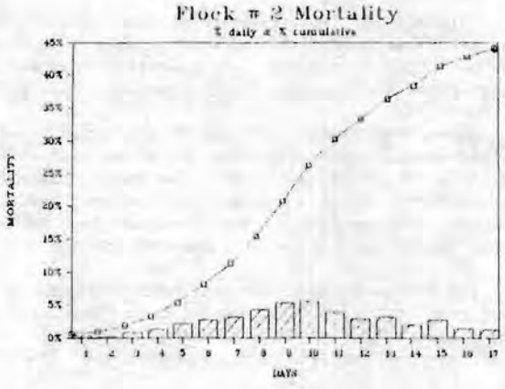

Fig. 2

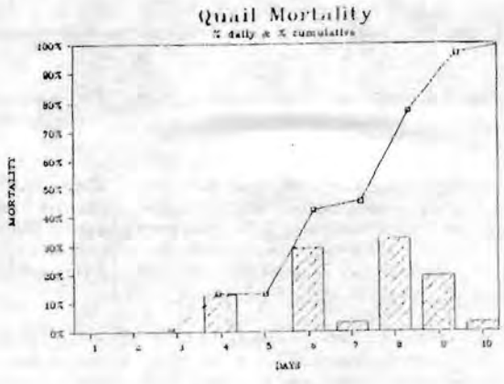

Fig. 3

MALIGNANT LYMPHOMA IN AN AFRICAN GREY PARROT

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A 1-1/2-year-old, African grey parrot (Psittacus erithacus) was presented to the Veterinary Medical Teaching Hospital, University of California, Davis, for evaluation of a severe right periorbital swelling. The parrot had been treated unsuccesfully for several months for a presumptive sinusitus, until a biopsy of the right periorbital tissues indicated the presence of an undifferentiated sarcoma. The bird was referred to the VMTH as a potential candidate for radiation therapy.

Physical Examination:

There was a 2 cm diameter area of soft tissue swelling around the right eye causing exopthalmus

The upper lid did not cover the cornea adequately and the nictitans function was impaired. An ulcer with mineralization was present in the dorsal quadrant of the cornea.

The remainder of the physical examination was normal. The CBC and serum chemistry values were normal. Radiographs were taken to look for a primary tumor or metastatic tumor sites. The radiologists noted an old malunion fracture of the left proximal radius and a loss of visualization of the caudal abdominal air sac, suspicious of an old adhesion of abdominal viscera to the left caudal abdominal wall. The spleen was not thought to be enlarged.

Based on our findings and the histopathology report of an undifferentiated sarcoma, radiotherapy was offered but with a guarded prognosis. There is no indication in the literature as to the radiosensitivity of normal avian tissues. Originally, we had planned to use iridium-192 implant in the tumor; however, after examining the bird, we felt the tumor was too extensive for interstitial radiation treatment. A computed tomography scan (CT) was done to help determine the extent of the mass. These films showed the tumor to involve most of the nasal sinus region of the right side of the head, and destruction of adjacent boney architecture.

Orthovoltage X-ray teletherapy was the recommended therapy. A total dose of 4000 rad was given to the region in 10 fractions of 400 rad/fraction on a M-W-F basis. There was dramatic regression of the size of the lesion within the first 3 treatments. The corneal ulcer healed when the exophthalmus was corrected. After 10 treatments, the periorbital swelling had decreased approximately 80%. The lids could open and close normally over the eye.

During the 21 days of hospitalization, the bird showed no other clinical abnormalities and continued to eat, drink and defecate normally. Due to the aggressive nature of the tumor, the prognosis was stil guarded despite the dramatic response to radiotherapy. The owner reported that 7-10 days after having the parrot home, he became very weak, unable to use his wings and his head hung low. He had to be force-fed for a 7-day period. He subsequently developed respiratory signs which consisted of ocular and nasal discharge. Seventy days following completion of the radiotherapy, the parrot was finally presented for a recheck at the VMTH.

The bird presented with right nasal discharge of a waxy yellow material. There was obvious loss of feathers around the right periorbital region, approximately 3.5 cm in diameter. There was ptosis of the right eye, erythematous lids, mild ocular discharge and a mild exophthalmia. The right cornea had an opacity in the medial quadrant that did not take up fluorescein stain. The left wing was dropped at the shoulder and held away from the body.

The radiographs of the skull showed soft tissue swelling of the right periorbital region. There were numerous pitting cortical destructive lesions in the left proximal ulna and radius. The proximal radial bone was sclerotic, as well as causing the radiohumeral joint to appear destructive. Lytic lesions were present in the left metacarpus. The left humeral head appeared sclerotic and enlarged. There were punctuated radiolucencis present in both proximal tibiotarsi.

Abdominal soft tissue changes were present radiographically as well. There was an obvious splenomegally and a soft tissue mass in the caudal abdominal region, in a pericloacal area. These findings were compatible with metastatic neoplasia. Euthanasia was advised, followed by necropsy.

The right infraorbital sinus was filled with a reddened gelatinous material. There was softening of the calvarium and upper mandible around the right eye. After decalcification processes and sectioning the skull, it was obvious that the right infraorbital sinus, the nasal cavity and the cancellous base of the upper mandible were replaced by a tan to cream hemogenous mass. The orbital tissues themselves did not show any gross evidence of alteration. The left ulna marrow cavity contained a yellow, tan exudate that penetrated through the cortex. The proximal left humerus was opaque and more solid than the metaphyseal and diaphyseal areas.

The liver was of expected size and on cut surface of the right lobe was a 0.2 cm diameter, soft, fatty, spherical white nodule. The spleen was 2-3 times normal size (2 x 1.5 x 1.0 cm). It was pale cream colored and had a fatty, lymphoid character on cut surface. No bursal reminant was present. There were well developed thymic nodules along both jugulars with petecchia throughout several lobules. There was a 0.2 cm grey irregular focus on the dorsomedial aspect of the right lung lobe and a subtle discoloration of the caudal margin of the left lobe. The posterior lobes of both kidneys were mildly swollen.

Specimens of all major organs were fixed in neutral-buffered 10% formalin, embedded in parafin, sectioned at 6 um, and stained with hematoxylin and eosin. An impression of the periorbital mass was stained by the *Ziehl-Neelsen* method for acid-fast bacilli.

Histologically, the infraorbital sinus and much of the skull on the right side was replaced by a mass of cells. The mass extended across the midline ventrodorsally, filling many of the air cells of the skull. Those not filled with mass were filled by protein rich edema fluid. Sections through the beak, just anterior to the cere showed the trabecular spaces of the mandible to be filled with these neoplastic cells whose morphology was similar to those in the sinus. The cells were fairly uniform, round to polyhedral, with wispy grey cytoplasm and indistinct cell margins. Nuclei were round to oval, occasionally folded with small basophilic nucleoli. Nuclear size was variable and frequent mitotic figures were seen.

The histopathologic appearance of the nodules seen grossly in the spleen, liver, ulna, thymus and lungs were similar. The spleen had only small areas of normal parachyma filled with plasma cells and these were compressed by lobules of neoplastic cells. The proximal humerus showed marked proliferation of woven bone. Marrow fat was necrotic and there was lipogranulomatous inflammation with numerous giant cells. No neoplastic cells were seen here.

The basic morphology was that of a round cell sarcoma, for which the differential includes neoplasia of the lymphoid cell line, histiocytic, immature myloid cells or mast cells. To further characterize the tumor, we did a Giemsa, Methyl Green Pyronin and Reticulum stain and submitted portions for electron microscopy. The Giemsa did not reveal any granules in the cytoplasm of the cells, ruling out a mast cell or mature myloid cell line. The Methyl green pyronin stained the cytoplasm of mature plasma cells well, but only faintly stained the cystoplasm of the neoplastic cells.

The electron microscopy is still pending, so we are left to consider lymphoid neoplasia and malignant histiocytoma. The incidence of histiocytoma is rare in most species and the electron microscopy will help characterize this further.

Another consideration was the histiocytic form of Mycobacterium tuberculosis in psittacines, often confused with histiocytic tumors. The morphology of the cells seen in this case were obviously anaplastic, plus the acid fast stains were negative.

Based on the gross and histopathological findings, the diagnosis is an undifferentiated, highly anaplastic, round cell sarcoma. Based on distribution and morphology, it is of lymphoid origin and so termed a malignant lymphoma involving the right periorbital region, spleen, liver, lung, ulna and thymus.

There are few reports of lymphoid neoplasia in cage birds.[1-7] Reports in the literature use terms such as malignant lymphoma, lymphoid leukosis, lymphoid lymphoma, lymphoblastic leukosis and Marek's disease. Leukosis can be used as a general term for an abnormal proliferation of leukocytes and/or their precursors. In poultry, the leukosis complex is divided into 3 entities, each caused by a virus or a closely related group of viruses.[8,9] Virus has not yet been isolated from lymphoid tumors in pet birds.[7-10] Some have suggested the term psittacine lymphoid leukosis, hinting at a presumptive viral etiology.[3]

Radiation therapy for neoplasia in birds is not well described. A dramatic reduction in mass size is common to mammalian lymphosarcomas treated with radiotherapy, as in this case. Chemotherapy using prednisolone has been applied in a psittacine lymphoid neoplasia with partial remission.[3] Perhaps a combination of radiation therapy and chemotherapy would have a longer remission time.

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EMTRYL TOXICITY IN COCKATIELS

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Introduction

In recent years giardiasis has increasingly been recognized as a disease problem in cockatiels and budgerigars. One drug that has commonly been used to control this disease is dimetridazole (Emtryl, Salsbury Laboratories, Inc.) (3,4,8). At the recommended dose of 0.1% Emtryl given as the sole source of drinking water for seven days no toxicity had been reported among budgerigars or cockatiels. In 1982 and 1983, however, isolated cases of tremors and ataxia were reported in nestlings of flocks treated with Emtryl (1,5). This study was conducted to determine Emtryl toxicity in hand-raised cockatiels.

Materials and Methods

Basic hand-rearing protocol: Cockatiel eggs were artificially incubated and hatched. Upon hatching the chicks were separated and placed in brooders. From day 1 to day 4 the chicks were fed a dry 20% protein purified diet mixed with distilled water to make a 7% solids diet (6) every two hours from 6 a.m. to 10 p.m. On the fourth day the percent solids was increased to 30% and chicks were fed every four hours. Each bird was weighed before and after each feeding to determine food intake.

Experiment 1: Chicks 4 to 11 days old

Three control chicks were fed as outlined above. The experimental chicks were fed as above except that three received diet mixed with a 0.1% solution of Emtryl instead of distilled water; three, diet mixed with an 8% solution of Emtryl; one, diet mixed with a 1.0% solution of Emtryl; and five, diet mixed with a 0.5% solution of Emtryl. Chicks were evaluated daily for behavior, activity level, hydration, rate of crop emptying, and character of feces. Necropsies were performed on all chicks that died and on one control chick. Brain, liver, and kidney were fixed in 10% buffered formalin for histopathology.

Experiment 2: Chicks 1 to 8 days old

Three chicks were fed as outlined previously with diet mixed with distilled water. Three chicks were given 0.1% Emtryl and one chick received 0.5% Emtryl. The chicks were evaluated as in Experiment 1.

Experiment 3: Chicks 24 to 31 days old

Cockatiel chicks were raised to 24 days of age as previously described. When the birds were 24 days old six birds were continued on the basic diet, five birds were given 0.1% Emtryl, and six birds received 0.5% Emtryl. The birds were evaluated as in Experiments 1 and 2.

Experiment 4: Adult cockatiels

Nine adult cockatiels were placed in individual wire cages in an aviary without light or temperature control. For seven days five birds were given access to 50 ml of tap water daily and four birds were given access to 50 ml of a 0.1% Emtryl solution daily. A pelletted diet (7) was supplied ad libitum. Daily water consumption was measured for each bird and maximum and minimum ambient temperatures were recorded daily. After seven days treatments were reversed such that the Emtryl treated birds now received tap water whereas the control birds received 0.1% Emtryl. At the end of seven days one bird from the Emtryl group was sacrificed for necropsy and histopathology.

Results

Experiment 1: Chicks 4 to 11 days old

Chicks receiving 0.1% Emtryl (recommended dose) showed no signs of toxicity, no differences from the controls. Chicks given 0.5% Emtryl and 1.0% Emtryl all became weak and inactive within 24 hours after receiving their first meal with Emtryl. By the second day all showed tremors. All had died by the third day. Necropsy showed multiple hemorrhages, bruising, and enlarged, pale kidneys. Histologic

examination was not performed. The birds receiving 8% Emtryl were weak and showed tremors within two to four hours of the first meal with Emtryl. Two of the three birds were dead by the end of four hours. No lesions were seen at necropsy.

Experiment 2: Chicks 1 to 8 days old

The 0.1% Emtryl chicks appeared normal for three days, but on the fourth day were weak and inactive and their growth rate lagged behind the control birds (Table 1). On the sixth day they began exhibiting tremors and two of the three chicks died. The third chick eventually recovered when Emtryl was discontinued on the eighth day. This sole survivor gradually caught up to the average control birds' body weight by nine days after the Emtryl was discontinued. Necropsy of the other chicks revealed multiple hemorrhages, pale livers, and enlarged and pale kidneys. Microscopic examination showed no lesions in the brain. The liver showed extensive hepatocellular necrosis and vacuolization, in a predominantly centrilobular distribution and dilated sinusoids containing fibrinous material. The kidneys revealed widespread intratubular mineralization, active tubular necrosis, and casts.

The chick given 0.5% Emtryl was weak and inactive by the beginning of the second day of treatment; it was dead by the end of the second day. No lesions were seen at necropsy.

Table 1

Experiment 2 Average Body Weights (g)

Group		Days of exposure							
		0	1	2	3	4	5	6	7
0.1% Emtryl (n=3)	mean	4.30	4.92	4.68	5.53	7.27	7.93	9.35	11.80
	SD	± .45	± .31	± .05	± .28	± .40	± 1.13	± .82	± 1.10
0.5 % Emtryl (n=1)		4.53	5.12	5.41	5.71	dead			
Control (n=3)	mean	4.38	4.78	5.36	6.61	9.22	12.80	16.68	21.05
	SD	± .26	± .39	± .32	± .24	± .26	± .60	± 1.49	± 2.83

Experiment 3: Chicks 24 to 31 days old

Chicks given 0.1% Emtryl showed no signs of toxicity and necropsy revealed no gross lesions. The chicks given 0.5% Emtryl became ataxic by the end of the first full day of treatment. They all exhibited extensor rigidity of their legs and necks. All six birds had died by the sixth day after treatment was discontinued. Necropsies revealed emaciation (6/6), multiple hemorrhages (6/6), pale livers (5/6), and pale kidneys (3/6). Microscopic examination of brain sections showed no lesions. Hemosiderin deposits were found throughout the liver parenchyma. The kidneys revealed widespread intratubular mineralization and casts and multifocal patches of tubular regeneration. No active necrosis was found in the kidneys.

Experiment 4: Adult cockatiels

No adverse effects were seen in any of the birds. Water consumption was high in both groups, but did not differ significantly betweeen control and 0.1% Emtryl birds (Table 2). Necropsy and histology revealed no gross or microscopic lesions.

Table 2

Experiment 4 Average Daily Water Consumption (ml)*

Group		Days of exposure							
		0	1	2	3	4	5	6	7
0.1% Emtryl (n=4)	mean	12.2	15.2	9.4	13.8	11.0	13.6	12.0	11.2
	SD	± 2.5	± 1.8	± 3.7	± 1.9	± 1.4	± 2.6	± 1.9	± 2.4
Control (n=5)	mean	16.3	19.3	8.3	15.5	12.0	14.0	13.0	12.3
	SD	± 6.3	± 1.3	± 1.3	± 2.9	± 2.9	± 2.8	± 1.8	± 2.8
Min/max temp		55/88	57/98	58/98	55/91	59/95	59/101	58/96	58/96

Table 2 continued

Table 2 continued

Group		Days of exposure (cont.)							
		8	9	10	11	12	13	14	15
Control (n=4)	mean	10.0	14.8	12.8	11.4	12.4	15.0	13.2	12.0
	SD		± 5.0	± 2.6	± 1.7	± 2.6	± 0.7	± 3.3	± 1.6
0.1% Emtryl (n=5)	mean	9.3	12.5	13.3	12.5	13.5	15.8	11.0	12.3
	SD		± 2.4	± 5.1	± 1.7	± 2.5	± 2.2	± 2.5	± 2.2
Min/max temp		54/91	56/96	57/99	58/97	60/98	61/104	61/109	62/107

* Treatments were reversed on day 8 with control birds receiving 0.1% Emtryl and experimental birds receiving tap water.

Discussion

At the commonly recommended dose of 0.1% Emtryl, dimetridazole appears to be innocuous to adult cockatiels, even when consumption is increased to three or four times normal due to high ambient temperatures. The 0.1% Emtryl also appears to be safe for older nestlings, but at only five times this level it is highly toxic. The recommended dose is very toxic to young nestlings, possibly due to the proportionally higher dose they receive. If we assume our feeding protocol mimics the parents' protocol, a 5 g chick in a flock being treated with 0.1% Emtryl would receive a dose of about 200 mg dimetridazole/kg/day. An adult weighing 100 g drinking 10 ml/day would receive only 20 mg/kg/day.

At only five times the commonly recommended dose of Emtryl all age groups of nestlings showed severe toxic signs. Two patterns of toxicity were seen. In very young chicks receiving 0.5% Emtryl and older chicks receiving the highest doses, signs developed early and death quickly followed onset of signs. Necropsy revealed no lesions. In very young chicks receiving 0.1% Emtryl and older chicks receiving 0.5% Emtryl the birds showed a more prolonged clinical course and necropsy revealed multiple hemorrhages, liver lesions, and kidney lesions. Histology showed evidence of hepatocellular and renal tubular damage. It is possible that acute toxicity is directed at the CNS whereas more chronic toxicity causes hepatic and renal damage. In mammals, related 5-nitroimidazoles are metabolized extensively in the liver and metabolites and unchanged drug are excreted in the urine (2,9). If birds handle dimetridazole similarly this could explain the damage seen in liver and kidney at high doses and at lower doses in younger chicks that may have immature enzyme systems and thus less capacity for metabolizing dimetridazole.

In view of the severe adverse effects of Emtryl in chicks and the narrow margin of safety, this drug should not be used during the breeding season. Treatment of adult birds does appear to be safe.

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SELECTED SOFT TISSUE - PET AVIAN SURGICAL PROCEDURES

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INTRODUCTION

IN RECENT YEARS, SURGERY IN PET AVIAN SPECIES HAS BECOME MORE AND MORE SOPHISTICATED. DEVELOPMENT OF NEWER GASEOUS ANESTHETICS HAS MADE COMPLICATED INTERNAL WORK RELATIVELY ROUTINE. THE PURPOSE OF THIS PAPER IS TO DESCRIBE A FEW OF THE PROCEDURES WHICH ARE NOW PERFORMED IN A PROGRESSIVE PET AVIAN PRACTICE.

ISOFLURANE ANESTHESIA

THE ANESTHETIC FOR ROUTINE AND INVOLVED SURGICAL PROCEDURES IS A NEW GASEOUS ANESTHETIC, ISOFLURANE (FORANE® - OHIO CHEMICAL). WE CONSIDER THIS PRODUCT THE ANESTHETIC OF CHOICE IN PET AVIAN MEDICINE. FIRST USED AND STUDIED IN BIRDS BY HARRISON, THIS GASEOUS ANESTHETIC GIVES EXCELLENT SURGICAL CONTROL. THE PRODUCT IS NOW USED EXTENSIVELY IN HUMANS BECAUSE OF PROBLEMS WITH DELAYED HEPATOPATHIES OF PATIENTS AND AN INCREASED CANCER RATE IN OPERATING ROOM PERSONNEL WORKING WITH HALOTHANE AND METOFANE.

BESIDES THE SAFETY OF THE GAS AND THE EASE OF ADMINISTRATION WITHOUT THE NECESSITY OF ENDOTRACHEAL INTUBATION, THE BIRDS RECOVER EXTREMELY FAST WITH LITTLE OR NO WING FLAPPING. THE PATIENTS WILL OFTEN STAND WITHIN SECONDS OF CESSATION OF THE GAS FLOW.

OTHER ANESTHETICS

OTHER ANESTHETIC REGIMENS CAN BE USED, OF COURSE, BUT DO NOT HAVE THE SAFETY AND OTHER ADVANTAGES OF ISOFLURANE. METOFANE INDUCTION BY CONE, FOLLOWED BY INTUBATION AND THE USE OF HALOTHANE BY AN OPEN BREATHING SYSTEM, IS ONE REGIMEN. OTHERS PREFER METOFANE INDUCTION AND METOFANE MAINTENANCE IN A CLOSED SYSTEM. GASEOUS ANESTHETICS ARE USUALLY CHOSEN BECAUSE OF RAPID RECOVERY TIME AND RELATIVE SAFETY AND CONTROL.

INJECTABLE ANESTHETICS ARE USED FOR CERTAIN PROCEDURES BUT ARE NOT AS EASY TO CONTROL AS THE GASES. RESPIRATORY AND CARDIAC DEPRESSION MAY BE COMPLICATION FACTORS. THE AUTHORS PREFER INTRAVENOUS ROMPUN-KETAMINE (EQUAL VOLUMES OF 100 MG/CC KETAMINE AND 20 MG/CC XYLAZINE) IF AN INJECTABLE MUST BE USED.

IN GASES OTHER THAN ISOFLURANE AND WITH THE USE OF INJECTABLES, WING FLAPPING ON RECOVERY IS PREVENTED BY WRAPPING THE PATIENT IN A LARGE TOWEL. BY THE TIME THE BIRD CAN WORK ITSELF OUT OF THE TOWEL, IT USUALLY CAN PERCH AND NOT INJURE ITSELF.

GASEOUS ANESTHETICS ARE PREFERABLE FOR BIRDS. IT IS EXTREMELY REASSURING TO HAVE A PATIENT "WALK OFF THE TABLE," RATHER THAN REMAIN COMATOSE FOR A PROLONGED PERIOD OF TIME FOLLOWING INJECTABLE ANESTHETICS.

PRE-OPERATIVE EVALUATION

THE CLINICAL PATHOLOGY LABORATORY PLAYS AN IMPORTANT ROLE IN THE PRE-OPERATIVE EVALUATION OF THE AVIAN PATIENT. THE PROBLEM OF SUBCLINICAL ILLNESS IN CAGED BIRDS AND THEIR ABILITY TO MASK SYMPTOMS MAKES DIAGNOSIS OF ILLNESS DIFFICULT.

IN AN ELECTIVE SURGERY, EVERY ATTEMPT IS MADE TO CORRECT ANY ABNORMALITIES BEFORE PROCEEDING. IN A NON-ELECTIVE SURGICAL PROCEDURE, THE ABNORMALITIES ARE TREATED AS PART OF THE POST-OPERATIVE CARE. IN A NON-ELECTIVE SURGICAL PROCEDURE, THE CLINICIAN IS ADVISED TO BE ESPECIALLY WARY IF AN SGOT (SAST) RISE HAS OCCURRED. WHILE NOT LIVER SPECIFIC, AN SGOT (SAST) RISE OFTEN INDICATES LIVER DISEASE WHICH MAY PORTEND POTENTIAL BLEEDING PROBLEMS. AN INJECTION OF VITAMIN K_1 (1-2 MG/KG) PRIOR TO SURGERY MAY HELP MINIMIZE POTENTIAL BLEEDING.

ANTIBIOTICS, STEROIDS AND FLUIDS

PRIOR TO AN ELECTIVE SURGERY, THE AUTHORS PREFER TO INITIATE ANTIBIOTIC THERAPY. INJECTABLE PENICILLIN DERIVATIVES SUCH AS PIPERACILLIN (PIPRACIL® - LEDERLE) AND CARBENICILLIN (GEOPEN® - ROCHE) USED IN COMBINATION WITH THE NEWER AMINOGLYCOSIDES SUCH AS TOBRAMYCIN (NEBCIN® - ELI LILLY) OR AMIKACIN (AMIKIN® - BRISTOL) ARE PREFERRED.

IF A PARTICULARLY RISKY SURGERY IS TO BE ATTEMPTED, ESPECIALLY IN SMALL BIRD PATIENTS, WE PREFER TO GIVE A SHOCK DOSE OF DEXAMETHASONE (1 MG/LB I.M.) PRIOR TO SURGERY. IN OUR EXPERIENCE, THIS SEEMS TO ENHANCE SUCCESS. WE HAVE MOST FREQUENTLY USED THIS TECHNIQUE WHEN REMOVING ABDOMINAL MASSES FROM BUDGERIGARS OR ATTEMPTING RISKY PROCEDURES SUCH AS EGG YOLK PERITONITIS CLEANUP OR ECTOPIC EGG REMOVAL IN SMALL BIRDS.

FLUID THERAPY IS SELDOM NECESSARY UNLESS BLOOD LOSS IS SEVERE. INTRAVENOUS FLUIDS MAY BE GIVEN TO THE ANESTHETIZED BIRD THROUGH THE ULNAR VEINS, USING 25 OR 26 GAUGE NEEDLES OR BUTTERFLY CATHETERS (ANGIOCATH® - DESERET). WE USUALLY USE LACTATED RINGERS SOLUTION OR A MIXTURE OF 50:50 5% DEXTROSE AND LACTATED RINGERS AT A VOLUME OF .05CC/GM/BODY WEIGHT. SOME OF THE FLUIDS CAN BE GIVEN I.V. AND THE REST SUBCUTANEOUSLY (AXILLARY AND INGUINAL AREAS). FLUIDS ARE GIVEN BY RAPID BOLUS ADMINISTRATION.

WARMTH IS CRITICAL TO RECOVERY IN THE AVIAN SURGICAL PATIENT. JUDICIOUS USE OF HEATED CAGES, HEATING PADS AND INCUBATORS IS IMPORTANT.

ELLMAN SURGITRON VS. CONVENTIONAL INSTRUMENTATION

THE USE OF THE ELLMAN SURGITRON UNIT, AS PIONEERED BY ALTMAN, HAS REVOLUTIONIZED PET AVIAN SURGERY. INCISIONS MAY BE MADE WITH MINIMAL BLEEDING USING THIS EQUIPMENT. CONVENTIONAL INSTRUMENTS ARE OFTEN USED IN COMBINATION WITH THE USE OF ELECTROSURGERY.

SURGICAL PROCEDURES

SELECTED SOFT TISSUE SURGICAL PROCEDURES USED IN PET AVIAN PRACTICE WILL NOW BE DISCUSSED. SURGICAL SEXING, BEAK REPAIR AND ORTHOPEDICS ARE NOT COVERED IN THIS PAPER.

ORAL ABCESSES AND CHOANAL INFECTIONS

A MYRIAD OF ORGANISMS CAN AFFECT THE PHARYNGEAL AREA OF BIRDS. GRAM NEGATIVE INFECTIONS, SOME GRAM POSITIVE INFECTIONS, VITAMIN A DEFICIENCY BREAK DOWN OF TISSUES, YEAST INFECTIONS (I.E. CANDIDIASIS), LOCALIZED FUNGAL INFECTIONS (I.E. ASPERGILLOSIS), PARASITIC CONDITIONS (I.E. TRICHOMONIASIS, NEMATODE CYSTS, ETC.) ARE ALL COMMON. CHOANAL PUS MAY BREAK OFF AND BECOME INHALED, RESULTING IN ACUTE DYSPNEA AND DEATH.

CARE MUST BE TAKEN TO LOCALIZE THE INFECTION WITH PROPER CLINICAL PATHOLOGY BACKUP AND SYSTEMIC AND LOCAL ANTIBIOTICS BEFORE SURGERY IS CONTEMPLATED (UNLESS INHALATION OF LOOSE PARTICLES OF PUS SEEMS IMMINENT). THE AUTHORS HAVE SEEN AND HEARD OF INEXPERIENCED VETERINARIANS RESTRAINING SERIOUSLY ILL BIRDS TO THOROUGHLY CURRETTE MOUTH ABCESSES (AS IN THE DOG AND CAT APPROACH) WITH RESULTING NIGHTMARISH HEMORRHAGE AND DEATH. BE CONSERVATIVE WITH YOUR APPROACH! ALWAYS ANTICIPATE HEMORRHAGE AND INTERNAL COMPLICATIONS AND STABILIZE A PATIENT BEFORE SURGERY. REMEMBER THAT A BIRD WITH AN ABNORMAL LOOKING MOUTH MAY HAVE A SIMILAR LOOKING LIVER.

WHEN CLEANING UP ORAL AND CHOANAL ABCESSES, FERRIC SUBSULFATE SOAKED CAUTERY STICKS CAN BE USED TO BLUNTLY DISSECT AWAY CASEOUS PUS WHILE AIDING WITH HEMOSTASIS AT THE SAME TIME.

SINUS SURGERY

INJECTING THE PARANASAL SINUSES WITH ANTIBIOTIC-SALINE MIXTURES IS COMMONLY PERFORMED. THE PARANASAL SINUSES CAN BE ENTERED BY INJECTING THROUGH THE SKIN (LATERAL WALL) AT A SITE MIDWAY AND SLIGHTLY VENTRAL TO A POINT EQUIDISTANT BETWEEN THE NARIS AND THE EYE. ANTIBIOTICS INJECTED INTO THIS AREA WILL PERFUSE THE PARANASAL SINUS AREA AROUND THE EYE AND CAN REACH AN AREA THAT A NASAL FLUSH MAY NOT BE ABLE TO REACH IN A CHRONIC CASE OF SINUSITIS.

SINUS AND RHINAL INFECTIONS ARE COMMON IN BIRDS AND IT HAS BEEN OUR EXPERIENCE THAT MOST ACUTE OR SEMI-ACUTE INFECTIONS WILL RESPOND TO NASAL FLUSHES AND ANTIBIOTIC THERAPY WITHOUT INVOLVED SURGERY. HOWEVER, IN CHRONIC CASES, INJECTING SINUSES MAY PROVE A USEFUL ADJUNCT TO THERAPY.

SINUS TREPHINATION IS RARELY USED BUT MAY BE NECESSARY IF INFECTION INTO THE POSTERIOR NASAL SINUSES OR PARANASAL SINUSES HAS BECOME EXTENSIVE. IN CASES OF CHRONIC SINUSITIS, PUS POCKETS OR MICRO-ABCESSES MAY FORM. THESE AREAS ARE OFTEN NOT TREATABLE WITH CONVENTIONAL ANTIBIOTIC THERAPY DUE TO THE LACK OF BLOOD PERFUSION. IF REPEATED SINUS FLUSHING (NASAL TECHNIQUE) AND ANTIBIOTIC THERAPY DICTATED BY REPEAT GRAM STAINS AND CULTURES AND SENSITIVITIES FAILS, AND OTHER POSSIBILITIES HAVE BEEN RULED OUT (I.E. ALLERGIES, FOREIGN BODY PARTICLE INHALATION, ETC.), THE SINUS TREPHINATION IS CONSIDERED.

THE PURPOSE OF SINUS TREPHINATION IS TO CREATE AN OPENING TO INSTILL FLUSHING AND ANTIBIOTIC SOLUTIONS TO AN OTHERWISE INACCESSIBLE AREA (USUALLY 1:10 SOLUTIONS BY VOLUME, I.E. 9.5CC LACTATED RINGERS AND .5CC GENTAMYCIN OR OTHER ANTIBIOTIC OR COMBINATION). THE OPENINGS ARE CREATED SO THAT THEY WILL STAY PATENT AT LEAST A WEEK, WHICH HAS BEEN AN EFFECTIVE TECHNIQUE FOR US.

THE SURGICAL TECHNIQUE IS AS FOLLOWS: THE PATIENT IS ANESTHETIZED WITH METOFANE BY "OPEN-DROP" METHOD (CONE) OR FORANE BY ANESTHETIC MACHINE TO FACILITATE QUICK RECOVERY. *DO NOT USE INJECTABLE* ANESTHETICS DUE TO THE POTENTIAL BLEEDING THAT MAY BE ENCOUNTERED AND POTENTIAL DANGER OF BLOOD INHALATION. THE SURGICAL SITES ARE AN AREA ABOUT 2/5 THE WAY BETWEEN THE EYE AND NARIS AND DORSAL TO THE PLANE OF THE EYE. A DREMEL TOOL IS USED TO FACILITATE CREATING THE OPENINGS, AFTER FIRST PLUCKING THE FEATHERS AND WIPING THE SKIN WITH ALCOHOL AND BETADINE (AVOID THE EYES - APPLY GENTOCIN OPTHALMIC FIRST). AN INITIAL INCISION IS MADE OVER BOTH SITES, USING FERRIC SUBSULFATE OR ELECTROCAUTERY TO STOP SKIN BLEEDING (CAN BE A PROBLEM). THE BONE IS EXPOSED AND THE SINUSES ARE INVADED. IF PROFUSE BLEEDING OCCURS, GEL FOAM PACKING MAY BE USED. THE OPENINGS ARE TESTED FOR PATENTCY AFTER FIRST OBTAINING SWAB SPECIMENS FOR POTENTIAL GRAM STAINS AND CULTURES AND SENSITIVITIES. PATENTCY IS TESTED BY INJECTING THE USUAL MIXTURE (LACTATED RINGERS - GENTOCIN) INTO THE OPENINGS. FLUID SHOULD BE EVIDENT THROUGH THE CHOANAL SLIT. BE CAREFUL ABOUT RAPIDLY INDUCING THE FLUID, AS THE PERIORBITAL TISSUE MAY SWELL. IF THIS HAPPENS, ABSORPTION IS RAPID. THE BIRD IS ALLOWED TO RECOVER IN A DARK CAGE. WATCH CAREFULLY FOR POST-

OPERATIVE BLEEDING AND APPLY FERRIC SUBSULFATE SWABS, IF NECESSARY, TO THE SKIN AND OPENINGS.

THE NEXT DAY, THE TREPHINATION SITES CAN BE USED TO FLUSH THE SINUSES. I WILL OFTEN FLUSH THE SINUSES TWICE DAILY AND APPLY AN OINTMENT AFTERWARDS, SUCH AS GENTOCIN OPTHALMIC. THE OPENINGS WILL SEAL RAPIDLY AND MAY REQUIRE FORCED OPENING TO RETAIN PATENTCY. ONCE THEY SEAL, HEALING IS RAPID AND USUALLY VERY LITTLE SCAR TISSUE IS NOTICEABLE AFTER HEALING HAS OCCURRED.

AIR SAC EXPLORATORY

THE EXPLORATORY LAPAROSCOPY IS BECOMING MORE AND MORE USEFUL AS A DIAGNOSTIC AID. IN CHRONIC RESPIRATORY DISEASE, SAMPLES FOR GRAM STAIN, CULTURE AND SENSITIVITY, OR BIOPSY, MAY BE TAKEN FROM THE AIR SACS DIRECTLY THROUGH AN ABDOMINAL INCISION. IT HAS BEEN OUR PREFERENCE TO USE OPEN DROP METOFANE FOR THIS PROCEDURE DUE TO ITS SAFETY, AS THESE PATIENTS ARE SEVERELY COMPROMISED. HOWEVER, FORANE APPEARS TO BE THE NEW ANESTHETIC OF CHOICE FOR THE PROCEDURE. THIS METHOD IS USUALLY USED ONLY IF CONVENTIONAL DIAGNOSTIC WORKUPS AND ANTIBIOTIC THERAPY HAVE FAILED TO SOLVE THE PROBLEM, OR IF RADIOGRAPHS HAVE OUTLINED AN OBVIOUS GRANULOMA OR INTERNAL ABCESS AND A DIRECT VISUALIZATION IS DESIRED.

RUPTURED AIR SACS

RUPTURED AIR SACS ARE SEEN FROM TIME TO TIME IN MANY AVIAN SPECIES. THE ETIOLOGY MAY BE EITHER TRAUMA OR INFECTION. THE USUAL TREATMENT INVOLVES TEARING THE BIRD'S SKIN WITH A LARGE (18GA) NEEDLE TO ALLOW THE AIR TO ESCAPE. MANY BIRDS WILL HEAL THIS WAY AND OTHERS REQUIRE REPEAT TREATMENTS. OF COURSE, THE BIRD SHOULD BE GIVEN THE USUAL WORKUP TO TRY TO DETERMINE ETIOLOGY AND PROPHYLACTIC ANTIBIOTICS ARE SUGGESTED, IN ANY CASE. SOME SMALL BIRDS SUCH AS BUDGIES OR CANARIES MAY ASSUME BIZARRE APPEARANCES WHEN VICTIMIZED BY THIS CONDITION. MOST WILL EVENTUALLY HEAL WITH TIME (I.E. 2 WEEKS). SOME CASES BEOME CHRONIC, PRODUCING LONG TERM OR PERMANENT "SUBCUTANEOUS EMPHYSEMA". IN SUCH CASES, LARGER OPENINGS IN THE SKIN CAN BE MADE AND SUTURED OPEN TO ENCOURAGE LEAKAGE OF AIR. THE AIR SACS MAY HEAL WITH TIME. WE HAVE NOT BEEN ABLE TO SUCCESSFULLY SUTURE AIR SAC TEARS. HARRISON HAS DESCRIBED SUCCESSFUL LIGATION OF TORN AIR SACS OF THE SUBMANDIBULAR AREA IN YOUNG COCKATIELS. HE ALLOWS THE BIRDS TO MATURE, THEN REPAIRS THE MATURE AIR SACS WITH A SINGLE LIGATURE.

CROPOTOMY PROCEDURES (INGLUVIOTOMY)

CROPOTOMIES (INGLUVIOTOMIES) ARE FREQUENTLY NECESSARY IN AVIAN MEDICINE. THE TWO MOST FREQUENT INDICATIONS ARE CROP IMPACTION AND FOREIGN BODY REMOVAL. OCCASIONALLY, ONE MAY ENTER THE CROP TO REACH THE PROVENTRICULUS. THE PROCEDURE IS PERFORMED WITHOUT ANESTHETIC AND PRODUCES NO APPARENT DISCOMFORT. THE SKIN IS WET DOWN WITH ALCOHOL AND A FEW FEATHERS PLUCKED AWAY (GO "WITH THE GRAIN"). ALCOHOL IS WIPED ON, FOLLOWED WITH BETADINE. THE FOREIGN BODY IS GRASPED INSIDE THE CROP AND AN INCISION IS MADE THROUGH THE SKIN. THE CROP IS THEN INCISED AND THE FOREIGN BODY GRASPED AND REMOVED. 3-0 OR 4-0 DEXON IS USED TO SUTURE THE CROP AND 4-0 DEXON OR SILK IS USED TO SUTURE THE SKIN. IN YOUNG BIRDS, IT IS PREFERABLE TO GO INTO THE CROP THROUGH THE LEFT SIDE IF A SIDE IS NOT EVIDENT, AS THIS IS THE EQUIVALENT OF THE FUNDUS OF THE STOMACH, AND GIVES ONE MORE EXPOSURE WITH LESS CHANCE OF INJURING A VITAL AREA. THE CROP WILL HEAL WITHOUT STRICTURES IF HANDLED THIS WAY. THE SKIN IS INCISED AND THE CROP WILL BULGE OUT OF THE INCISION WHEN GENTLY SQUEEZED.

VENTRICULOTOMY (GIZZARDOTOMY)

WE HAVE FOUND THE VENTRAL, MIDLINE APPROACH THE MOST EFFICACIOUS FOR REACHING THE GIZZARD. AFTER A STANDARD PREPARATION, A MIDLINE INCISION IS MADE BETWEEN THE PUBIS AND EXTENDED APPROXIMATELY 2 INCHES. THE ABDOMINAL WALL IS CAREFULLY OPENED WITH SCISSORS, TAKING CARE NOT TO TRAUMATIZE INTESTINAL LOOPS. AFTER THE ABDOMINAL AIR SACS ARE OPENED, THE VENTRICULUS IS GENTLY EXTERIORIZED USING ALLIS TISUE FORCEPS. A SMALL INCISION IS MADE IN THE MUSCULAR WALL OF THE DISTAL GIZZARD AND SPREAD 0.5 INCH BY BLUNT DISSECTION. THE INSIDE OF THE GIZZARD IS EASILY ACCESSIBLE. 4-0 DEXON IS USED IN CLOSURE.

CLOACA-PEXY

THIS SURGERY IS USED TO REPAIR CHRONIC PROLAPSE OF THE CLOACA. A MIDLINE INCISION IS MADE AND THE CLOACA IS OUTLINED BY HAVING AN ASSISTANT WEARING A FINGER COT PUSH THE CLOACA INTO THE INCISION SITE. FROM HERE, IT IS CAREFULLY SUTURED TO THE ABDOMINAL WALL USING 3-0 SURGICAL WIRE AND 3-0 DEXON IN A CROSSING PATTERN. CARE MUST BE TAKEN NOT TO PENETRATE THE CLOACAL WALL.

DIGESTIVE TRACT BIOPSIES

BIOPSY TECHNIQUES ARE BECOMING MORE AND MORE POPULAR. LIVER BIOPSIES ARE PROBABLY THE MOST USEFUL DIAGNOSTICALLY. A MIDLINE INCISION IS MADE AND A WEDGE OF LIVER IS REMOVED. BLEEDING IS USUALLY MINIMAL BUT CONSIDERATION SHOULD BE GIVEN TO POTENTIAL PROBLEMS IF THE LIVER IS COMPROMISED. A PROPHYLACTIC INJECTION OF VITAMIN K_1 (1-2 MG/KG) 12-24 HOURS PRIOR TO BIOPSY MAY BE CRUCIAL WHEN BLEEDING DISORDERS EXIST. BLEEDING POTENTIAL CAN BE CHECKED BY PLUCKING A FEW FEATHERS PRIOR TO BIOPSY.

CLOACAL POLYPS AND GROWTHS

CLOACAL GROWTHS AND POLYPS HAVE BEEN SEEN FOR YEARS IN VARIOUS PSITTACINE SPECIES. SOME FEEL THESE MAY BE OF VIRAL ORIGIN AND VACCINE RESPONSIVE. SURGICAL PROCEDURES RANGE FROM SIMPLE LIGATION AND EXCISION, ELECTROCAUTERY, TO CRYOSURGERY. THE METHOD OF REMOVAL DEPENDS ON THE EXTENT OF INVOLVEMENT OF SURROUNDING TISSUES. THE AUTHORS PREFER ELECTROCAUTERY AND HARRISON HAS HAD SUCCESS WITH CRYOSURGERY.

VIABILITY OF COCKATIEL (NYMPHICUS HOLLANDICUS) EGGS STORED UP TO TEN DAYS UNDER SEVERAL CONDITIONS

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Introduction

Artificial incubation provides the basis for the commercial poultry industry. With the increasing interest in captive propagation of exotic species, artificial incubation is becoming more prevalent in the exotic bird industry as well.

Hatching eggs can be stored under appropriate conditions for a week or more prior to incubation without a loss in hatchability. Most information concerning length of storage comes from precocial species important to the poultry and gamebird industries. Little or no information is available on the storage time of hatching eggs from altricial species common to the cage bird industry.

A preliminary study suggested that the maximum storage time for cockatiel eggs prior to incubation was about 3-4 days, after which hatchability declined to less than 50%. This current study was undertaken to evaluate the storage time of cockatiel eggs under several test conditions.

Materials and Methods

Sixty-five pairs of cockatiels were housed in individual breeding units, one pair per cage. Feed and water were provided ad libitum.

Eggs were collected daily between 1600 and 1700 hours and marked with a felt tip marker to indicate parentage and the date egg was laid. Within 3 hrs of collection, eggs were weighed. All cracked eggs were discarded.

Eggs were fumigated using 1.22 ml formalin (37% formaldehyde) and 0.6 grams potassium permanganate per cu ft for 20 minutes. After fumigation the eggs were distributed into three treatment groups such that no pair produced significantly more eggs for one group than another.

Treatment 1 eggs were placed small end down on cardboard egg flats. These eggs were not turned during storage. Treatment 2 eggs were turned 45^{0} from the vertical once per day. Treatment 3 eggs were placed small end down on cardboard egg flats, bagged in self-sealing freezer bags, and turned 45^{0} from the vertical once per day.

The three groups were stored at a temperature and relative humidity of 55^{0}F and 60 percent, respectively. Eggs were stored from 0-10 days prior to incubation. There were five separate egg settings.

All eggs were weighed immediately prior to incubation to determine weight loss during the storage period.

Eggs were incubated in a Jamesway 252 incubator at a temperature of 99.5^{0}F and a humidity of 87.0^{0} wet bulb. The eggs were set in chicken trays modified to accommodate the small cockatiel eggs. Eggs were turned automatically every two hours.

At seven days of incubation the eggs were candled and all infertile and early dead embryos were removed for break out examination. On day fifteen of incubation the eggs were candled and any additional dead embryos were removed and examined. All eggs remaining after the second candling were weighed again to determine weight loss between setting and transfer to a Lyon's glass top hatching machine. The hatching unit was maintained at a temperature and humidity of 98.5^{0}F and 88-90^{0} wet bulb. Eggs that failed to hatch by the 19th day of incubation were removed and examined. Infertile eggs, age at death, and embryonic abnormalities were recorded.

Results and Discussion

Figure 1 shows the pooled hatchability data of the 5 settings by treatment groups relative to storage time. There are no differences in hatchability between the treatment groups, perhaps reflecting sample size. However, hatchability decreased within treatment groups as storage time increased. Hatchability declined in treatments 1 and 2 after four days of storage. This decline in hatchability continued, ultimately reaching 0 at nine and ten days of storage, respectively.

Hatchability remained high in treatment 3 until after the sixth day of storage and did not decline to 0 as was observed in treatments 1 and 2. This demonstrated that bagging eggs during the storage period can successfully increase the storage time. Storage of the eggs in plastic bags appears to reduce the rate of dehydration and pH change due to CO_2 loss from the egg. Hatchability began to decrease when weight loss reached 0.04 grams per egg. Treatment 1 lost 0.04 grams per egg by 4 days of storage, treatment 2 by 5 days, and treatment 3 by 6 days, paralleling their decline in hatchability.

Figures 2-5 represent embryonic mortality after 1, 5, 7 and 10 days of preincubation storage. No morphological abnormalities were found among the treatment groups. Embryonic mortality is represented

as percent mortality (number dead/number survivors on that day) at a given age (days) of embryonic development.

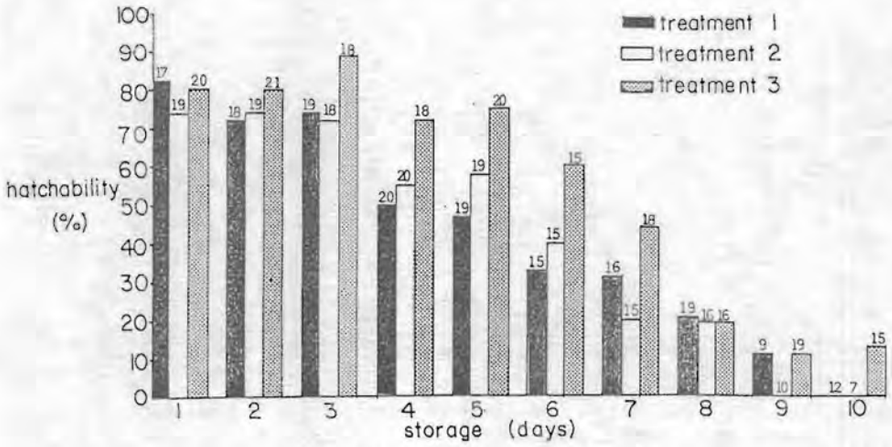

Figure 1. Pooled hatchability (%) data of the 5 settings by treatment groups relative to storage time. Numbers above bars are the numbers of fertile eggs for that treatment on that day.

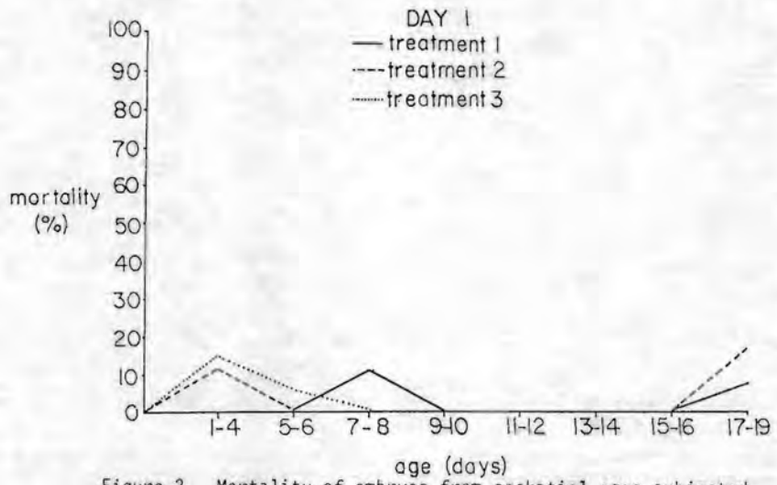

Figure 2. Mortality of embryos from cockatiel eggs subjected to 1 day of preincubation storage.

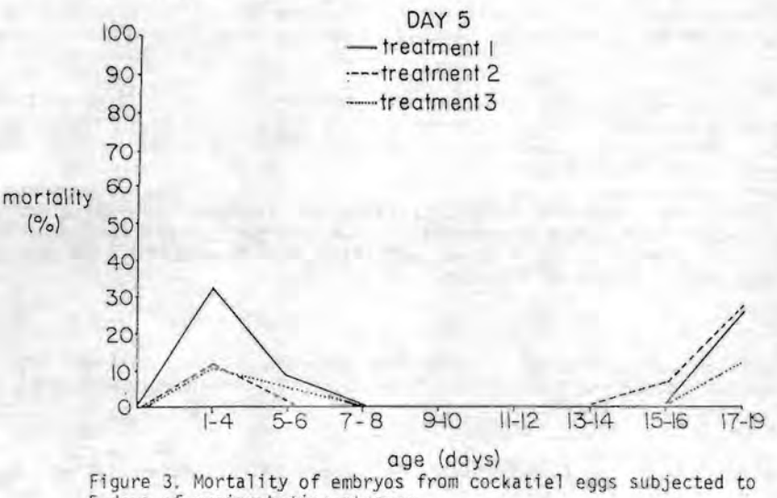

Figure 3. Mortality of embryos from cockatiel eggs subjected to 5 days of preincubation storage.

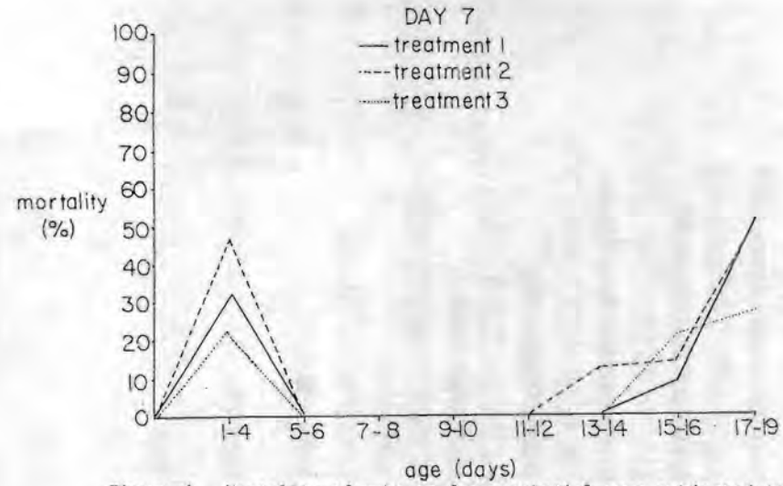

Figure 4. Mortality of embryos from cockatiel eggs subjected to 7 days of preincubation storage.

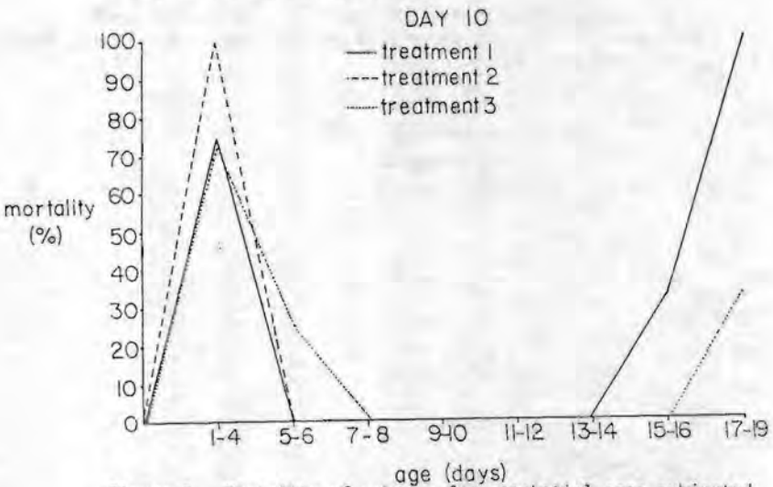

Figure 5. Mortality of embryos from cockatiel eggs subjected to 10 days of preincubation storage.

Figure 2 demonstrates that there were essentially no differences among the treatment groups in the early (days 1-4) and late (days 17-19) mortality peaks. The mid peak (days 7-8) mortality associated with treatment 1 is unexpected and was not observed in the other treatment groups.

Figure 3 demonstrates an increase in the early embryonic mortality of treatment 1 after 5 days of preincubation storage, which one would expect in eggs subjected to extended preincubation storage periods.

Figure 4 shows an increase in both the early and late mortality periods of treatment groups 1 and 2, resulting in a decline in hatchability. Treatment group 3 had a lower mortality in both periods than treatments 1 and 2. Although this was not statistically significant because of sample sizes, bagging appears to improve viability during storage.

Figure 5 demonstrates the embryonic mortality among the treatment groups after 10 days of preincubation storage. Treatments 1 and 2 demonstrate a high embryonic mortality, resulting in zero percent hatchability. Treatment 3 shows a lower mortality than observed in treatments 1 and 2, resulting in eggs hatching after 10 days of storage.

Summary

Hatchability of cockatiel eggs declines after 4 days of preincubation storage. Bagging eggs in self-sealing plastic bags can reduce the loss of viability during the preincubation storage period.

Acknowledgement

The authors acknowledge the guidance, assistance, and review of this manuscript by U.K. Abbott and C.R. Grau.

PROTEIN REQUIREMENT OF GROWING COCKATIELS

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Introduction

A 20% protein diet was used to study solids-water relationships in the feeding of cockatiel chicks (Roudybush and Grau 1983) based on similar diets used for Japanese Quail (Vohra 1972). The success of these hand-feeding trials with cockatiels permitted the present study of the protein requirements for growth of chicks from hatching to four weeks of age. Growth results were obtained from diets containing 5% to 35% protein provided by isolated soybean protein supplemented with methionine. The diets also contained 3.75% lipid and variable amounts of corn starch.

Methods

The diets were essentially the same as those used by Roudybush and Grau (1983) except that part of the regular corn starch was replaced by a modified food starch that produced a thick mixture with cold water, thus avoiding cooking. The final wet mixture was warmed to 40.5°C before feeding. The solids contents were 7% for the first three days, then 30% to the end of the trial. The composition of the 20% protein diet is given in Tables 1, 2, and 3. Diets with other protein levels were identical except for isolated soybean protein, methionine, and corn starch. The ratio of methionine to protein was kept constant.

Table 1

Composition of the 20% protein diet.

Ingredient	g/kg diet
Soybean oil, crude	37.5
Cellulose	50.0
Calcium carbonate, $CaCO_3$	10.0
Dicalcium phosphate, $CaHPO_4 \cdot 2H_2O$	30.0
Vitamin mixture *	1.25
Mineral mixture **	13.1
Choline chloride (60%)	4.2
Isolated soybean protein (87% protein)***	234.8
DL-Methionine	3.5
Corn starch	575.65
Modified food starch ****	40.0
Total	1000.0

* See Table 2 for vitamin mixture composition
** See Table 3 for mineral mixture composition
*** Purina Protein 500E - Isolated Soy Protein. Ralston Purina Co., Checkerboard Square, St. Louis, MO 63164.
**** Instant Clear Jel - Food Starch - Modified. National Starch and Chemical Co., Bridgewater, NJ 08807.

Table 2: Vitamin Mixture

Vitamin	mg/kg diet
Vitamin B_{12} (1 mg/g in mannitol)	14.0
Menadione (vitamin K source)	4.9
Niacin	231.3
Riboflavin	18.7
Vitamin E (227,000 IU/lb)	238.3
Folic acid	4.7
Thiamine	9.3
Biotin	0.9
Vitamin A (30,000 IU/g)	624.0
Vitamin D_3 (320,000 IU/g)	13.1
Pyridoxine (Vitamin B_6)	18.7
Pantothenic acid (calcium salt)	75.0
Total	1252.9

Table 3

Ingredients	mg/kg diet
Magnanese sulfate, $MnSO_4 \cdot H_2O$	297.00
Copper sulfate, $CuSO_4 \cdot H_2O$	97.00
Cobaltous acetate, $Co(C_2H_3O_2)_2 \cdot 4H_2O$	20.0
Potassium iodate, KIO_3	9.00
Magnesium sulfate, $MgSO_4 \cdot 7H_2O$	3970.00
Potassium chloride, KCl	2970.00
Dibasic potassium phosphate, K_2HPO_4	4950.00
Sodium molybdate, $Na_2MoO_4 \cdot 2H_2O$	9.00
Sodium selenite, $Na_2SeO_3 \cdot 5H_2O$	0.66
Zinc oxide, ZnO	120.00
Ferrous sulfate, $FeSO_4 \cdot 7H_2O$	644.00
Total	13086.66

Two experiments were performed. In the first the protein levels in percent of the diet were 5, 10, 15, 20, and 25, with 6-13 chicks per group. In the second the protein levels were 15, 18, 20, 25, and 35% with 12-16 chicks per group.

Results

Differences in growth rates among the groups fed the various protein levels began to be apparent early and by 7 days of age the 5% and 10% protein groups were different from higher levels, as shown in the growth curves of experiment 1 (figure 1). In experiment 2 (figure 2) protein levels were 15% to 35%. The two experiments were performed several weeks apart but the results were similar. The response curves of figure 3 show clear differences among the groups by 14 days, when growth was most rapid. The 15% protien level resulted in the slowest growth ($P<.01$), with 18% and 35% protein growing slower than 20% and 25% protein. The differences remained evident at 21 days of age, but by 35 days there were no differences among groups fed 15% or more protein.

The mortality for all groups combined was 17.3% for the first four days and 34.5% for 28 days. There were no differences among the treatments except possibly the 5% protein level group in which only

one chick survived out of six.

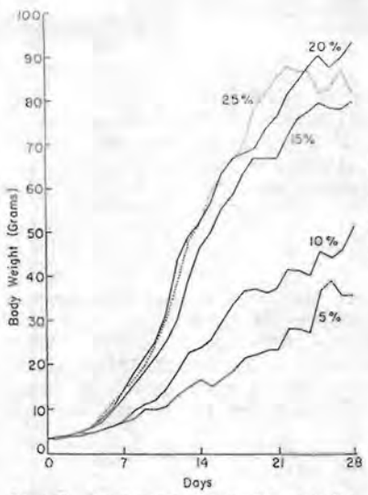

Figure 1. Growth curves of cockatiel chicks fed various levels of protein. The initial numbers of chicks per group were: 5%, n=6; 10%, n=7; 15%, n=12; 20%, n=12; and 25%, n=7.

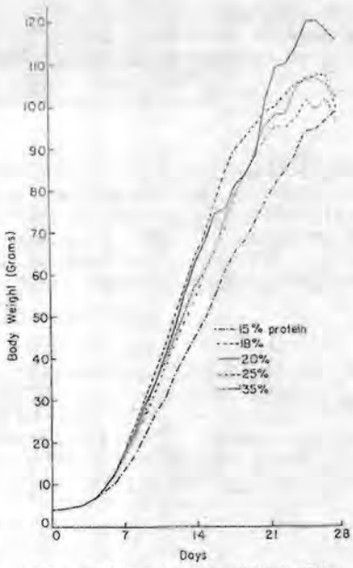

Figure 2. Growth rates of cockatiel chicks. Protein levels and inital numbers: 15%, n=12; 18%, n=12; 20%, n=13; 25%, n=16; 35%, n=12

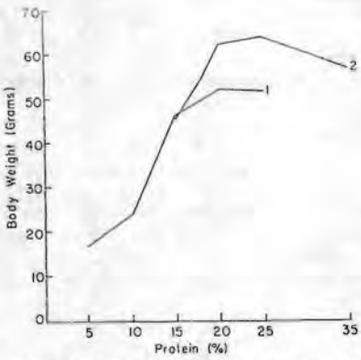

Figure 3. Growth response curves for experiments 1 and 2 based on body weight at 14 days of age.

Discussion

The difficulty of establishing the minimum dietary requirement for a nutrient is clearly shown by the data presented here. For rapid growth cockatiel chicks require 20% protein, but if eventual adult weight is used as the criterion, nutrient requirements must be set lower, possibly less than 15% protein. The time that such a lower protein diet must be fed is significantly longer, however, and thus time becomes a factor to be considered. Application of these observations to a commercial operation, whether chicks were parent-fed or hand-fed, would probably favor the higher protein level if the differences in costs of the diets were small because of the time saved to produce salable young. Feeding a marginal level of a nutrient may increase the risk of permanent stunting or susceptibiltiy to disease or stress; however, data on this possibility are not yet available.

Cockatiel chicks tolerated a protein level of 35%, but they grew more slowly than with 20% protein. No permanent stunting was apparent.

Summary

The protein requirement of cockatiels from hatching to 28 days of age is 20% of the diet.

1. Roudybush, T.E. and C.R. Grau. 1983. Solids in diets for hand raising cockatiels. Proc. 32nd Western Poultry Disease Conference, Davis CA. Feb. 8-10, 1983. pp. 94-95.

2. Vohra, P. 1972. Magnesium requirement for survival and growth of Japanese quail (Coturnix coturnix japonica). Poultry Sci. 51:2103-2105.

HAND-FED VS. PARENT-FED COCKATIELS: A COMPARISON OF CHICK GROWTH

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Introduction

Avian nutrition has primarily been studied using chickens or other precocial birds as experimental subjects. Research with altricial birds was begun at the Department of Avian Sciences in 1979. Cockatiels were selected due to their practical size, moderate price, and sexual dimorphism. For the past 3 years, nutrient requirements of hand-fed chicks have been studied using growth and survival as the criteria for evaluation. The aim of this study was to establish a standard growth curve from parent-fed cockatiel chicks to be used as a basis for comparison with hand-fed birds.

Materials and Methods

To evaluate the growth responses of hand-fed cockatiel chicks, parent-fed chicks were also weighed daily. Growth results from hatch to 35 days are compared in this paper.

Parent-fed Chicks

Breeding pairs of cockatiels, which previously had their eggs removed for artificial incubation, were allowed to continue to lay, hatch their eggs, and rear chicks. A 20% protein, crumbled diet (Table 1) and water were available to the breeding birds ad libitum. There were twenty-seven parent-fed chicks in this group. Most had food in their crops when weighed.

Hand-fed Chicks

Hand-fed chicks were the control birds from 4 nutrient requirement experiments (described here as Trials 1-4, see Table 2). A purified diet containing 20% protein (Grau and Roudybush, 1985) was fed in all trials. Chicks were fed with sterile plastic disposable syringes. For Trial 1, hatch times were recorded from 6 am to 10 pm. Any chicks hatching after 10 pm or before 6 am were recorded as hatching at 6 am. For Trials 2-4, hatch times were recorded around the clock. The diet, consisting of 7% solids and 93% water, was fed every 2 hours from 6 am to 10 pm beginning at hatch. When a change was made to 30% solids and 70% water, feedings were cut back to every 4 hours from 6 am to 10 pm. As the chicks grew and it took longer for their crops to empty, the number of feedings per day was reduced. The crops of birds in Trial 1 were filled to capacity causing some birds to regurgitate. Birds in Trials 2 and 3 were fed until the crop appeared rounded. The crops of Trial 4 chicks were filled to just below the bend in the neck initially, and then the same as in Trials 2 and 3 (see Table 2 and Fig. 1). Chicks were weighed daily before the morning feeding when their crops were empty.

The chicks from Trial 3 were weighed before and after each feeding to determine daily food intake and body weight gained. The ratio of food consumed to weight gained (feed conversion) was calculated. To evaluate the relative consumption during growth, the ratio of dry food intake to mean body weight was also calculated.

Table 1. Composition of crumbles diet for cockatiels

Ingredients	per 1000g diet
Corn, yellow, ground	817.7 g
Soybean protein, isolated, 87% protein	144.6
Calcium carbonate, $CaCO_3$	6.4
Dicalcium phosphate, $CaHPO_4 \cdot 2H_2O$	25.7
DL-Methionine	2.6
Mineral mixture (see below)	1.2
Vitamin mixture (see below)	1.8
Total	1000.0 g

Mineral mixture

Potassium chloride, KCl	990
Manganese sulfate, $MnSO_4 \cdot H_2O$	141.2
Copper sulfate, $CuSO_4 \cdot 5H_2O$	5.4
Potassium iodate, KIO_3	0.6
Sodium selenite, $Na_2SeO_3 \cdot 5H_2O$	0.25
Zinc oxide, ZnO	39.6
Total	1177.05 mg

Vitamin Mixture

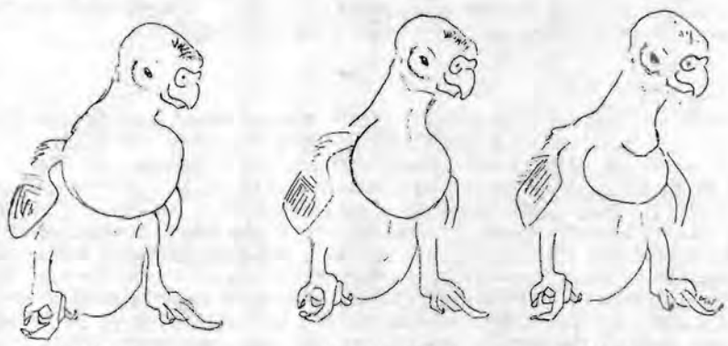
Vitamin A	2500 IU
Vitamin D_3	500 IU
Vitamin B_{12}	0.009 mg
Vitamin K	0.8 mg
Niacin	30 mg
Riboflavin	3 mg
Folic acid	0.3 mg
Biotin	0.015 mg
Pantothenic acid	7 mg
Choline chloride	1020 mg
Total	1800 mg

Table 2

Hand-feeding Trials

Trial	n	Diet* dilution and feeding schedule	Appearance of crop
1	14	7% solids, 93% H_2O from hatch to Day 4; 30% solids, 70% H_2O from morning of Day 4 to weaning.	Crops filled until they ballooned outward.
2	14	7% solids, 93% H_2O from hatch until 48 hrs after 1st meal. 30% solids, 70% H_2O from 48 hrs to weaning.	Crops filled until the wrinkles in them disappeared.
3	10	Same as Trial 2.	Same as Trial 2.
4	8	Same as Trial 2.	Crops filled to below the bend in the neck for 9 days, then as in trial 2.

* Grau and Roudybush, 1985.



Trial 1 Trials 2 & 3 Trial 4

Figure 1. Crop appearance of cockatiel chicks after hand feeding.

Results

The growth of parent-fed chicks differed from Trial 1 hand-fed chicks (Fig. 2) in two ways. First, during the initial 4 days, parent-fed birds tripled their weights while hand-fed birds only increased their weights by 50%. Second, peak body weights of parent-fed birds exceeded their five-week weights by 12%, whereas peak weights of hand-fed birds exceeded their five-week weights by 50%. By 34 days of age both groups of birds reached approximately the same mean weights.

Chicks that were fed 30% solids at 48 hours of age (Trial 2) showed a 15% increase in weight from day 2 to 3. Chicks being fed 7% solids at 48 hours of age (Trial 1) only increased their weight by 7.8% (see Fig. 3 inset).

Figure 4 shows feed conversion and dry food intake/body weight ratios for Trial 3 birds. For days 1 and 2 the ratio of dry food intake to body weight was low, feed conversion was poor, and birds did not grow. Increased dry food intake and improved feed conversion resulted in a period of rapid body weight gain during days 4 to 13.

During the rapid growth phase, Trial 2 birds showed more fluctuations in growth than Trial 1 birds (Fig. 3). Additionally, Trial 2 birds did not exhibit the high peak weights seen in Trial 1.

The initial cautious feeding during Trial 4 while a new feeder gained experience resulted in slow growth until day 9 when relative meal sizes were increased (Fig. 5). After the increase in meal size, growth increased and paralleled that of the parent-fed birds. Body weights of Trial 4 chicks and parent-fed chicks were similar at 35 days.

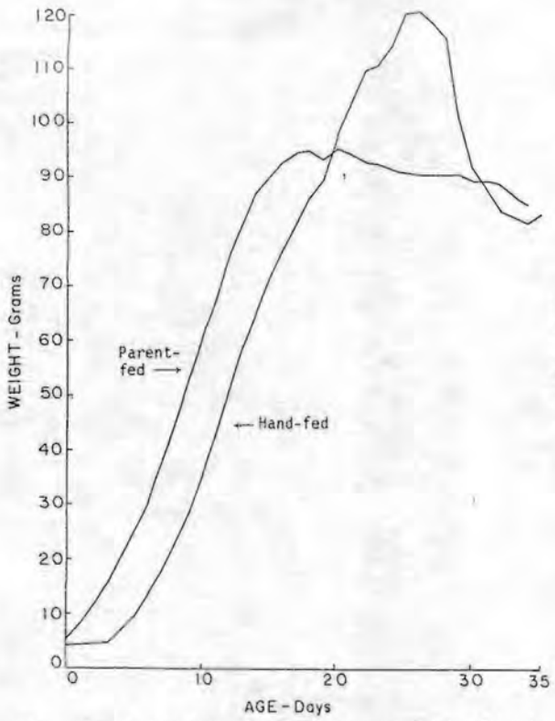


Figure 2. A comparison of growth of parent-fed (n=27) and hand-fed (Trial 1) (n=14) cockatiel chicks from hatch to 35 days of age.

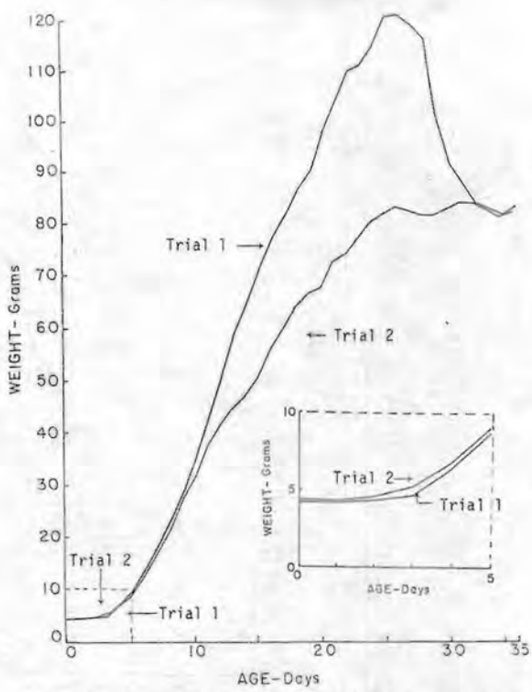


Figure 3. A comparison of growth of Trial 1 and Trial 2 hand-fed chicks from hatch to 34 days of age. The inset shows an enlarged portion of days 0-5 (n=14/trial).

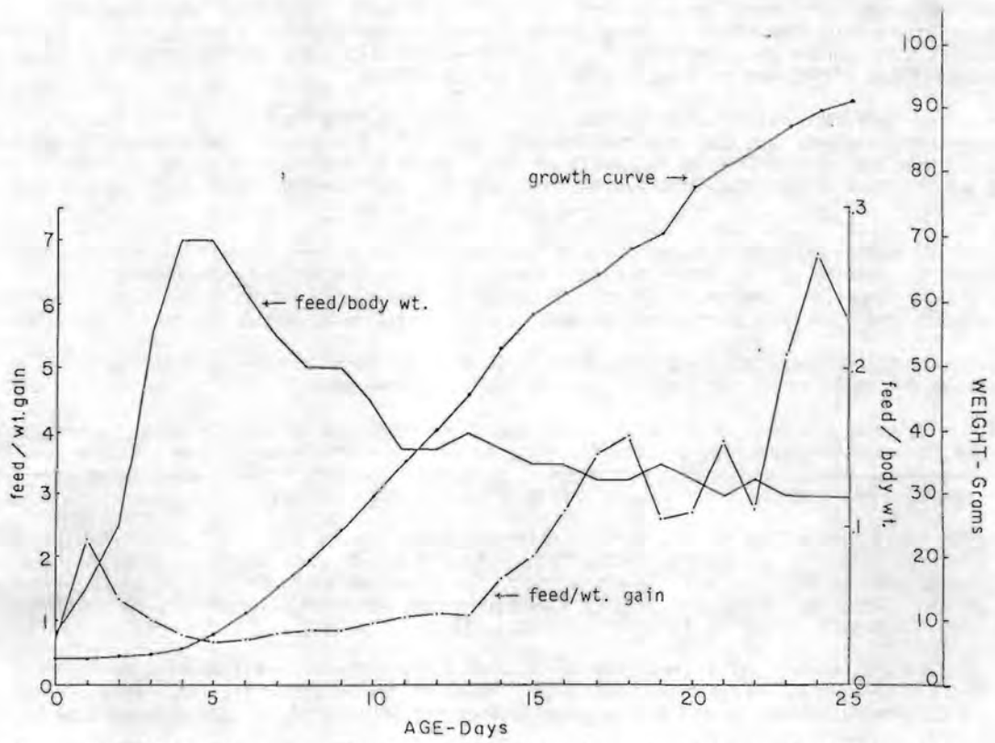


Figure 4. Relationship between feed conversion (food intake/weight gained), feed intake/body wt. and growth of Trial 3 cockatiels (n=10).

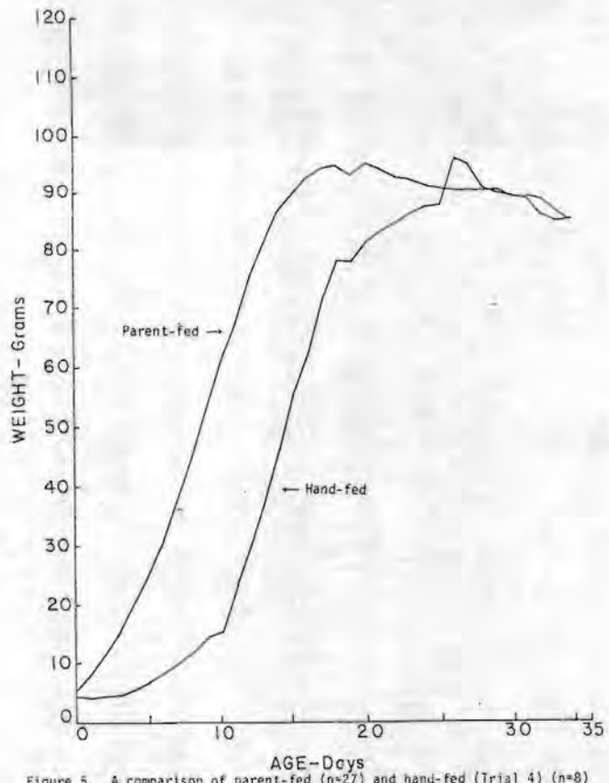


Figure 5. A comparison of parent-fed (n=27) and hand-fed (Trial 4) (n=8) cockatiel chicks from hatch to 34 days of age.

Discussion

Utilization of food eaten by a growing animal can be partitioned into two components: 1) food used to maintain body functions - the maintenance requirement and 2) food above the maintenance requirement that can be used for growth. Feed conversion (the ratio of food intake to body weight gain) measures the efficiency of food utilization by the animal.

When the feed conversion value is low, food utilization is efficient. As food intake increases, maintenance requirements are met and food becomes available for growth. This happened on day 2 of Trial 3. There was an increase in the ratio of food intake to body weight caused by the increase in dietary solids from 7% to 30%. Food became available for utilization beyond maintenance and growth occurred.

Prior to day 2, food intake was low and feed conversion values were high. This indicates that when birds are fed 7% solids, they are only receiving enough energy for maintenance and limited growth. Unfortunately, the level of solids cannot be increased prior to two days of age because levels greater than 10% have been shown to cause mortality in young chicks (Roudybush and Grau, 1983).

Two ways to increase dry food intake would be to feed more frequently than every two hours or feed through the night until the level of solids can be increased.

The high peak weights of Trial 1 birds may have been due to overfeeding. When crops are overfilled, birds sometimes regurgitate. Chicks from Trial 1 after about 3 weeks of age would often regurgitate food; other times they would attempt to regurgitate unsuccessfully. Because less food was fed per meal, weight overshoot and regurgitation did not occur in Trial 2.

Growth rates may differ due to feed intake variations even if groups of chicks are fed the same diet. This was demonstrated by the fluctuations in rapid growth rate shown in Trial 2. These birds were fed by 2 people, feeding different amounts, in contrast to birds in Trial 1 which were fed by a single person. The growth curve for Trial 1 birds showed a smoother slope during the period of rapid growth than the growth curve for Trial 2 birds (Fig. 3)

Although birds from Trial 4 grew very slowly for 9 days, growth was increased by increasing meal size. Trial 4 chick body weights eventually met those of the parent-fed birds. This indicates that slow initial growth, if recognized and corrected, does not necessarily affect body weights at 35 days of age.

Conclusion

When cockatiels were hand-fed a nutritionally complete diet at a level of 30% solids, growth was parallel to parent-fed birds. Initial growth was limited when chicks were fed only 7% solids. The cause of growth variations between parent-fed chicks and the four trials of hand-fed chicks was probably due to differences in feed intake. However, within the broad limits of feed intake described in these trials, 35-day weights were unaffected.

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LYSINE REQUIREMENT OF COCKATIEL CHICKS

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Introduction

Lysine may be the single most important amino acid that affects the protein nutrition of captive birds fed practical diets. It is the essential amino acid most often limiting in cereals and other seeds which comprise the main sources of nutrition for many caged birds. A deficiency of lysine in the diet of young domestic birds such as chickens, quail and turkeys results in poor growth and in failure to deposit the normal dark melanin pigments of feathers. Because body size and feather color are important considerations when assessing the value of caged birds, lysine nutrition had a high priority among problems that needed study.

This project was designed to determine the quantitative lysine requirement of young cockatiel chicks, based primarily on growth. Feather pigmentation was also studied. The basic approach was to feed newly hatched chicks diets in which the amino acids were provided by mixtures of pure amino acids in place of protein. The lysine content of the diet could thus be varied from low to high levels and the effects on growth and feathering determined.

Materials and Methods

The management techniques used here were virtually identical with those we developed to determine solids requirements (Roudybush and Grau, 1983). The basal diet, presented in Tables 1, 2, and 3, did not include lysine, which became the single variable in two feeding trials with groups of cockatiel chicks.

Table 1

Lysine Diets

Ingredient	g/Kg
Soybean Oil	37.5
Cellulose	50.0
$CaCO_3$	10.0
$CaHPO_4 \cdot 2H_2O$	30.0
Vitamin Mix[1]	1.25
Mineral Mix[2]	12.3
Choline Chloride (60%)	4.2
Amino Acid Mix[3]	239.0
Corn Starch	458.35
Instant Clear Jel[4]	130.0
Na_2CO_3	15.0
NaCl	0.4
Total	988.0

Experiment 1

	Lysine (%)			
	0.1	0.4	1.0	2.0
Basal	978.0g	978.0g	978.0g	978.0g
Lysine HCl	1.1	4.4	11.0	22.0
Starch	20.9	17.6	11.0	0.0
	1000.0g	1000.0g	1000.0g	1000.0g

Experiment 2

	Lysine (%)				
	0.2	0.4	0.6	0.8	1.2
Basal	988.0g	988.0g	988.0g	988.0g	988.0
Lysine	2.0	4.0	6.0	8.0	12.0
Starch	10.0	8.0	6.0	4.0	0.0
	1000.0g	1000.0g	1000.0g	1000.0g	1000.0g

1 Grau and Roudybush 1985
2 See Table 2 for mineral mixture.
3 See Table 3 for amino acid mixture.
4 Instant Clear Jel-Food Starch-Modified. National Starch and Chemical Co., Bridgewater, NJ 08807.

Table 2

Mineral Mixture

Ingredient	mg/Kg diet
Manganese sulfate $MnSO_4 \cdot H_2O$	297.00
Cupric sulfate $CuSO_4 \cdot 5H_2O$	97.00
Colbaltous acetate $Co(C_2H_3O_2)_2 \cdot 4H_2O$	20.00
Potassium iodate KIO_3	9.00
Magnesium carbonate $MgCO_3$	2752.70
Dibasic potassium phosphate K_2HPO_4	4950.00
Sodium molybdate $Na_2MoO_4 \cdot 2H_2O$	9.00
Sodium selenite $NaSeO_3 \cdot 5H_2O$	0.66
Zinc acetate $Zn(C_2H_3O_2)_2$	323.70
Ferrous sulfate $FeSO_4 \cdot 7H_2O$	644.00
Total	12,271.06

Table 3

Amino Acid Mix

Ingredient	g/Kg diet
L-Arginine[1]	11
Glycine	6
L-Serine	3
L-Histidine[1]	5
L-Leucine	11
L-Methionine	5
L-Cystine	3
L-Phenylalanine	6
L-Threonine	7
L-Tryptophan	2
L-Valine	8
L-Glutamic acid	127
L-Alanine	11
L-Aspartic acid	11
L-Proline	11
L-Isoleucine	7
L-Tyrosine	5
Total	239

1 Supplied as the hydrochloride in experiment 1, but as the free base in experiment 2.

The levels of L-lysine added to the dry basal diet varied from 0.1% to 2.0%. Each group of 12-17 chicks was fed its diet from hatching to 14 days of age or longer. One of these experiments was concluded at 14 days, but then the chicks were fed either the same diets or were shifted to alternatives in order to observe recovery or, in the case of one group, the effects of imposing a deficiency half way through the growth period. Each bird was weighed each day before the first feeding at 6 a.m. Observations of feather pigmentation of all chicks were made from the time juvenile feathers could first be seen through the skin at 10 days until the experiments were concluded at fledging.

Results

In the first experiment, the groups were fed the four levels of lysine to 14 days, at which time the results of feeding the diets were clear. The lowest level of lysine (0.1%) permitted only poor growth and survival, but 0.4% permitted much better growth, as shown in the growth curve of figure 1. The groups fed 1.0% or 2.0% lysine grew well but not as rapidly as similar chicks fed 20% protein from isolated soybean protein supplemented with methionine, as presented in the report of our first study (Roudybush and Grau 1983).

After 14 days, when the group fed 2.0% lysine was shifted to the diet containing 0.4% lysine, growth continued at approximately the same rate for 8 days, essentially in the same pattern as the group fed 1.0% lysine for the whole period. Meanwhile the group that was shifted from 0.4% lysine to the 20% protein diet grew rapidly and continued to gain up to the weight expected by fledging (80-90 grams).

The levels of lysine that had been chosen for the second experiment were based on the results of the first study. The lowest level (0.2%) permitted only slow growth (figure 2), but was better than 0.1% lysine in experiment 1. Groups fed higher levels grew faster until the level of 0.8% lysine was reached and when maximum growth was obtained. As in the first experiment, body weight at 14 days of age was used as the criterion for determining the lysine requirement, as shown in figure 3.

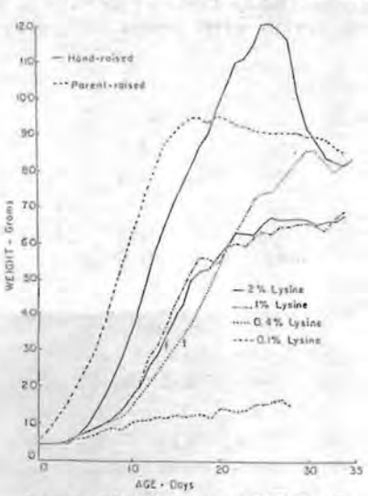

Figure 1. Growth curves of cockatiel chicks fed various levels of lysine in experiment 1. Chicks fed 0.4% lysine from hatching were switched to a 20% protein diet at 16 days of age. Chicks fed 2% lysine were switched to 0.4% lysine at 14 days of age.

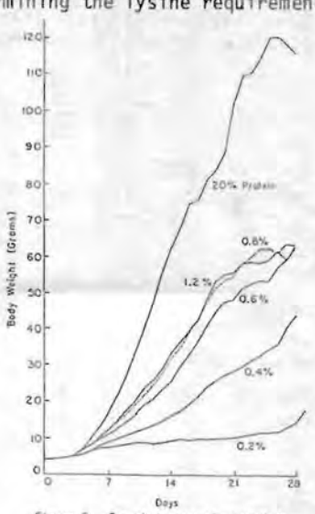

Figure 2. Growth curves of cockatiel chicks fed various levels of lysine in experiment 2.

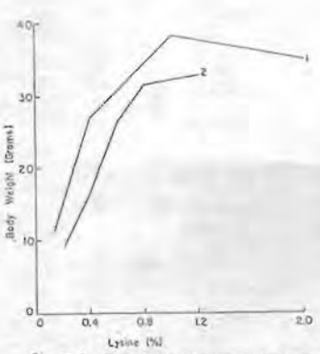

Figure 3. Growth response curves for experiments 1 and 2 based on body weights at 14 days of age.

None of the groups fed the amino acid-containing diets grew as well as those fed the protein-based diet. No observable differences accounted for this effect.

With regard to feather pigmentation, no white or light feathers or parts of feathers were observed in areas that are normally dark. These observations were made in birds in which growth was responding to wide variations in the lysine concentrations of the diets.

Discussion

Until the experiments reported here were performed no data were available on the qualitative or quantitative requirements of cockatiels or other altricial birds for any amino acid. The results obtained with respect to growth effects are similar to those obtained with poultry and rats, with cockatiels apparently unable to synthesize lysine and thus completely dependent on the diet to supply it. After feeding several dietary levels to groups of cockatiels and noting the minimum concentration in the diet that permitted maximum growth, it was concluded that cockatiel chicks require 0.8% lysine in the solids portion of the diet when that diet contains approximately 20% protein. This fairly high requirement is not easily met by seeds alone, thus lysine is a nutrient that is marginal in many diets for breeding cockatiels that are feeding chicks.

The lack of effect of lysine deficiency on feather pigmentation is in marked contrast to that documented in turkeys, chickens and quail, in all of which lysine deficiency results in formation of feathers that lack melanin pigment. In those birds, abnormal white feathers are a diagnostic sign of lysine deficiency. When we began the lysine study we fully expected to see some effect of lysine deficiency in feathers, so the lack of effect was indeed surprising. Because so may different dietary levels of lysine were fed and such wide differences in growth were obtained, we believe it unlikely that any other level would have resulted in white feathers. At the lowest lysine level, feather growth of survivors was poor, but at moderate levels while growth was slow feathers did develop, and all appeared to have normal pigment.

The growth obtained with the amino acid based diet was less than that when soybean protein was fed, but this is not different from observations with other young birds. In order to achieve good growth in chicks, careful balancing by trial and error of the various amino acids was required, and even then the intact protein produced better growth.

Summary

The lysine requirement of cockatiel chicks that were hand-fed from hatching was found to be 0.8% of the dry portion of the diet. This diet, which contained amino acids equivalent to 20% crude protein, was fed at 7% solids for 3 days, then 30% solids to weaning. Poor growth was obtained with low levels of lysine, but feather pigmentation was normal at all lysine levels, and thus the effects of lysine deficiency on the melanin formation in cockatiels appear to differ strikingly from turkeys, quail, and chickens. It is concluded that satisfying the lysine requirement of young cockatiel chicks may be difficult with breeding diets based primarily on seeds.

1. Grau, C.R. and T.E. Roudybush. 1985. Protein requirement of growing cockatiels. Proc. 34th Western Poultry Disease Conference, Davis, CA March 4-6, 1985 in press.

2. Roudybush, T.E. and C. R. Grau. 1983. Solids in diets for hand raising cockatiels. Proc. 32nd Western Poultry Disease Conference, Davis, CA. Feb. 8-10, 1983, pp. 94-95.

IMPROVING REPRODUCTION IN CAPTIVE COCKATIELS VIA ENVIRONMENTAL MANIPULATION

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Introduction

Reproductive potential in hole-nesting birds such as cockatiels is often limited by nest-site availability. Thus, reproductive activity in captive cockatiels can be stimulated or discouraged by presenting or withdrawing nest-boxes. Whether environmental manipulation can be used to augment the reproductive stimulus of the nest-box has been studied by only a few investigators (Woodard and Morrissey, 1980, referred to by Grau and Roudybush, 1984). In diverse species environmental factors such as daylength, light intensity, temperature, humidity and availability of nutrients, whether providing initial predictive cues or essential supplementary information, can influence the progression of the reproductive cycle (Wingfield, 1983). Following a period of adverse weather or inadequate food supplies, increases in these factors tend to accelerate or strengthen the reproductive effort, as measured by date of first nest occupancy, date of first egg, clutch length, and egg size.

To increase the reproductive efficiency of our cockatiel colony we devised a program of environmental changes so the presentation of the nest-box would follow a series of changes, each having the potential of strengthening reproductive effort. Lacking clues as to which factor(s) might stimulate reproductive effort and lacking resources to test each factor separately and with proper controls, we adopted a shot-gun approach and simultaneously altered several parameters. It must be remembered that the results reported below were obtained without satisfactory controls and should be interpreted with appropriate caution.

Materials and Methods

Thirty-six pairs of cockatiels were housed in wire cages (30 x 30 x 60 cm) in a room provided with artificial lighting, heating, and evaporative cooling. In 1982-83 the diet consisted of mixed seed, mineral blocks, and vitamin supplementation in the drinking water. No environmental changes were made. Reproduction was elicited by presenting nest-boxes constructed from stainless steel or plywood. The photoperiod provided a minimum of 14 hr light, but was supplemented by natural daylight through ventilation screens in each end of the room. In 1984 the diet was changed to a crumbled diet (Roudybush et al., 1984) and the room was made light proof during the cooler portions of the year (November through March). The effect of the change in diet on the results reported below is not known.

The program of environmental changes we devised and tested in 1984, Trial #4, is presented in Table 1. Birds were first exposed to a 10-12 week period of time with no minimum temperature control or humidity supplementation and with environmental conditions shown in Table 1, Day 0 ("winter"). Five factors were then changed over a two-week period as indicated.

In 1984, in Trial #5, the same procedure was followed with the exception that during the "winter" one half of the pairs received only millet, while the other half received the conventional crumbled diet.

Results

A summary of the results of five trials -- three trials before environmental manipulation was used and two trials after -- is presented in Table 2. We observed a striking increase in three of the parameters measured after environmental manipulation: (1) percent of all pairs laying increased about two to four times; (2) mean clutch size increased by three to four times ; and 3) the number of eggs laid within 21 days of nest-box presentation increased by three to seven times. Chick weight at hatch also increased by more than 15%. In contrast, latency to first egg, fertility, and hatchability were similar in the five trials. No effect of "winter" diet (millet vs crumbles) was detected in 1984, Trial #5.

Discussion

As these results were obtained without suitable controls, we must consider other factors that might account for the success we observed. First, the flock aged by two years during the course of this work and experience is known to improve reproductive performance. Second, in 1984 each of the pairs in both trials were proven breeders; in 1982-83 the history of numerous pairs was unknown. Third, the diet changed between 1982-83 and 1984. While both diets were considered to be nutritionally adequate, they had not been tested for effects on reproduction. Fourth, these factors may have interacted to produce these results. Breeding in an outdoor aviary at our facility was also substantially improved in 1984 compared to previous years, although the improvement was not as great as that reported above. In addition, 1984 trials at the outdoor aviary took place at a time when environmental conditions were changing from the extremes of winter and summer to milder conditions of spring and fall.

In summary, while these results may have resulted from an interaction of the uncontrolled factors mentioned above, the uniform and dramatic increase observed in two successive trials suggests that environmental manipulation *per se* may substantially improve reproductive performance in captive cockatiels.

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1. Roudybush, T.E., C.R. Grau, T. Jermin and D. Nearenberg. 1984. Pelleted and crumbled diets for cockatiels. Feedstuffs 56:18-20.

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Table 1. Chronology of environmental changes.

Day	Min temp (°C)	Misters[a]	Light[b] Intensity (lux)	Light[b] Photoperiod (L:D)	Diet[c]	Nestbox
0		off	50-250	9:15	millet	down
1	20.6	off	50-250	9:15	millet	down
2	20.6	off	50-250	9:15	millet	down
3	21.7	off	100-500	9:15	millet	down
4	22.8	off	500-120	9:15	millet	down
5	23.9	on	500-1200	10:14	millet	down
6	25.0	on	500-1200	11:13	millet	down
7	26.1	on	500-1200	12:12	crumbles	down
8	26.1	on	500-1200	13:11	crumbles	up
9	26.1	on	500-1200	14:10	crumbles	up
10	26.1	on	500-1200	15:9	crumbles	up
11	26.1	on	500-1200	15:9	crumbles	up
12	26.1	on	500-1200	15:9	crumbles	up
13	26.1	on	500-1200	15:9	crumbles	up
14	26.1	off	500-1200	15:9	crumbles	up

[a] Humidity misters on from 1300-1700 hr daily
[b] Midpoint of photophase at 1330 hr. Illumination from incandescent lamps at intensities of 50-500 lux and from both incandescent and fluorescent lamps from 500-1200 lux. Fluorescent lamps on 1/2 hr later and off 1/2 hr earlier than incandescent lamps.
[c] Roudybush et al. (1984).

Table 2. Egg production in cockatiels before and after environmental manipulation (n = 36 pair/trial).

Date nest-boxes presented	Percent of all pairs laying[a]	Elapsed days to first egg[b]	No. eggs in first sequence[b,c]	No. eggs within 21 days
Before environmental manipulation				
May 8, 1982	42	12.9 ± 1.0	3.6 ± 0.3	45
Aug 5, 1982	33	14.5 ± 1.4	3.1 ± 0.3	32
Sep 21, 1983	19	16.5 ± 0.8	N/A	19
After environmental manipulation				
Feb 21, 1984	92	12.1 ± 0.5	12.5 ± 1.2	158
Oct 3, 1984	94	13.0 ± 0.4	11.5 ± 1.2	147

[a] Within 21 days of nest-box presentation.
[b] Mean ± SE.
[c] Number of eggs laid before first pause of 4 or more days (mean ± SE). Individual hens were still laying without pause of 4 or more days at conclusion of trial.

PARROT REOVIRUS AND PAPOVAVIRUS INFECTIONS AND FEATHER AND BEAK SYNDROME

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Parrot Reovirus Infection. Reoviruses have been isolated from many species of non-domestic birds (3, 10,11,12,15,16,17) but not until 1984 was a reovirus definitively identified as a pathogen in parrots thru fulfillment of Koch's postulates (7).

The incubation period of the experimentally-produced disease in African grey parrots is 5 to 6 days. The clinical illness is brief (6 to 24 hours) and is characterized by lassitude or somnolence, anorexia, mild to severe diarrhea, and agonal regurgitation of crop fluid. The brevity of the clinical disease permits no appreciable weight loss.

Necropsy reveals hepatomegaly. There is a fine multifocal to reticular pattern of yellow-tan regions of necrosis on a darker brown ground color. The spleen is of normal size to enlarged and may contain foci of necrosis and hemorrhage. In many cases the prominent post mortem lesions are petechial, ecchymotic and suffusion hemorrhages in the subcutis, muscle fasciae, epicardium, and various serosal surfaces.

Histologically there are multiple, non-zonal foci of hepatic parenchymal coagulation necrosis; the foci frequently coalesce to form an extensive reticular pattern. Mononuclear cell infiltration is usually evident at the periphery of the necrotic regions. The spleen contains foci of necrosis which may be limited to the perivascular reticulum cell sheaths or widely extend into adjacent lymphoid regions. Enteritis characterized by mucosal hyperemia and mononuclear hypercellularity of the lamina propria occurs to varying degrees of severity.

Intravascular thrombocyte aggregates may be found in small blood vessels and capillaries in many organs but are most frequently and readily observed in lung capillaries. In experimentally-infected parrots there was a decrease in fibrinogen and an increase in fibrin degradation products between preinoculation and agonal blood samples. Thus, the possibility should be considered that disseminated intravascular coagulation and the accompanying consumption coagulopathy may be involved in the pathogenesis of the hemorrhagic lesions commonly observed in this disease.

The gross and microscopic lesions of parrot reovirus infection resemble, qualitatively and quantitatively, those in some cases of acute bacterial septicemia and are thus not pathognomonic of the disease.

The parrot reovirus can be isolated on the chorioallantoic membrane (CAM) of chicken embryos and in chick embryo fibroblast (CEF) and chicken kidney (CK) cell cultures. On the basis of reciprocal immmunofluorescence tests the parrot reovirus appears to bear but slight antigenic similarity to a field isolate (FDO) of fowl reovirus.

In our laboratory rapid diagnosis of parrot reovirus infection is made by the fluorescent antibody test on impression smears of the liver and spleen.

Generalized Parrot Papovavirus Infections (GPPI). Budgerigar fledgling disease has been well-characterized as a generalized papovavirus infection (1,2,5,6). In 1984 there were two reports of "papova-like" virus infections in nestlings of other parrot species (4,9); a "papova-like" virus was observed ultrastructurally but was not isolated.

Since 1982 we have diagnosed GPPI by virus isolation or demonstration of virus in tissue by specific immunofluorescence in domestically bred nestlings of several Austral, Asian, and Neotropical parrot species (7).

Affected nestlings and recent fledglings evidence subacute to acute illness most often characterized by no more than depression and anorexia for a few hours to 24 hours prior to death. Clubb (4) observed reduction in daily weight gain, prolonged crop-emptying time, regurgitation of crop contents, dehydration, and increased bleeding tendencies.

Necropsy reveals: pallor of most tissues; subcutaneous, fascial, and serosal hemorrhages; varying degrees of hepato- and splenomegaly; ascites; and (in a minority of cases) swollen, moist kidneys. The plumage defects which are common in infected budgerigars are rare in nestlings of other species.

Infection of cells with this papovavirus results in pronounced nuclear enlargement (karyomegaly) with vacuolation or, less frequently, with homogeneous basophilic inclusion body formation. The latter is most readily observed in infected squamous epithelial cells in the skin, feather follicles, and esophageal mucosa. Pronounced vesicular karyomegaly of the splenic perivascular reticulum cell sheaths and of endothelial cells in other organs is common. Impression smears of the spleen often reveal the karyomegalic cells and provide a presumptive diagnosis. Extensive multifocal to pan-hepatic liver necrosis is common and in many cases only the bile ducts are spared. Membranous glomerulopathy has been observed in conures, eclectus parrots, and cockatoos and is more pronounced in birds that died as late nestlings or fledgelings rather than as young nestlings.

The virus is readily visualized by electron microscopy in the nuclei of infected cells. In our laboratory rapid definitive diagnosis of the infection is accomplished by the fluorescent antibody test on spleen and liver impression smears. The virus is readily isolated in budgerigar embryo fibroblast cell cultures; primary isolation in CEF cultures or on chicken CAM is less reliable.

Cockatoo Feather and Beak Syndrome. The cockatoo feather and beak syndrome (FBS) has been observed in captive and wild cockatoos (7,8,13,14). The definitive gross and histopathologic description of this disease was reported by Pass and Perry (14).

Feather and beak syndrome is a disease of young cockatoos and possibly of other species. Birds less than 2 years old are at greatest risk; however, occasional birds of up to 4 years of age may be affected.

The disease is manifest in regenerating feathers; clinical onset is thus observed during moult. Most cases are observed in recently imported cockatoos undergoing the post juvenile moult. Some domestically bred cockatoos, however, have manifested the disease during the postnatal moult.

In some birds with FBS overgrowth and/or degeneration of the rhamphotheca and claws is also observed.

The clinical course of the disease is variable in rate of progression. Some birds commence moult, develop lesions of FBS and within 3 to 6 months are essentially naked save, perhaps, for a few primary and secondary feathers on each wing. Other birds evidence slower progression of the disease over periods one to three years. Whether of rapid or slow progression the disease is fatal.

Any and all feather tracts may be affected and assymmetry of tract involvement is the exception rather than the rule. Affected regenerating feathers are invariably stunted to a greater or lesser degree. The feather sheath is thickened (hyperkeratotic) and persists - sometimes completely enclosing the still furled feather within as a hypermature "pinfeather" or opening only at the distal extreme to expose the distal end of the feather. The enclosed, compressed feather is usually malformed by curling or twisting as it is projected into the restricting sheath. Some feathers and their sheaths have one or more annular constrictions and in others, there is hemorrhage in the distal portions of the pulp and inspissated blood between the more distal keratinized pulp caps.

Histologic examination of affected follicles and regenerating feathers reveals various degrees of focal to diffuse degeneration and necrosis of follicle and pterylogenic epithelium. There is dysgenesis of the keratinized regions of the calamus, rachis, barbs or barbules being produced by the affected regions of epithelium. The feather sheaths are, in most regions, clearly hyperkeratotic. Similar degenerative and necrotic changes are observed in the rhamphothecal dermis and deep layers of the epidermis.

In most affected feathers and rhamphothecae there are cells containing - and usually distended and distorted by - deeply basophilic granules and globules which vary markedly in size. Thin sections of plastic embedded feathers were examined with the electron microscope and the basophilic globules were found to contain numerous paracrystalline arrays, chains, and dense whorles of small (16 to 19 nm) virus-like particles.

Although neither experimental transmission of this disease, nor isolation of an infectious agent from its lesions has been accomplished, an infectious etiology is suspected.

In our laboratory we have produced an FITC conjugate (Cornell #85-69) of serum immunoglobulins from an umbrella cockatoo afflicted with feather and beak syndrome. The conjugate has been applied to frozen cross sections of that bird's affected regenerating feathers and demonstrates specific affinity for degenerating and necrotic pterylogenic epithelial cells and for dense globular aggregates in the pulp and epithelium which correspond to the basophilic globules observed by light microscopy. The same conjugate produces specific fluorescence in feathers from other affected cockatoos but not in feathers from a normal cockatoo nor from an Amazon parrot.

Pass and Perry (13) suggest that the same disease which affects cockatoos also affects other species. Such may be the case. A peachfaced lovebird with progressive feather loss of several months duration was submitted to our laboratory. The beak was normal. The changes in regenerating feathers were identical in all regards to those of typically affected cockatoos and electron microscopy revealed random aggregates, whorles, and paracrystalline arrays of small virus-like particles in the basophilic globules. No specific fluorescence could be demonstrated, however, with the cockatoo fluorescent antibody conjugate (#85-69).

It remains to be determined whether the specific immunofluorescence demonstrated by the cockatoo fluorescent antibody conjugate is reflective of an immune response to an infectious agent or of an autoimmune response to an endogenous substance which has been rendered autoantigenic by the disease process.

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AVIAN PARAMYXOVIRUSES

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INTRODUCTION

Newcastle disease virus (NDV) is not only responsible for a devastating disease in most avian species but is particularly important for its grave economic threat to poultry farmers throughout the world. The most virulent form of the disease was reported as a virus infection as early as 1927 (13) and for many years NDV was considered to be the only paramyxovirus normally affecting avian species. However, in 1956 another paramyxovirus, serologically distinct from NDV, was isolated from chickens in Yucaipa, California (10). This virus was shown to be of low virulence for poultry and despite evidence obtained during the 1960s that infections were widespread in North America (11) little importance was attached to the virus.

A third serotype of paramyxovirus was isolated from turkeys in Ontario in 1967 and Wisconsin in 1968 (26).

During the late 1970s a complete change took place in the understanding and significance of avian paramyxoviruses. Considerable interest arose at that time in the presence of influenza A viruses in avian species and a number of large scale surveys were undertaken in different parts of the world to examine the ecology of influenza viruses in birds. Such surveys resulted not only in the isolation of numerous influenza A viruses but paramyxoviruses were found to be present in large numbers. Not all the paramyxovirus isolates fell into the existing serogroups so that, at the present time, the number of recognised serotypes has increased to nine.

CLASSIFICATION

Under the present classification avian paramyxoviruses form part of the Paramyxovirus genus of the Paramyxoviridae family. NDV is the prototype virus of the genus which also includes mumps virus and the mammalian parainfluenza viruses.

Serological differentiation of the avian paramyxovirus isolates by haemagglutination inhibition (HI) tests (1), immunodouble diffusion tests (6, 17), serum neutralization tests (26) and neuraminidase inhibition tests (16) and comparison of other properties such as structural polypeptides (2) have indicated nine distinct serotypes to date.

It has been generally accepted that the serotypes should be labelled PMV-1 to PMV-9 and, in addition, the individual isolates have been named using the rules recommended for influenza A viruses (27). A new isolate is therefore named:- 1) serotype; 2) species or type of birds from which it was isolated; 3) geographical location of isolation - country or state; 4) reference number or reference name, if any; 5) year of isolation. For each serotype general usage or nomination has resulted in certain viruses being regarded as prototype strains, these are listed in Table 1 using the recommended nomenclature.

TABLE 1

AVIAN PARAMYXOVIRUS ISOLATES REGARDED AS PROTOTYPE

PMV-1	Newcastle disease virus
PMV-2	chicken/California/Yucaipa/56
PMV-3	i) turkey/Wisoncsin/68 ii) parakeet/Netherlands/449/75
PMV-4	duck/Hong Kong/D3/75
PMV-5	budgerigar/Japan/Kunitachi/75
PMV-6	duck/Hong Kong/199/77
PMV-7	dove/Tennessee/4/75
PMV-8	goose/Delaware/1053/76
PMV-9	duck/New York/22/78

Variation within serogroups

Isolates forming serotypes PMV-4, PMV-5, PMV-6, PMV-8 and PMV-9 have shown no major variation

in serological tests, although in some of these groups very few isolations have been made.

The PMV-1 serotype, from which most isolates have been made, has also been considered to form a serologically homogeneous group, based on HI tests. Classical NDV isolates have shown some variation in more stringent serological tests (22) but these have been considered to be minor variations of little practical importance. Recently, Russell and Alexander (21) prepared mouse monoclonal antibodies against NDV strain Ulster 2C and examined their ability to bind to MDBK cells infected with isolates of NDV. A total of 40 isolates was examined and these could be placed into eight distinct groups of isolates binding the same monoclonal antibodies. Viruses placed in the same group shared both biological and epidemiological properties. It was further shown that isolates of the virus responsible for disease in racing pigeons which spread across Europe in 1981-1983 were identical in their monoclonal antibody binding pattern and formed a ninth distinct group (7). The racing pigeon isolates and four other isolates from wild ducks, although showing different binding patterns, were the only viruses tested that did not bind monoclonal antibodies directed to the haemagglutinin antigen. This enabled a simple HI test with one of the monoclonal antibodies to be employed to distinguish between pigeon PMV-1 isolates and classical NDV.

Considerable variation in the PMV-2 serogroup may be detected by HI tests and two viruses may show quite wide serological differences. However it frequently occurs that both these viruses may be closely related to a third virus. This may be an indication that such variants have arisen by gradual selection. Other isolates have shown asymmetrical relationships with different PMV-2 isolates. Other tests, such as immunodouble diffusion, have tended to confirm the relatedness of putative PMV-2 isolates.

PMV-3 viruses isolated in North America were indistinguishable by several serological tests (26). However, isolates from psittacines have tended to show much closer relationships with each other than with the turkey isolates. Isolates from British turkeys made in 1981 and 1982 were demonstrably more similar to North American turkey isolates than psittacine isolates (3).

Relationships between serotypes

No attempt has been made to produce a definition of what constitutes sufficient antigenic variation to warrant classification of a new avian paramyxovirus serotype. Generally the serotypes have been formed as the result of obvious grouping of isolates in serological tests. Such grouping has usually been done by HI tests, but where other serological tests have been applied these have confirmed the current groupings. However some minor cross-relationships have been reported between viruses from different serotypes. Generally such relationships have been far more apparent in HI tests than other criteria used for classification. To this extent very minor cross-relationships have been reported between PMV-1 and PMV-4 isolates, PMV-2 and PMV-6, PMV-1 and PMV-8, PMV-1 and PMV-9, PMV-4 and PMV-8 and PMV-3 and PMV-9. However the most marked cross-relationship has been seen between PMV-1 and PMV-3 viruses.

Several studies with both turkey and psittacine PMV-3 isolates have shown low-level cross reactions with PMV-1 viruses in HI and serum neutralization tests (1). But more importantly, Alexander et al (4) showed that chickens infected with PMV-3 viruses were, to some extent, protected against challenge with virulent NDV and that survivors showed a rise in HI titres to both viruses. This relationship has been further confirmed by serological results obtained from field infections of British turkeys with PMV-3 virus during 1981-1983. PMV-3 infections in unvaccinated turkeys resulted in high PMV-3 HI titres but no positive NDV titres were recorded. In birds that had been vaccinated against NDV prior to infection with PMV-3 a slight depression of the PMV-3 HI titres was noted and NDV HI titres were boosted considerably in direct correlation to the PMV-3 titres (7).

The cross-relationship between PMV-1 and PMV-3 viruses has, on some occasions, given rise to difficulties in typing isolates but generally the use of adequate control sera and viruses has minimised this problem.

HOSTS AND DISTRIBUTION

a) Captive caged birds

The countries reporting isolations of avian paramyxoviruses, other than NDV, from captive caged birds and the types of birds involved are summarised in Table 2. Apart from the PMV-5 viruses which caused an epidemic of severe disease among budgerigars in Japan during 1974-1976 (20) and the isolation of a PMV-7 virus from a pigeon held in quarantine in England virus isolations from captive caged birds have been from only three serotypes: PMV-1, PMV-2 and PMV-3.

Isolations of paramyxoviruses from birds dying in quarantine in Great Britain have been investigated in detail since 1976 and are probably representative of the situation seen in most countries importing large quantities of caged birds. Two conclusions could be drawn from the results: 1) PMV-2 viruses usually infect passerines and have been only isolates from psittacines that have been in contact with passerines. PMV-3 viruses have been isolated much more frequently from psittacines than from passerines. Similar results have been reported for the USA (23).

2. PMV-2 viruses appear to be endemic in the indigenous birds trapped in some countries for export as caged birds. For example many of the isolates made in Great Britain have been from birds origi-

nating in Senegal where surveys have shown PMV-2 viruses to be present in birds trapped for export.

TABLE 2

Avian paramyxovirus in captive caged birds

Serotype	Country	Type of bird
PMV-2	Japan	imported passerines and psittacines
	N. Ireland	imported finches
	England	imported passerines and psittacines
	USA	imported passerines and psittacines
	Senegal	indigenous caged passerines
	Indonesia	indigenous caged passerines and psittacines
	Kenya	caged passerines
PMV-3	USA	imported psittacines and passerines
	Netherlands	Zoo psittacines
	England	imported psittacines and passerines
	Federal Republic of Germany	imported psittacines and passerines
	Japan	imported finch
PMV-5	Japan	budgerigars (Melopsittacus undulatus)
PMV-4) PMV-6) PMV-7*) PMV-8) PMV-9)	No known isolations from captive caged birds	

* A PMV-7 isolate was obtained from a pigeon dying in quarantine in England

b) Wild birds

The isolation of viruses from free-flying birds clearly depends on whether or not such birds are captured and sampled. In some countries extensive surveillance schemes have been undertake, primarily concerned with the ecology of influenza viruses but which have led to the isolation of numerous paramyxoviruses. In many cases such surveys have been restricted to specific types of birds, such as waterfowl and this must be borne in mind when considering the available data on the host and geographical distributions of avian paramyxoviruses summarised in Table 3.

There have been no reports of isolations of viruses of PMV-3, PMV-5 or PMV-9 serotypes from feral birds.

PMV-2 viruses have widespread geographical distribution amonst passerines. Only in Israel have other wild bird species been shown to be infected and this may reflect spread from domestic turkeys during the severe epidemic that occurred there (19).

PMV-4 and PMV-6 viruses have been isolated exclusively from waterfowl and the worldwide spread of these viruses probably reflects the migratory nature of such birds.

PMV-7 viruses, and those placed provisionally in this serotype, have been isolated only from members of the Columbidae family in USA, England and Japan.

There have been only two reports of isolations of PMV-8 viruses, from a goose in Delaware, USA and ducks in Japan.

c) Domestic poultry

PMV-4, PMV-6 and PMV-9 viruses have been isolated from domestic ducks and geese probably due to infections resulting from spread from wild waterfowl. There has also been a single report of an isolate of PMV-6 subtype from turkeys in Canada. Apart from these isolations widespread infections of domestic poultry with avian paramyxoviruses other than NDV have been restricted to viruses of PMV-2 and PMV-3 serotypes (Table 4).

The first isolation of a PMV-2 virus was from chickens in California, USA but later work showed the virus to be more prevalent in turkeys (11). More recent evidence suggests that PMV-2 viruses may be widespread and endemic in turkeys in the USA. Viruses of the PMV-2 subtype have also been isolated from turkeys in Canada, Italy, France and Israel in recent years. In Israel numerous PMV-2 virus isolations have been made from turkeys experiencing a serious disease epidemic.

PMV-3 viruses were first shown to be present in turkeys in Canada in 1967 and USA 1968 (26) and there is evidence that this virus is still causing problems in turkeys in the USA. In 1981 a PMV-3

TABLE 3

Isolations of avian paramyxoviruses from feral birds

Serotype	Country	Type of bird
PMV-2	German Democratic Republic	passerines (various species)
	Senegal	indigenous captive birds (passerines)
	Indonesia	indigenous captive birds (passerines and psittacines)
	Czechoslovakia	Wrens (Troglodytes troglodytes)
	Israel	Cattle egret (Anas platyrhyncos) Coot (Fulica atra)
	Japan	passerines (Emberiza spodocephala)
	India	resident and migratory birds
PMV-4	USA	ducks (various species)
	Czechoslovakia	coots, mallards, pheasants (Phasianus colchicus)
	Federal Republic of Germany	coots and mallards
	Japan	ducks (various species)
	England	imported, semi-wild duck (Calonetta leucophrys)
	New Zealand	Mallard
PMV-6	Canada	ducks (various species)
	Federal Republic of Germany	ducks (various species)
	Japan	ducks (various species)
	Czechoslovakia	ducks (various species)
PMV-7	USA	doves (Zenaidura macroura)
	England*	doves (Streptopelia decaocto)
	Japan*	pigeon (Columba livia)
PMV-8	USA	goose (Branta canadensis)
	Japan	duck (Anas acuta)
PMV-3) PMV-5) PMV-9)	No known isolations from feral birds	

*Viruses isolated in England and Japan have been only provisionally placed in the PMV-7 group

virus was isolated from turkeys in Great Britain and more recently viruses of this subtype have been reported in turkeys in France (9).

TABLE 4

Isolations of avian paramyxoviruses from domestic poultry

Serotype	Country	Type of bird
PMV-2	USA	turkeys, chickens
	Canada	turkeys, chickens
	USSR	chickens
	Japan	chickens
	Italy	turkeys
	Israel	turkeys, chickens
	India	"domestic birds"
	France	turkeys
PMV-3	Canada	turkeys
	USA	turkeys
	England	turkeys
	France	turkeys
PMV-4	Hong Kong	ducks, geese, chickens
PMV-6	Hong Kong	ducks, geese
	Canada	turkeys
PMV-9	USA	ducks
PMV-5) PMV-7) PMV-8)	No known isolations from domestic poultry	

DISEASE

a) Disease in wild birds

Apart from the isolation of viruses from birds found dead there is little evidence of any associated disease with avian paramyxoviruses isolated from wild birds. Most isolations from this source have come from trapped healthy birds or hunter-killed birds.

b) Caged birds

Most of the avian paramyxoviruses isolated from caged birds have been from those held in quarantine, generally as a result of sampling all the birds or those that die. As a result there have been few reports of specific clinical disease signs associated with such isolations.

PMV-3 virus infections have been related to a disease epidemic occurring in psittacines in aviaries in the Netherlands (25). The disease seen was one of nervous signs similar to the neurological form of NDV and could be reproduced experimentally in some psittacine species. PMV-3 virus was also isolated from peach-faced love birds (Agapornis roseicollis) dying in quarantine in the USA after signs of general lethargy. Post-mortem examination of these birds showed swollen spleens and kidneys (15). A major epidemic restricted to budgerigars was seen in Japan in 1974-1976 which was characterized by inappetence, diarrhoea, ruffled feathers and mortality up to 100%. The virus isolated was typed as PMV-5 and in experimentally infected budgerigars depression, dyspnoea, diarrhoea, torticollis and death were seen (20).

c) Poultry

Apart from NDV the vast majority of reports of avian paramyxovirus infections of domestic poultry have related to PMV-2 or PMV-3 viruses (Table 4). Exceptions have been the reports of isolates of PMV-4 and PMV-6 viruses from symptomless domestic chickens, geese and ducks in Hong Kong (24) and PMV-9 from a domestic duck in the USA. These are the only reports of avian paramyxoviruses other than NDV from domestic waterfowl. There has also been a single report of a PMV-6 virus infecting turkeys in Canada with associated clinical signs of slightly elevated mortality and respiratory disease.

The first PMV-2 virus isolate to be reported was from chickens in the USA from birds also infected with infectious laryngotracheitis virus (10). PMV-2 infections of chickens associated with respiratory disease and air sacculitis have been reported from Japan and USSR. However, turkeys appear to be the most susceptible and worst affected hosts for PMV-2 viruses. In the USA, the presence of PMV-2 antibodies have been frequently reported as present in turkey flocks that had suffered from respiratory distress or egg production problems, but no conclusive evidence was obtained that PMV-2 viruses were solely responsible for the disease signs (12). Similar results were obtained in Italy where a virus of PMV-2 serotype was isolated from turkeys showing respiratory signs (14). Lang et al (18) reported severe disease problems associated with PMV-2 infections of turkeys in Canada consisting of:- severe respiratory disease, sinusitis, low egg production, enteritis and elevated mortality. However on each occasion other organisms were also isolated and it was concluded that PMV-2 viruses were responsible for extreme exacerbation of the disease resulting in a more severe and chronic illness. Probably the most severe series of disease outbreaks with which PMV-2 viruses have been associated occurred during the epizootic of turkeys and chickens in Israel in 1978-1980. The disease in turkeys was characterized by clinical signs varying from mild rales to severe respiratory disease with sinusitis, conjunctivitis and pneumonia, low egg production was also seen in breeder birds. Morbidity was normally close to 100% but mortality varied from 5-90%. The disease in chickens was similar but less severe. PMV-2 virus isolations of serological evidence of PMV-2 viruses were seen on affected farms but Pasteurella multocida, Mycoplasma gallisepticum and Alkaligenes faecalis were also frequently isolated (19). PMV-2 viruses have also been implicated in outbreaks of turkey rhinotracheitis in France where Chlamydia infections may have had an exacerbative role (9).

The first isolation of PMV-3 viruses were from turkeys in North America showing respiratory disease, general depression and a 60% drop in egg production (26). In more recent years PMV-3 infections in turkeys in the USA have been associated with egg production problems. PMV-3 infections of turkeys have been seen in turkeys in Great Britain since 1981. A limited serological survey indicated that such infections were present in breeder turkeys in Great Britain. Infections have been mainly associated with falls in egg production and a high level of white-shelled eggs, hatchability and fertility have been only rarely affected (7). PMV-3 viruses were also isolated from turkey flocks in France showing egg production and quality problems (9).

Experimental infections of chickens and turkeys with PMV-2 and PMV-3 viruses isolated from domestic poultry have failed to produce any disease signs under laboratory conditions. This finding and the considerable variation seen in disease signs associated with field infections of poultry with avian paramyxoviruses confirm the necessity of other organisms working synergistically to produce serious disease. As a consequence a complete spectrum of disease signs ranging from minor respiratory or egg production problems to devastatingly high mortality have been associated with avian paramyxovirus infections. The data available on field disease was indicated that the presence of such organisms as: Pasteurella, E.coli, Alkaligenes faecalis, Mycoplasma, Chlamydia, Aspergillus, Coccidia and a large number of viruses including other paramyxoviruses and influenza viruses may produce a

synergistic effect with avian paramyxoviruses resulting in a much more severe disease. Live vaccination may also be an important consideration as could a compromised or deficient host immune system. Physiological conditions, such as egg production or other stresses to the birds during infection such as overcrowding, extreme heat or cold or large fluctuations in temperature may all combine to produce more severe disease.

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GENETIC STRATEGY OF NEWCASTLE DISEASE VIRUS

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Newcastle disease virus appears to exist in vertebrate hosts as a cluster of genetically distinctive and mutually tolerant subpopulations. Forty isolates recovered from chickens and associated birds during the 1972 epizootic of Newcastle disease in California contained two or more subpopulations that were distinguishable if one hundred plaques of each isolate was examined. Similar diversity has been observed in strains isolated before 1972 (2,9) and has been found in those isolated since (5). Each cluster probably arises by mutation from a stem virus that may or may not continue to persist. The number and size of these subpopulations remains relatively unchanged (7) as the virus moves from host to host as long as the means of transfer and the species of host remains unchanged. However, when a new species of host is encountered or a new means of transfer occurs, changes do take place in the relative size of the subpopulations and Frank Sterner, one of my students, has found that one or more of the subpopulations may disappear and new ones may arise. The existence of genetically heterogenous populations of pathogenic viruses in infected individuals and the transfer of these population complexes to susceptible hosts has epidemiological and evolutionary significance.

In order to evaluate the probability that natural infections are initiated by transfer of genetically distinctive virions representative of all or most members of a strain cluster, one needs to examine the circumstances of aerosol transmission (10). Within 48 hours after infection, the air surrounding the infected bird is being continually charged with infective virus and as the epizootic develops an infective cloud envelopes the entire flock. If measured samples of the air are drawn into a container in which the virus can be trapped, the number of virions per liter of air can be counted. Such counts have shown that air of houses with infected birds contains enough virus so that each and every bird breathing the air captures several virions in its respiratory tract every minute. Potentially, a susceptible bird could receive thousands of virions.

Many virions are trapped on nasal hairs and in mucus of the upper respiratory tract and removed. Others are blocked or inactivated by natural inhibitors. One hundred times more virus of one clone of a Newcastle disease vaccine virus, for example, was neutralized by a normal nasal inhibitor than was true of another clone obtained from the same vaccine virus (4). The probability of successful infection of a new host is different for each genotype in a cluster.

For a period of time following the initiation of infection in a cell by the first arriving virion, newly arriving virions will be able to find and infect cells. This has been demonstrated by investigators who have shown that the course of disease in chickens exposed for a few hours to an aerosol of lethal virus can be altered if the birds are then exposed to a avirulent virus. Because of the relatively long period of susceptivity (6-12 hours), it is possible for a cluster of distinct subpopulations to be transmitted from host to host with little loss of genetic diversity.

Most subpopulations in a cluster exist at ratios to each other that range from 1:1 to 1:50 and perhaps some subpopulations are present at even greater ratios. One or two subpopulations usually dominate the cluster and when there are more than four subpopulations, the remainder usually are small minority. Among velogenic strains, clusters of subpopulations represented by 4 to 7 distinguishable plaque types are not uncommon (2). Less virulent strains have fewer subpopulations. However, genetically distinct subpopulations exist that cannot be distinguished by plaque markers and it is possible to isolate them in a state of considerable purity. A minor thermostable subpopulation of about 1,000 virions in a population of 1,000,000,000 can be selected by subjecting the culture several times to heat shock (3). Some investigators would explain this selection as evidence of an induced mutation. However, if a strain of Newcastle disease virus of low pathogenicity is subjected to several cycles of adsorption on brain cells, a process that does not induce mutation, a nonbinding line that is apathogenic for chickens and the cell-binding line that is highly pathogenic is obtained in 3 or 4 passages (8). This is easily explained if one assumes a small prexisting population of a neurotropic mutant.

One of my students, Frank Sterner, who derives clones by a procedure that minimizes clumping of virions, does not find the high rate of mutation reported by many investigators. Using this method, he does not find the higher mutation rate for red plague clones as compared to clear plague clones which we had previously observed. Consequently, we suspect that other investigators are selecting pre-existing mutants from cultures that are not the homeogenous clones they thought them to be.

Tolerance appears to be essential for the persistence of the subpopulations in a cluster. Attempts to assemble synthetic clusters using clones obtained from unrelated strains have failed within a few passages. The lack of tolerance among NDV strains is readily demonstrated by the exposure of a monolayer of chicken embryo fibroblasts to a virulent plaquing strain following a prior infection with an avirulent non-plaquing strain (1,6). Striking differences were observed

between the ability of a particular avirulent strain to tolerate or to reduce the plaque titer of a particular virulent strain.

Some interdependence may also exist as an occasional clone, representative of a subpopulation of a strain that was isolated from a diseased chicken and which is readily passaged in cell culture and chicken embryos, appears unable to initiate a productive infection in chickens when introduced alone. Possibly such clones are dependent upon sister clones for some essential product.

During an epizootic, the subpopulations in a cluster appear to be transferred from host to host and this transfer increases the chance that virus will be able to persist in partially immune host populations, or if the virus infects an alternate host species such as the pigeon, it will give rise to a successful communicable disease. Viruses that can bring along some of the mutants that have arisen during replication when they infect a new host have a greater chance of surviving rapid changes in the epidemic climate and consequently have an evolutionary advantage.

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Avian Paramyxovirus Type 1 Virus (Newcastle Disease) in Pigeons in the United States: A Preliminary Report on the Characterization of the Virus

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In early April 1984, a "new disease" was recognized in pigeons in the eastern United States. The disease is clinically similar to a condition which has been described in pigeons throughout most of Europe since 1981. Clinical signs include ruffled feathers, torticollis, loss of balance, walking in circles, unwillingness and inability to fly, and moderate to high mortality. The disease spreads slowly; consequently, clinical disease may be observed within a pigeon loft for several weeks.

The clinical disease was first reported in racing pigeons as early as April 1984, in the Brooklyn and Queens boroughs of New York City. Owners of affected lofts were initially reluctant to acknowledge the presence of the disease syndrome, and several weeks elapsed before specimens were submitted for diagnosis. The first submission to the National Veterinary Services Laboratories (NVSL) was received on May 17, 1984, and consisted of six dead pigeons. Avian paramyxovirus type 1 (PMV-1) virus was isolated from five of the pigeons. The isolates were antigenically similar, but not identical, to Newcastle disease virus (NDV) as determined by hemagglutination-inhibition tests. Isolates of PMV-1 were confirmed by reisolation from the original specimens. Based on the mean death times of the isolates in embryonating chicken eggs and cloacal inoculation of susceptible six- to eight-week-old chickens, the virus was characterized as a lentogenic strain of avian PMV-1. Chlamydia psittaci was also isolated from one of the five pigeons from which PMV-1 was isolated. Mycoplasma columbinum and Mycoplasma columborale were isolated from the respiratory tissues of several others.

From May 1984 to January 1985, PMV-1 was isolated at the NVSL from 22 laboratory submissions from New York, New Jersey, Vermont, and Maryland. Twenty-one of the isolations were submissions from privately owned racing pigeons; however, one of the isolations was from a feral pigeon.

Pathogenicity studies were initiated due to the antigenic differences between the pigeon PMV-1 and prototype strains of NDV and the inability of pigeon PMV-1 to cause clinical disease in susceptible chickens. Adult pigeons negative for antibody to NDV were inoculated intramuscularly (IM) or intravenously (IV) with 0.2 ml of a 10^{-1} dilution of the isolates. The intravenous pathogenicity index (IVPI) was determined using 8 six-week-old White Leghorn chickens for each isolate. Daily observations over a 10-day period were made. Each bird was scored as normal (0), sick (1), paralysed (2), or dead (3) and the mean score for each isolate was calculated (Table 1). If all chickens would have died within 24 hours post inoculation, a maximum IVPI score of 3 would have been assigned. If all chickens had remained normal through the observation period, a score of 0 would have been assigned. The intracranial pathogenicity index (ICPI) was similarly determined using 10 one-day-old chicks with the exception that an observation period of eight days was used. Each day the birds were scored as normal (0), sick (1) or dead (2). If all chicks died within 24 hours post inoculation, a maximum ICPI score of 2 would have been assigned. If all chicks remained normal during the observation period, a score of 0 would have been assigned (Table 1). Of the 20 pigeon PMV-1 isolates evaluated, an average of 63% of the inoculated pigeons died. Mortality rates in pigeons ranged from 25 to 100% (Table 1). The mean time of death for inoculated pigeons was 9.5 days, with a range of 4 to 25 days.

The duration of virus shedding by pigeons was determined for 3 pigeon isolates from the United States (U.S.), 1 pigeon isolate from the United Kingdom (U.K.) (kindly provided by Dr. D. J. Alexander, Weybridge, Surrey) and 2 chicken lentogenic ND viruses (Table 2). Pigeons inoculated with the 3 U.S. pigeon isolates consistently shed virus thru 10 days post inoculation (dpi), and 1 isolate was shed for up to 17 days. Pigeons inoculated with the isolate from the U.K. shed virus for 20 dpi. There was only one isolation on day 10 from one of the pigeons inoculated with the two chicken NDV isolates. Pigeons inoculated with the chicken NDV isolates failed to seroconvert to NDV, whereas all of the pigeons seroconverted following inoculation with the U.S. and U.K. pigeon viruses.

A limited experiment was performed to evaluate contact transmission of pigeon PMV-1 from pigeons to chickens. Four chickens were placed in direct contact with four inoculated pigeons (case no. 84-46798). All birds were swabbed twice weekly for 42 days. One of the four chickens died on 2 dpi of an undetermined cause. Two of the three remaining chickens began shedding virus at 13 days post contact. One of the chickens consistently shed virus from day 13 thru day 34. Virus was isolated from the other chicken only at 13 dpi. One of the three chickens did not shed detectable virus at any time during the 42 day period. Although virus was isolated from only 2 of the 3 chickens, all three were positive for antibody to NDV at 42 dpi and remained clinically normal.

In summary, clinical disease was not observed in chickens inoculated with pigeon PMV-1 by the cloacal, intranasal, and caudal-thoracic-air-sac routes; however, severe disease and death was observed when chickens were inoculated by the IV route. The pigeon PMV-1 viruses isolated from the U.S. appear to be antigenically and biologically similar to each other and to at least one of the U.K. isolates tested. Additional comparisons of U.S. and U.K. pigeon PMV-1 viruses using monoclonal antibodies are pending. The threat posed by pigeon PMV-1 viruses to the poultry industry is difficult to evaluate; however, it seems unlikely that this virus would cause a problem in chickens that are immunized against NDV. The obvious approach to control of the disease is through an organized and effective vaccination program for racing and show pigeons.

Table 1. Pathogenicity of pigeon PMV-1 isolates

Isolate	Pigeons # Dead/# Inoc.	Chickens (Cloacal) # Dead/# Inoc.	IVPIa	ICPIb	MDTc
84-42949	5/8	0/4	0.0	1.24	>100
84-44407	8/8	0/4	1.33	1.36	>100
84-46798	3/4	0/4	1.05	--	>100
84-81385*	3/8	0/4	0.87	1.45	>100
85-06044	4/5	0/4	0.0	--	>100
85-08308	5/5	0/4	0.18	--	98
85-08727	5/5	0/4	0.42	--	>100
LaSota (NDV)	0/5	0/4	--	0.21	>100
84-44404**	0/8	0/4	0.05	--	>100

* United Kingdom isolate
** Illegal Belgian NDV vaccine used to vaccinate pigeons in New York
a Intravenous pathogenicity index, six-week-old Leghorn chickens
b Intracranial pathogenicity index, day-old Leghorn chickens
c Mean death time hours, embryonating chicken eggs

Table 2. Pigeon PMV-1 virus-shedding in pigeons as determined by cloacal swabbing

Isolate	Days Post Exposure (# swabs pos/# swabs taken) 4	7	10	13	17	20	24
84-42949	6/8	5/5	4/5	2/5	2/5	0/4	0/4
84-44407	7/8	5/5	2/2	-	-	-	-
84-46798	2/4	3/3	1/2	0/1	0/1	0/1	0/1
84-81385*	8/8	8/8	6/6	2/5	3/5	1/5	0/5
LaSota (NDV)	0/4	0/4	0/4	0/4	0/4	0/4	0/4
84-44404**	0/8	0/8	1/8	0/8	0/8	0/8	0/8

* United Kingdom isolate
** Illegal Belgian NDV vaccine used to vaccinate pigeons in New York

AVIAN PARAMYXOVIRUS TYPE 1 (NDV) INFECTIONS IN PIGEONS AND POULTRY

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INTRODUCTION

The object of this paper is to review the events that occurred in Great Britain during 1983-1984 when a variant avian paramyxovirus type 1 (A/PMV-1) distinguishable from more classical Newcastle disease virus (NDV) strains was responsible for epizootics in pigeons and fowls.

Pigeons (Columba livia) and other members of the Columbidae family are well known to be susceptible to infection by paramyxoviruses of the A/PMV-1 serotype (which includes NDV strains). Usually reports of outbreaks of disease amongst pigeons, particularly domesticated pigeons, have appeared during widespread epizootics of NDV in domestic poultry and it has been assumed that the pigeons were affected due to contact with diseased poultry (9, 10, 12, 14). Pigeons associated with such epizootics have usually shown clinical signs closely resembling the disease in poultry. Non-epizootic lentogenic A/PMV-1 viruses have also been isolated from pigeons with mild respiratory disease (16).

A disease of racing and show pigeons, resembling the neurotropic form of ND in chickens, was first reported in Europe in 1981 (15) and spread rapidly through the racing pigeon population across the continent during 1981-1983 (6).

CLINICAL SIGNS IN PIGEONS

The clinical signs seen in pigeons closely resembled the neurotropic form of ND in chickens (7) although respiratory signs were usually absent. At the onset of disease owners often reported general loss of condition, anorexia and excessive drinking. This was usually followed by profuse watery diarrhoea, frequently green in colour. Very occasionally no further disease signs were seen, but more usually nervous signs consisting of torticollis, drooping of wings, leg paralysis, difficulties in alighting, tremors, inco-ordination, abnormal flying and inability to peck grain were seen in various combinations and degrees of severity. The nervous signs usually persisted for a considerable period, in the more severe cases resulting in complete paralysis and death. More often the affected birds were culled by the owners. There were also some reports of gradual improvement and return to normal in less severely affected birds. The disease spread slowly among the birds in a loft and estimations of morbidity and mortality were difficult to make. In the more severely affected lofts up to 80% of the birds showed disease signs.

CHARACTERIZATION OF VIRUS ISOLATES

a) Serological differentiation

The vaccination and diagnostic procedures used for NDV are based on the concept of antigenic homology in the A/PMV-1 group. Although it has long been known that stringent serological tests involving neutralization did indicate some variation the concept of homology has been supported by haemagglutination inhibition (HI) tests.

Russell and Alexander (11) prepared mouse monoclonal antibodies to NDV strain Ulster 2C. The nine monoclonal antibodies prepared recognised seven distinct epitopes on the virus involving the HN (two), F, NP and P polypeptides. The ability of these monoclonal antibodies to bind to MDBK cells infected with 40 different strains of NDV was assesed and these viruses could be placed into eight distinct groups on their ability to cause binding of the same monoclonals. Viruses placed in the same group tended to share epidemiological and biological characteristics. Conventional serology had indicated that the A/PMV-1 isolates from pigeons represented a variant A/PMV-1 strain but monoclonal antibody binding tests were able to show that these viruses were distinguishable from all other viruses tested forming a ninth group (3). In addition, the pigeon isolates and four isolates from ducks were the only viruses which did not bind the monoclonal antibodies directed against the haemagglutination antigen. The failure of these monoclonal antibodies to inhibit haemagglutination by the pigeon viruses (Table 1) enabled a rapid and simple HI test to be employed to distinguish between pigeon A/PMV-1 and classical NDV isolates (3, 6).

In an international collaborative study 53 viruses isolated from pigeons from 15 countries, 12 European, Israel, Japan and Sudan, were examined in monoclonal antibody binding tests and shown to be indistinguishable (4). This study confirms the spread of the disease in pigeons which can now be considered to have reached panzootic proportions.

b) Pathogenicity indices

Fifty-one isolates made from pigeons in Great Britain during 1983, 39 in 1984 and 53 European isolates were tested in intracerebral pathogenicity index (ICPI) tests in day-old chicks and intravenous pathogenicity index (IVPI) tests in six-week-old chickens. The 1983 British isolates showed

ICPI values ranging from 1.15 to 1.68 mean 1.47 and IVPI values of 0.00 - 1.81 mean 0.49 (5). The European isolates showed ICPI values of 1.06 - 1.79 mean 1.44 and IVPI values of 0.00 - 2.44 (6). These results are summarized in Table 1.

Alexander and Parsons (1) made a detailed study of the pathogenicity of A/PMV-1 viruses from pigeons. They showed that isolates with low IVPI values in chickens gave much higher indices in intravenously infected adult pigeons. Virulence for chickens was increased by passage through chickens and regardless of initial IVPI value indices of 2 or more were recorded for all isolates tested after 3 or 4 passages. Passage through chickens also resulted in some increased in the pathogenicity for pigeons. Alexander et al (6) suggested that the marked variation in the IVPI values on initial testing may be due to some selection pressure at isolation, such as more than one passaged through chick embryo cells which may result from inoculating only small amounts of virus in the primary samples.

The results obtained with pigeon isolates would suggest that the ICPI test was initially the best measure of potential pathogenicity for chickens.

DISEASE OUTBREAKS IN BRITISH RACING PIGEONS

The spread of disease in racing pigeons in continental Europe was first noted in Great Britain in early 1983 but despite a ban on races from the continent, in March 1983, the disease was confirmed in a pigeon loft in Cornwall in early July. Subsequently 192 lofts were confirmed as infected with the disease during July-December 1983. The close season for racing and showing pigeons is October to March in Britain and up to the end of June 1984 only 84 outbreaks were confirmed. However from July to December 1984 a further 774 lofts were confirmed as having the disease, giving a total of 858 for 1984.

Tracing of the outbreaks in pigeons during 1983 and 1984 indicated that the overwhelming majority resulted from contact with infected birds during transportation to and holding at release points for races. Some outbreaks could be traced to training flights, visits to and from infected lofts, purchase of infected birds, introduction of infected stray birds into a loft and, in two cases, probable contact with infected feral birds.

INFECTIONS OF CHICKENS IN GREAT BRITAIN

In September 1981 a "stamping out" policy for Newcastle disease was adopted in Great Britain and vaccination made illegal. By early 1984 the national poultry flock could be considered fully susceptible to NDV although no outbreaks had been confirmed during the period since the cessation of vaccination.

In February 1984 birds in several houses on a multi-house site of about 200,000 egg-laying hens were confirmed as infected with NDV and all birds on the site slaughtered. The affected birds had shown an egg production drop of 83% to 23%, an evlevated mortality and nervous signs were seen in some birds. Use of monoclonal antibodies showed the virus from this outbreak to be indistinguishable from the A/PMV-1 viruses isolated from racing pigeons (Table 1). Contact with racing pigeons seemed most unlikely and there was no evidence to suggest direct contact with infected feral birds had occurred. However, it was known that the A/PMV-1 virus was at that time affecting the feral pigeon population, estimated as 30,000, in the Liverpool dock area. Subsequent investigations showed that food from stores at Liverpool and closely situated docks, had been fed untreated to the affected hens and that these stores were demonstrably infested with diseased pigeons. Virus was isolated from carcases of pigeons found dead amongst the food and from samples of food itself.

Between the end of February and July 1984 23 outbreaks of NDV were confirmed in fowls in Great Britain and, with one exception, viruses isolated from these outbreaks were shown to have identical monoclonal antibody binding patterns to the racing pigeon A/PMV-1 isolates (4). Of the 22 outbreaks due to pigeon-associated virus 19 were in broiler breeder or egg-laying hens and three in broiler chickens. Epizootiological tracing showed that birds in 14 outbreaks had direct of indirect contact with food from the infested food stores at Liverpool and Birkenhead docks. Five outbreaks had very close direct contact with other infected premises. Two of these were broiler flocks that had received chicks hatched from eggs from affected hen s under circumstances that indicated that the virus may have been passed from the laying flock. The source of virus from the three remaining outbreaks associated with pigeon virus and the other outbreak was obscure.

The clinical signs produced in adult fowls infected with the pigeon-associated virus were usually first noticed as a small decrease in egg production which progressed until in some cases, production ceased completely. Eggs laid during this period were often white and soft-shelled. On several occasions it was reported that loose droppings or diarrhoea was seen and there was an increase in mortality with nervous signs in a small proportion of birds. In all cases it was noticeable how slowly the disease spread amongst a flock and on some multihouse sites there was complete failure of the disease to spread from one house to another. This was consistent with the assumed faecal/oral route of transmission.

Disease signs in broilers infected with the pigeon-associated virus were: elevated and rising mortality generally associated with nervous signs which consisted of difficulties in walking and eating, leg paralysis, weakness and lethargy. As with laying birds spread was very slow both within a flock and between different flocks on the same site.

The unrelated outbreak in broilers appeared to be due to infection with a strain of NDV of lower virulence than the pigeon virus but with exacerbation due to Escherichia coli involvement. These birds showed high and rising mortality with diarrhoea and respiratory involvement. Post-mortem examination revealed intestinal haemorrhages, tracheitis, pneumonia and pericarditis, findings which were absent from broilers infected with the pigeon-associated virus.

The link between the infested food stores and the disease outbreaks indicated the source of the virus. However this implies that virus present in the food would survive the milling process and any storage that may occur. It is well known that NDV will survive for long periods in avian faeces (8) and Alexander et al (2) have demonstrated, under laboratory conditions, that infection may be established in adult hens receiving infectious pigeon faeces presented in food. In Britain rations for broiler breeder or egg-laying hens are usually only mixed at the mills and undergo no further treatment, whereas broiler food is usually pelleted. The pelleting process involves heating to 80°C which is held for about 30 seconds and this may be sufficient to kill any contaminating A/PMV-1 virus. It is significant, therefore, that no primary outbreak occurred in birds receiving pelleted food (4).

Stringent controls were placed on food stores at Liverpool and closely situated docks and no outbreaks were seen after July 1984. Despite the prevalence of the disease in pigeons throughout the world, particularly in Europe, and the known spread to feral pigeons in some countries (6) no other country has reported spread of disease to poultry. A possible explanation for this may be the ND vaccination programmes used in most other countries.

The 23 outbreaks in Great Britain in 1984 resulted in the slaughter of over 800,000 birds and the expenditure of over £2 million in compensation and other costs. The compensation paid was covered by an insurance policy, the premiums for which were financed by the Poultry Industry. As a result of the outbreaks the Industry decided not to renew the insurance and in September 1984 the "stamping out" policy was abandoned and vaccination resumed.

TABLE 1 CHARACTERIZATION OF VIRUSES FROM PIGEONS AND POULTRY

Isolates	Haemagglutination inhibitions titres[a] with: Chicken Serum	Monoclonal antibody to HN-1	Monoclonal antibodies[b] reacting	non reacting	Pathogenicity[c] in chickens ICPI	IVPI
1983 British pigeon isolates (51 tested)	64-256	<10	481,38,479	14/32/86,445, 688,424	1.1-1.7(1.44)	0.0-1.8(0.48)
European pigeons isolates (53 tested)	64-512	<10	481,38,479	14/31/86,445, 688,424	1.1-1.8(1.44)	0.0-2.4(0.67)
British chicken isolates (20 tested)	128-512	<10	481,38,479	14/32/86,445, 688,424	1.5-2.0(1.73)	0.5-2.4(2.1)
Chicken Outbreak 2	1024	640	14/32/86,38, 479,688,424	445,481	0.7	0.0
Feral pigeons at Liverpool docks (3 isolates)	256-512	<10	481,38,479	14/32/86,445, 688,424	1.3-1.5(1.39)	0.0-0.9(0.58)
Food at Liverpool docks (2 isolates)	256	<10	481,38,479	14/32/86,445, 688,424	1.4-1.6(1.48)	0.7-1.3(1.01)
NDV-F	1024	640	14/32/86,38, 479,424	445,481,688	NOT DONE	NOT DONE
NDV-B_1	1024	640	14/32/86,38, 479,688,424	445,481	NOT DONE	NOT DONE
NDV-Ulster 2C	512	640	ALL	-	NOT DONE	NOT DONE

a: Reciprocal of dilution causing inhibition of 4HA units of virus. Mouse monoclonal antibodies were treated with receptor destroying enzyme.

b: Mouse monoclonal antibodies were prepared against NDV-Ulster2C (Russell and Alexander 1983). Reactivity was estimated by the ability of antibodies to bind to MDBK cells infected with the virus which were detected by an indirect immunoperoxidase test.

c: ICPI = intracerebral pathogenicity index in day-old chicks. IVPI = intravenous pathogenicity index in six-week-old chickens. Means are shown in brackets.

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DIGEST OF THE DISCUSSION -
Paramyxovirus Workshop

The digest is taken from a tape and memory. It may have differnt emphasis than that which other listeners or participants recall, but may be useful to those not in Davis on March 3, 1985. The editor's (ASR) apologies for omissions,repetitions or misinterpretations - we try! but will perhaps have to try harder.

For errors or misinterpretation of the following paraphrase of the discussion blame the WPDC secretary not the speakers. Following the reports by Drs. Alexander, Hanson and Senne, the question of Nomenclature came up; some felt that that the two terms "Newcastle or PMV-1 from pigeons", used interchagably implied greater identity than was actually the case. It was suggested that "PMV-1 from pigeons" be used as the term for pigeon outbreaks whereas 'Newcastle disease virus" be used as the term for isolates from chickens and turkeys. It has been demonstrated that there are differences which, though slight, show up using monoclonal antibodies or fingerprinting; but differences in target cell activity (respiratory, nervous tissue or intestinal epithelium) though demonstrable are not great enough to indicate the isolates are different antigenic types of paramyxoviruses.

It was also pointed out that amongst the Newcastle disease viruses isolated from pigeons or from other birds there is a very wide range of pathogenicity and target cells. For instance the Largo strain, a highly pathogenic isolate from a cage bird in Largo, Florida is very different from the Ulster strain which was isolated from and has a proclivity for the gut and is very mild or B-1 which invades and provides immunization thru the respiratory epithelium. With regard to pathogenicity or virulence of the isolates, it has been interesting in going over some of the older literature to find that the primary isolate in the United Kingdom was described by Doyle in 1926 as one which caused high mortality and was highly pathogenic for pigeons. Dr. J.R. Beach indicated that the isolates of Avian Pneumoencephalitis in California were different from Newcastle disease virus in that though they caused mortality in some chicken flocks and usually a drop of egg production, they did not cause any mortality, signs or disease in infected or challenged pigeons.

Dr. Yoram Weisman from Israel presented data showing the isolations that they have made from pigeons (mostly show pigeons since they do not have many racing pigeons in Israel) cross-protected and cross-reacted with the Israeli mild B-1 type vaccine as well as with their highly pathogenic chicken isolates, used as reference strains. Isolations were made from an eagle (species undetermined) which showed typical torticollis and died, from falcons, from show pigeons and from wild or feral pigeons. Some of the isolates were submitted to Dr. Alexander for typing who reported that two from pigeons were the same virus and were the same as the PMV-1 isolated from pigeons in Great Britain, whereas another one was typical Newcastle virus, La Sota type.

Dr. Weisman concluded his remarks indicating that there was cross reaction between the various isolates although there were also higher titers between anti-sera and isolates using homologous material. However it was pointed out earlier that it is usually true that you get a slightly higher titer between homologous material as contrasted to heterologous material of the same basic sero-type. It was also pointed out that they had recommended to the pigeon producers and growers and fanciers in Israel that they should vaccinate pigeons with oil emulsion inactivated vaccine after priming them with a mild live virus similar to the B-1 or La Sota. This seemed to give quite a good protection and the most recent isolates reported just prior to leaving for the WPDC came from a flock of birds - (pigeons) which had not been vaccinated. Dr. Weisman also pointed out that they had two isolates at three week intervals from the same flock or from the same owner and that the first isolate was typically PMV-1 (as indicated by Dr. Alexander) whereas the second isolate was typically Newcastle, La Sota type.

During the course of the workshop the matter of the effect of the vaccination on fertility was brought up, since this is a major concern of pigeon fanciers. It was pointed out that this simply may be a side result since the inactivated vaccines do or may cause some irritation at the point of injection which may lead to reluctance on the part of the hen to accepting her nest mate rather than an effect of the vaccine per se on the semen or ovules. In connection with the vaccination procedures for pigeons, Dr. Eskelund presented limited data on the efficacy of a killed or inactivated oil emulsion vaccine prepared from the PMV-1 isolate from New York. The indication was that this was efficacious, that two doses are superior to one and that under certain circumstances it might be available with USDA, Biologics, okay.

The workshop participants voiced concern because of lack of information on the activity of pigeoneers and the possibility of spread of a more than normally pathogenic Newcastle disease virus (PMV-1) by feral or racing pigeons to domestic poultry. Dr. Simon Shane from Louisiana pointed out that either legally or illegally large amounts of vaccine would probably be used on pigeons in the next several months or year and it would be well to initate, if possible, a widespread survey of the pigeon population to determine whether or not the virus or at least antibodies to the PMV-1 viruses are present in the racing pigeon flocks, the show birds and feral pigeons. As was pointed out, the racing pigeon fraternity, in training the bird for races and likewise in the races themselves, use many procedures which commercial poultrymen would not. They mix goups from numerous sources as much as possible in order to "even up" the odds in races and for training; they ship or truck them long distances and then fly them hundreds or even thousands of miles causing stress. Those which are coming down with the disease are likely not to return home and co-mingle either with other domestic flcoks or with feral pigeons in various areas. It was further pointed out that by immunization there was a good chance that the pigeon flocks would not only not become as readily infected but at least would not shed the virus if they did become infected for as long or in as great amounts.

Further it was pointed out that the infection had spread from original sources in Europe and London and likewise in the United States by the shipment of presumably infected pigeons, into lofts containing

some suseptible healthy birds, with the result in death losses of up to 50%. This was reported in the state of Connecticut by Dr. Van der Heide.

It was pointed out that although many of the pigeon isolates were of relatively low pathogencity for chickens by the cloacal swab route but highly pathogenic for pigeons when administered by the intravenous or intramuscular route, they nonetheless had high intracerebral pathogenicity indices and intravenous pathogenicity for chickens. The standard procedure for classifying Newcatle disease virus from chickens or pet birds as "velogenic" is to inoculate four, 6-8 week-old chickens by the cloacal swab. It was further pointed out that in assessing the virulence of isolates (or challenging to find the protective effect of vaccines) the route chosen for pigeons was the intravenous route because this was the only route by which high mortality, even with virulent strains, could be assured. Dr. Weisman pointed out in his discussion that the signs caused by typical virulent Newcastle disease virus from chickens and the PMV-1 isolates from pigeons were indistinguishable, being torticollis, leg weakness and instability, diarrhea and death.

During 1984, especially in the New York area, a national organization of racing pigeoneers attempted to curtail racing or to not race the birds unless they were vaccinated. This did not work out too well since the response was not enthusiastic. Attempts to encourage vaccination of racing pigeons met with less than enthusiastic response due in part to recommendations made by various racing pigeon people and some veterinarians dealing with pigeons against the use of vaccines because of "sterility problems". There is no evidence at all that vaccination does effect fertility except possibly as suggested earlier because of the irritation and painful effect leading to lower acceptance rate on the part of the hen from the cock.

Dr. Alexander pointed out that having endorsed vaccination for pigeons, after about a three month hiatus when no vaccine was available legally in the United Kingdom, there was a fair degree of acceptance of this idea and that there was apparently good protection against overt disease. One problem he pointed out, however, was that having vaccinated the birds at one time, pigeoneers frequently felt no urge to repeat the procedure. For example if a loft of birds were vaccinated in 1983 they did not need to be vaccinated or the flock did not need to be vaccinated in 1984. As a consequence when the 1984 cases started coming in about 80+% were in young birds which had never been vaccinated; no vaccination was done that year. He also indicated despite the fact that there had not been any losses in vaccinated or properly vaccinated pigeons or in lofts where vaccination had been practiced some of the racing pigeon clubs, particularly those around London recommended against vaccination. He also mentioned then that of the first 60 pigeon cases which were diagnosed in 1984, 50 of them were from the area where vaccination had been either poorly done or not done in which vaccination was recommended against.

It was suggested that one of the problems in the United Kingdom were unvaccinated susceptible chicken and turkey flocks; fecal contamination of feedstuffs or direct co-mingling of infected feral pigeons with poultry was associated with rather severe losses in a poultry population in which no vaccine use was permitted. The matter of vaccination versus immunization was emphasized; it was pointed out that it is necessary to apply effective vaccine properly and to develop a good vaccination program for use in commercial chickens and turkeys. The potential spread of PMV-1 from pigeons to domestic poultry should be borne in mind and may change the epidemiology somewhat. Dr. Edson mentioned that breeder turkeys were usually vaccinated against Newcastle disease, but that commercial (turkey) meat flocks were seldom vaccinated and that this therefore might affect the flocks. It was also pointed out that not all the racing pigeons got home but that particularly the sick ones are very likely to drop out and might mingle with other groups of feral or confined birds.

Amongst the pigeoneers it was suggested that veterinarians encourage pigeon fanciers to vaccinate, pointing out its efficacy, particularly prior to the racing season and the training flights.

Diagnosis in a diagnostic laboratory would depend on serology, history of the flock (especially of vaccination) and possibly virus isolation. Usual HI tests do not, of course, reveal the source of the virus stimulating the titer (vaccine, field infection etc.), but paired serum samples from the same flock or birds with a rise in titer suggests reinfection due to re-vaccination, re-exposure to a field virus regardless of source (that is pigeons or chickens). Characterization would depend on more precise and sophisticated procedures, but no basic change in diagnostics should be necessary.

The sessions closed with comments by Bob Hanson somewhat like this: "We have been hearing the description of a disease in pigeons which was recognized for the first time a relatively few years ago and, which, as has been pointed out, spread in a rather frightening fashion to most of the world where pigeons are being raised and handled. The manner in which pigeons are handled to show them or to race them certainly helps disseminate the disease. We are dealing with a virus which we know, "Newcastle disease virus", which has a tremendous potential for showing different types of behavior in chickens; it varies all the way from an avirulent infection to a highly pathogenic, fulminating disease.

"It behaves differently in different species so that a disease that is highly pathogenic for chickens, for example and will kill chickens in no time at all, but in some of the wild species of birds in which the virus can be isolated it is avirulent. So that the degree and nature of virulence of this disease relates only to the host in which it is described. If you manipulate it in the laboratory even a few passages you can make changes in that population and change its behavior. So the primary thing is that it is a very adaptable agent, it can take advantage of the situation; and what we are talking about is more of a potential problem than a current, real problem as far as the poultry industry is concerned. But certainly if there is a new way for an old disease to get around we have to pay attention to that fact. I think that that is a primary concern that we have; it is getting around in a way that is new and we do not know what implication that will have to the control of the disease in poultry."

SUMMARY OF THE AVIAN PARAMYXOVIRUS WORKSHOP

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On March 3, 1985 a rather large group of interested individuals gathered on the UCD campus to participate in a workshop on avian paramyxoviruses. The workshop was most timely because of the recent emergence of pigeon paramyxovirus problems in Europe, especially Great Britian. Urgency was added for the workshop as it became known that the pigeon virus had gained access to the U.S., causing disease in pigeons in the states of New York, Vermont, New Jersey, Connecticut, and in the Baltimore-Washington area.

Fortunately, two of the world's experts on the avian paramyxoviruses were speakers at the workshop. First Dr. Dennis Alexander, Central Veterinary Laboratory, Weybridge, England, led off the meeting with a review of the avian paramyxoviruses. He was followed by Dr. R.P. Hanson, University of Wisconsin, long noted for his Newcastle contributions, who discussed the influence of mutations on the evolution of the Newcastle viruses.

It was entirely appropriate that such a workshop be held in California, for it was in this state that Dr. Beach published on the unique "pneumoencephalitis" that occurred in the 1940's. It differed markedly in clinical signs from the classical Newcastle disease described in 1926 by Doyle; it was subsequently identified as Newcastle disease. Newcastle is a type 1 avian paramyxovirus.

It was also in California that Dr Ray Bankowski in 1956 isolated a new virus from chickens called Yucaipa virus. We now know that this is a type 2 avian paramyxovirus.

Also in 1972-73 in California, the first extensive state/federal/industry eradication effort was mounted to rid the area of Viscerotropic Velogenic Newcastle disease virus (VVND). The effort will go down in disease control history as the most massive, but successful disease eradication effort in poultry.

Finally, an event took place during that VVND eradication effort that has had a great influence on acquiring information to improve our understanding of both the ortho- and paramyxoviruses. The event was the recovery by Slemons et al of influenza isolates from the feces of apparently healthy waterfowl. Many previous attempts to recover such viruses from ducks had been made from the respiratory tract. After this finding in California, the widespread prevalence of the influenza viruses in waterfowl began to be realized. During the searches for influenza viruses, a large number of avian paramyxoviruses have been recovered from waterfowl.

PMV-3 has been a problem in the turkey industry for over 15 years. Both PMV 2 and 3 can cause mild respiratory disease and egg production problems (declines, pigment loss, thin shells) if they are not complicated by other co-infections. When PMV-3 is complicated by co-infections of Alcaligenes faecalis, Pasteurella, or Mycoplasma, considerable mortality can result. Other complicating factors that influence the response of poultry to the avian paramyxoviruses include the reactions from the use of live vaccines, physiologic and enviromental stresses and a compromised immune system.

Dr. Hanson discussed the probable strategy that has enabled the Newcastle disease viruses (Avian PMV-1) to evolve rapidly as opportunities arose. He proposed that the ND viruses are generally a very heterogenous population of viruses with a fairly constant character as long as the environment remains stable. However, when the environmental pressures change to favor the emergence of minority populations, the character of the overall virus population changes. Even plaque purified Newcastle virus cultures are now considered to be more of a population of viruses than a clone of virus particles. This is due to the clumping of virions preventing the picking of a homogenous population when a plaque is picked. Similarly, birds are generally exposed not to a single virion but to a large number of varied virions as the disease progresses through the avian population. Dr. Hanson pointed out that cultures of Newcastle virus that are classified as virulent or highly pathogenic also have avirulent virions in their population. Likewise, avirulent cultures may have highly virulent virions in their population that, through special manipulations, can be recovered.

He believes that the rapid changes that occur in Newcastle viruses during an epidemic are not due to mutations but to the emergence of minority populations brought on by enviromental pressures or opportunities. These virions may go on to eventually predominate to make the virus which is recovered at the end of the epidemic quite different from the virus isolated at the beginning. These later viruses are generally less virulent, kill slower and are therefore shed longer. They are, therefore, more likely to eventually dominate than those viruses which kill their hosts rapidly.

Dennis Senne of the National Veterinary Services Laboratory in Ames presented information on the characterization of 24 isolates of pigeon PMV-1 Newcastle virus submitted from New York, New Jersey, Vermont and Maryland lofts. He stated that the virus is sometimes without significant HA activity on initial isolation in chicken embryos. The virus grows well in chicken kidney cells. All of the 20 isolates tested produced mortality in pigeons that averaged 63%. Disease was produced in chickens only upon I.V. administration. When infected pigeons were housed with chickens, the virus was successfully transmitted to the chickens as evidenced by recovery of the virus from the chickens from days 13 to 34. The mean embryo death times of the pigeon isolates was over 100 hours which, by the currently accepted criteria, would result in them being classified as lentogens. Infected pigeons shed virus for as long as 20 days with 4 to 17 days being usual. The mean death time in pigeons was 9.45 days with a range of 4 to 25 days.

35th WESTERN POULTRY DISEASE CONFERENCE (WPDC)
held jointly with the 11th Convention of
the Asociacion Nacional de Especialistas en Ciencias Avicolas (ANECA)
April 29 - May 2, 1986
Puerto Vallarta, Jalisco, MEXICO

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