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Historical Article—

Pullorum Disease: Evolution of the Eradication Strategy

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SUMMARY. The history of pullorum disease is closely intertwined with the history of avian health research and that of the poultry industry. The seriousness of the disease galvanized the attention and brought together, for the first time, the pioneers of poultry health research to work cooperatively on different aspects of the disease. Control of the disease made it possible for intensive poultry production to develop as the basis for the modern poultry industry. During the early 1900s, bacillary white diarrhea (BWD) was a devastating disease of young chickens threatening the developing poultry industry. Dr. Leo F. Rettger isolated and described the bacterial pathogen, *Salmonella enterica* serotype Pullorum, for the first time in 1900. BWD was renamed pullorum disease in 1929. In subsequent years, Rettger and coworkers were able to reproduce the disease and fulfill Koch's postulates. Rettger *et al.* also showed that *Salmonella* Pullorum was vertically transmitted, which was the first time that a pathogen was shown to be vertically transmitted. The development of serologic tests was of crucial importance because it led to the development of effective eradication methods to identify carrier birds and to exclude these birds from the breeder flocks. The negative impact of pullorum disease on the poultry industry ultimately was one of the major reasons that the National Poultry Improvement Plan (NPIP) was developed by scientists, the poultry industry, and the United States Department of Agriculture (USDA). Needless to say, the work of the pioneering researchers formed the basis for the control of the disease. The NPIP started in 1935, with 34 states participating in testing 4 million birds representing 58.2% of the birds hatched. The program rapidly expanded to 47 states by 1948 and tested more than 30 million birds. In 1967, all commercial chicken hatcheries participating in the NPIP were 100% free of pullorum and typhoid disease caused by *Salmonella enterica* serotype Gallinarum. This historical overview of pullorum disease describes in some detail the progress made, especially during the early years, toward controlling this disease using methodologies that were often very basic but nonetheless effective. One has to admire the ingenuity and persistence of the early researchers leading to their achievements considering the research tools that were available at the time.

RESUMEN. *Artículo histórico*—Pulorosis: Evolución de las estrategias de erradicación

La historia de la pulorosis está estrechamente relacionada con la historia de la investigación en salud aviar y de la industria avícola. La severidad de la enfermedad despertó la atención y reunió, por primera vez a los pioneros de la investigación en salud avícola para trabajar de manera cooperativa en diferentes aspectos de la enfermedad. El control de la enfermedad hizo posible que la producción avícola intensiva se desarrollara como base de la industria avícola moderna. A principios de la década de los 1900, la diarrea blanca bacilar (con las siglas en inglés BWD) era una enfermedad devastadora de pollos jóvenes que amenazaba la industria avícola en desarrollo. El Dr. Leo F. Rettger aisló y describió el patógeno bacteriano, *Salmonella enterica* serotipo Pullorum, por primera vez en 1900. La diarrea blanca bacilar pasó a llamarse pulorosis (pullorum disease) en 1929. En los años siguientes, Rettger y sus colaboradores pudieron reproducir la enfermedad y cumplir los postulados de Koch. Rettger y col. también mostraron que *Salmonella* Pullorum se transmitía verticalmente, y fue la primera vez que se demostró que un patógeno se transmitía verticalmente. El desarrollo de pruebas serológicas fue de crucial importancia porque condujo al desarrollo de métodos de erradicación efectivos para identificar aves portadoras y eliminar a estas aves de las parvadas reproductoras. El impacto negativo de la pulorosis en la industria avícola fue, en última instancia, una de las principales razones por las que los científicos, la industria avícola y el Departamento de Agricultura de los Estados Unidos (USDA) desarrollaron el Plan Nacional de Mejoramiento Avícola (NPIP). Es importante decir que el trabajo de los investigadores pioneros formó la base para el control de la enfermedad. El Plan Nacional de Mejoramiento Avícola comenzó en año 1935, con 34 estados participando en el análisis de 4 millones de aves que representaban el 58.2% de las aves producidas. El programa se expandió rápidamente a 47 estados en 1948 y evaluó a más de 30 millones de aves. En 1967, todas las plantas incubadoras de pollos comerciales que participaban en el Plan Nacional de Mejoramiento Avícola estaban 100% libres de pulorosis y tifoidea aviar causada por *Salmonella enterica* serotipo Gallinarum. Esta reseña histórica de la pulorosis describe con cierto detalle el progreso realizado, especialmente durante los primeros años, hacia el control de esta enfermedad utilizando metodologías que a menudo eran muy básicas no obstante efectivas. Es admirable el ingenio y la persistencia de los primeros investigadores que los llevaron a sus logros considerando las herramientas de investigación que estaban disponibles en ese momento.

Key words: history, pullorum disease, *Salmonella* Pullorum, white bacillary diarrhea

Abbreviations: BWD = bacillary white diarrhea; KSAC = Kansas State Agricultural College; NECAD = Northeastern Conference on Avian Diseases; NCLWBWD = Northeastern Conference of Laboratory Workers in Bacillary White Diarrhea; NPIP = National Poultry Improvement Plan; R.O.P. = Record of Performance; PT = pullorum typhoid; WPIA = Washington Poultry Improvement Association

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This article is based on the History Lecture (unpublished) presented by Dr. Kakambi V. Nagaraja at the annual meeting of The American Association of Avian Pathologists (AAAP) held on August 2019 in Washington, DC. The presenter of the History Lecture usually writes a History Article based on the presentation for publication in *Avian Diseases*. Sadly, Dr. Nagaraja passed away unexpectedly on July 22, 2020, and did not write the article. Based on the recommendations of the Advisory Board of Avian Diseases and The History of Avian Medicine Committee of the AAAP, which plans the lecture and designates the speakers, we, Schat and Saif, authored the article based on the material presented by Dr. Nagaraja. We followed the outline used by Dr. Nagaraja in his PowerPoint presentation and adhered closely to the text of his presentation to the point of using Dr. Nagaraja's own words in some parts of the article.

Pullorum disease, or bacillary white diarrhea (BWD) as it was known in the early 1900s, was a devastating disease threatening the development of the commercial poultry industry at that time (1,2). The isolation and characterization of the causative organism, the first recognition of vertical transmission of an avian pathogen, the development of serologic methods to identify carriers, and the subsequent control and eradication of the disease as a threat to the commercial poultry industry form a fascinating part of the history of veterinary medicine. It is amazing that the early researchers like Dr. Rettger and others were able to achieve this with the tools available during the early 1900s.

The research on pullorum disease also led to the first organization of poultry health workers in the United States when in 1928 the Northeastern Conference of Laboratory Workers in Bacillary White Diarrhea was formed. This organization still exists and is now known as the Northeastern Conference on Avian Diseases (NECAD). The history of NECAD will be described by Calnek and Schat in a future history paper. The need to completely eradicate pullorum disease also led to the formation of the National Poultry Improvement Plan (NPIP), the history of which will be described briefly at the end of this paper.

THE EARLY YEARS

The early history of pullorum disease is closely associated with the research conducted by Dr. Leo F. Rettger. Dr. Rettger was born in Huntingdon, Indiana on March 17, 1874 and passed away on January 7, 1954. His obituary has been published in the *Journal of Bacteriology* (3). He received his BA and MA degrees from Indiana University in 1896 and 1897, respectively. In 1902 he received his Ph.D. degree from Yale University, where he was a faculty member from 1902 to 1942. In addition to his work on pullorum disease, he published many other papers on bacteria and bacterial diseases. A search of pubmed.ncbi.nlm.nih.gov for L.F. Rettger yielded 76 publications in peer-reviewed journals, with only five papers on pullorum disease, but many of his results were reported in bulletins published by the Storrs Agricultural Experiment Station. Interestingly, the paper describing the disease for the first time in 1900 is not listed in PubMed. In this paper Rettger (4) wrote "Late in the summer of 1899 I had the privilege of observing a peculiar epidemic which occurred among young chickens two to three weeks old." A hen and 17 chicks had been purchased and, starting 3 days after the purchase, the first chick got sick and died. Over the next 10 days, 13 of the remaining chicks got sick, often with diarrhea, and 10 chicks died. Postmortem examination showed emaciated chicks with empty and pale intestines and a pale liver with some red patches. Histology revealed the presence of bacteria in the liver. Rettger isolated a Gram-negative, motile *Bacillus*, which he described in great detail using different culture methods. However, Rettger and Harvey (5) noted later that the *Bacillus* was not motile, which has been confirmed since then by many studies (6). To fulfill Koch's postulates, Rettger inoculated four chicks by subcutaneous injection with 0.5 to 1 ml of pure bouillon cultures. The husbandry during the experiment was interesting:

> *During the course of the experiment they were kept in separate and well-ventilated boxes and supplied twice a day with fresh water and food (moist bread, cracked corn, etc.). Sand and pebbles were also thrown into the box occasionally.*

All four chickens got sick and two died within 5 days. Pure cultures of the original bacillus were obtained, and liver sections showed the presence of many bacteria. Rettger (4) concluded that the disease was a form of septicemia. Shortly afterwards, young chicks on three adjacent farms in Winona, Indiana were affected with the same disease, causing approximately 80% mortality (7). The first cases occurred in chicks hatched by the mother hen, but chicks hatched in an incubator were also developing white diarrhea. Chicks from the next hatch in the same hatcher also developed white diarrhea, but after disinfection of the hatcher with formalin vapor, the next two broods remained free of the disease. Transmission experiments using feed spiked with 24-hr bouillon cultures caused disease, and transmission to contact chickens indicated a fecal-oral route of transmission, although the possibility of egg transmission was not ruled out (5,8). The name "bacillary white diarrhea" (BWD) was mentioned for the first time in 1909 (8). This name was changed in 1929 to pullorum disease during the second meeting of the Conference of Laboratory Workers in Pullorum Disease Eradication (CLWPDE) (9). Rettger (8) also decided on a name for the bacillus:

> *It will suffice here to recall that the organism, which after long deliberation I have called Bacterium pullorum, has every mark of belonging to the colon-typhoid-dysentery group.*

The name was changed to *Salmonella* Pullorum in 1925 by the Society of American Bacteriologists (10). Subsequently this name came into general use by, e.g., Segelin (11), although Bushnell and Hinshaw (12) and Roberts and Card (13) used *Salmonella pullora*, which is interesting because Bushnell *et al.* (10) mentioned that the name was changed to *S. pullorum*, which is currently named *S. enterica* serotype Pullorum. In this paper we will use *Salmonella* Pullorum.

After the first description of BWD by Rettger (4), it became clear that the problem was widespread in the United States with outbreaks in, e.g., Louisiana (14), New York (15), Maryland (16), and in Ontario, Canada (quoted by Rettger and Harvey) (5). Milks (14) reproduced the disease and reported the results for all the birds as follow: "The other was sick a few hours before death; was stupid and weak." Within 26 yr, the first review papers were published by Beaudette in 1925 (2) and Bushnell *et al.* in 1926 (10), attesting to the importance of pullorum disease. Pullorum disease was not restricted to chickens. In 1928, Hewitt (17) described the first case of pullorum disease in two turkey poults. These poults were hatched in an incubator previously used to hatch chicks. It was assumed that the incubator was not properly disinfected after chicks had been hatched

Table 1. Experimental infection of young chickens with *Salmonella* Pullorum (26)

No of birds	Challenge at age in hours	% mortality
Experiment 1		
26	Control	11.50
26	36	33.50
26	60	23.33
26	84	19.25
26	108	7.70
Experiment 2		
50	Control	16.66
50	24	72.00
50	48	38.00
50	72	30.00
50	96	20.00
50	120	16.00

before placing the turkey eggs. Over the next few years, many more cases were reported in turkeys (18 and references therein).

CHARACTERIZATION OF *SALMONELLA* PULLORUM

After the first reports of the isolation of *Salmonella* Pullorum by Rettger (4,7) some doubts were expressed about the characterization of this pathogen, especially in relation to *Bacterium sanguinarium*, later renamed as *S. enterica* serotype Gallinarum, which was isolated from older chickens with sudden mortality. Inoculation of hens with the isolated bacteria caused mortality, with lesions mostly in the liver. Because there were also abnormalities noted in the blood, Dr. Veranus Moore named the disease "infectious leukemia" (19), but it was also referred to as fowl typhoid. The isolated bacteria failed to produce gas when cultured in bouillon containing sugars, but acid was produced in the presence of selected sugars. Rettger and coworkers (8,20) reported that some of his isolates produced gas in bouillon with dextrose and mannite while other isolates did not produce gas but did produce acid. Clearly, the differentiation of *Salmonella* Pullorum and *Salmonella* Gallinarum was extremely challenging in the early 1900s. Smith and Tenbroeck (21) noticed the similarities between the two bacterial strains and compared five *Salmonella* Pullorum isolates, including the original Rettger isolate, with two fowl typhoid isolates. The two fowl typhoid isolates and the original Rettger *Salmonella* Pullorum isolate did not produce gas in 1% dextrose and mannite bouillon, while the other four *Salmonella* Pullorum isolates did produce gas. The two fowl typhoid isolates did produce acid in these cultures in contrast with the five pullorum strains. These authors also tried to differentiate the fowl typhoid isolates from the *Salmonella* Pullorum by raising antisera in rabbits using heated cultures of human typhoid strain μ, fowl typhoid, and one of the gas-producing pullorum strains. All sera agglutinated the three types of bacteria, and they concluded that agglutination tests could not be used to differentiate the bacterial strains. The absence of gas production by the original Rettger isolate was hypothesized to be caused by artificial cultivation. Smith and Tenbroeck also dismissed Hadley's statement (22) that the fowl typhoid bacillus probably belongs to the fowl cholera group as follows: "(this statement) is disproved by so many facts that it need not be specially considered." Rettger and Koser (23) compared three strains of both *Salmonella* Pullorum and *Salmonella* Gallinarum in more detail using bouillon containing 1% peptone and 0.25% Liebig's meat extract as the base medium. To study acid and gas formation, they added dextrose, levulose, lactose, maltose, galactose, inulin, raffinose, salicin, saccharose, dextrin, dulcite, adonite, glycerine, or mannite to the base medium. Most of these medium compositions did not differentiate between the two groups, but there were a few differences. *Salmonella* Gallinarum but not *Salmonella* Pullorum produced acid in media containing maltose, dextrin, or dulcite. Using the methyl-red test developed in 1915 by Clark and Lubs (24) caused a clear rose-red color in the acid producing media, while the media turned yellow in the absence of acid production. The absence of turning rose red was subsequently confirmed for the additional 13 *Salmonella* Pullorum isolates available to Rettger and Koser. Another difference between *Salmonella* Pullorum and *Salmonella* Gallinarum is the absolute absence of gas production in the later, while the former produced gas in media containing dextrose or levulose and while gas production was somewhat variable using mannite or galactose. Subsequently, all 16 *Salmonella* Pullorum isolates were tested in medium containing either dextrose or mannite: 15/16 produced gas with dextrose and 14/16 produced gas with mannite. The strain that was negative for gas production failed to produce acid and was negative in the methyl-red test and was therefore grouped with other *Salmonella* Pullorum isolates. This was a recent isolate suggesting that the absence of gas production was not related to artificial cultivation. They confirmed the observation by Smith and Tenbroeck (21) that agglutination tests were unable to differentiate between the two groups. Interestingly, Hadley (25) proposed in 1917 to divide the pullorum strains into *Salmonella* Pullorum A producing gas and *Salmonella* Pullorum B lacking the ability to produce gas. This division was not accepted by most other researchers (10). It is amazing how these early researchers were able to characterize and differentiate different bacteria.

AGE RESISTANCE

Early studies had suggested that only young birds get diseased with BWD but that older birds are not affected. Rettger *et al.* (26) challenged chicks at different times posthatch and noticed that infecting young birds shortly after hatching would cause disease and mortality, which dropped off when chicks were challenged after 100 hr of age (Table 1). Jones (15) confirmed that *Salmonella* Pullorum caused pullorum disease and that the disease was associated with young chicks, but surviving chicks could become carriers with pathological changes of the ovaries. However, age resistance is not absolute. Jones (27) reported an interesting case in which approximately 7% of the 700 hens between 1 and 2 yr of age died with acute infection with *Salmonella* Pullorum. The following quote from this paper is a good illustration of how an offer of assistance of one poultry man to another poultry man can cause a disaster:

> *A neighbor had been meeting with serious losses in his young chicks. The cause of this trouble was supposed to be improper incubation of the eggs and sudden chilling of the chicks. The poultryman volunteered to incubate a number of the neighbor's eggs and rear the chicks for him. Nearly all of the chicks hatched from these eggs died within ten days of bacillary white diarrhea. The eggs that failed to hatch were fed on March twelfth to the poultryman's adult hens. On March twenty-eighth two or three of the hens that had eaten the eggs died and from then on for a period of a month fifty fowls died of the disease.*

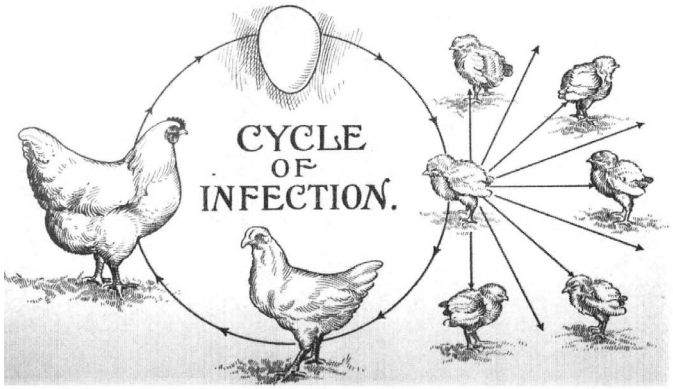

Fig. 1. Original diagram showing how BWD perpetuates itself in the breeding stock (31,33).

Jones was able to isolate pure cultures of *Salmonella* Pullorum from the dead hens as well as from chicks hatched from these eggs. Rettger *et al.* (28) confirmed that feeding eggs contaminated with *Salmonella* Pullorum could cause mortality in adult chickens. Shortly afterward, Hadley *et al.* (25) also reported a case of mortality in adult hens caused by *Salmonella* Pullorum. Subsequently, Tittsler and others (29 and references therein) also reported outbreaks in adult birds. It is important to mention here that pure cultures in that period may not always have been *Salmonella* Pullorum, and perhaps *Salmonella* Gallinarum was responsible for some of the outbreaks reported in (29). Interestingly, Beaudette (30) reported several instances of mortality in young chicks from which *Salmonella* Gallinarum was isolated. Thus, age-related susceptibility to pullorum disease *vs.* fowl typhoid was no longer a way to differentiate disease caused by *Salmonella* Pullorum or *Salmonella* Gallinarum without isolation of the causative organism (30).

VERTICAL TRANSMISSION

The elucidation of the transmission of *Salmonella* Pullorum was essential for the development of control measures. As mentioned before, the first cases were described in broods (4,7), suggesting that the infection may have come from the hen without solving the actual mode of transmission. Subsequent studies reported cases with chicks hatched in incubators (5). Feeding experiments using feed laced with cultured *Salmonella* Pullorum showed that oral infection caused BWD and that there was horizontal transmission from infected to contact chicks (5,8). Evidence for the possibility of vertical transmission was in the first instance based on several indirect observations. Especially Jones (15) but also Rettger and Stoneburn (20) noted a strong correlation of the introduction of pullorum disease in new locations with the arrival of newly hatched chicks that were directly sent from the hatchery. There was the presence of unabsorbed and often abnormal yolk, varying in size from a pea to a full-sized yolk, with color varying from yellow to brownish green or nearly black; *Salmonella* Pullorum could easily be isolated from these yolks (15,20). Postmortem examination of hens producing chicks with pullorum disease frequently showed abnormal ova from which *Salmonella* Pullorum could be isolated (16,20,31,32). Rettger and Stoneburn (20) made the following conclusion in 1909:

The finding of this organism in (a) the ova in the ovaries of the hens, (b) the yolk of fresh eggs, (c) eggs incubated for varying lengths of time and (d) yolk sacs of fully-developed chicks still within the shell, appeared to us to be conclusive evidence that the original source of infection is the hen.

This was the first time that evidence of vertical transmission of a pathogen was described in birds. In a follow-up study, these investigators (31) used trap nesting and were able to identify hens producing sometimes up to 70% infected eggs, while other hens produced far lower numbers of infected eggs. Postmortem exam of hens producing infected eggs showed variable numbers of diseased ova, while hens producing only negative eggs did not show pathology. Chicks surviving infection would become carriers, with *Salmonella* Pullorum present in the ovary, thus perpetuating the infection cycle (Fig. 1). The idea of vertical transmission must have been rather controversial because the same figure was published by the same author (Rettger) in 1914 (33). Additional data to support vertical transmission were reported in 1914 (34). A large group of 1- to 2-day-old chicks were infected with *Salmonella* Pullorum and another group was kept as control. Eighty-eight of the infected chicks survived until the termination of the experiment after about 1 yr, with 24% showing a positive agglutination test and lesions in the ovary. Only one of the 57 controls showed a positive agglutination test and pathology in the ovary. Interestingly, Rettger called this germinal transmission, which we now know is incorrect, but made sense in the early 1900s. Unfortunately, the infrequent number of positive eggs did not allow screening of eggs as a reliable method to eliminate carrier hens (32,33,34). Hinshaw *et al.* (35) confirmed that transmission from infected chicks to uninfected chicks is possible in forced-air-draught–type incubators. Their methods included adding feather down with dried-on *Salmonella* Pullorum to the incubator and inoculation of pipped eggs with *Salmonella* Pullorum. Both type of experiments resulted in horizontal transmission within the incubator.

Soon after the first description of vertical transmission of a pathogen in birds, Rettger and Scoville (36) suggested that vertical transmission occurred in a bacterial disease of ducklings, which was called "Keel." Beaudette (30) suggested in 1924 that vertical transmission was also occurring with *Salmonella* Gallinarum.

THE DEVELOPMENT OF SEROLOGIC TESTS

Because screening of eggs to identify positive hens was not feasible, the development of serologic tests became essential. This may sound like a simple development in the 21st century, but in the early 1900s there were only a few examples of serologic tests. Jones (37,38) noticed the use of agglutination tests for the detection of glanders, an infectious disease mostly in horses caused by the bacterium *Burkholderia mallei*, and contagious abortion in horses. Based on this information, he developed the first macroscopic agglutination test to identify adult chickens positive for *Salmonella* Pullorum. To prepare antigen, he used a mixture of different *Salmonella* Pullorum isolates harvested from slant agar tubes and resuspended the bacteria in 0.85% NaCl with the addition of 0.5 percent phenol. Suspensions were used with or without heat treatment for 1 hr at 60 C and filtered through cotton filters to remove clumps of bacteria. To obtain serum was another interesting part of the Jones papers. He offered the methods to collect 3–5 ml of blood: 1) cutting off several spikes of the comb using a sharp knife, 2) chiseling off the back toe, and 3) severing one of the wing veins close to the second joint. These methods certainly would raise

Fig. 2. The first meeting of Northeastern Conference of Laboratory Workers in Bacillary White Diarrhea. Paige Laboratory, Massachusetts Agricultural College, Amherst, April 24, 25, and 26, 1928.

concerns today and would not be permitted by the Institutional Animal Care and Use Committee. The test itself consisted of mixing 3 ml of antigen with different serum dilutions in tubes, incubating the tubes for up to 72 hr at 37.5 C, and examining the tubes for clumping of bacteria. The first results showed a high correlation between positive sera and lesions in the ovaries. Jones first tested 12 birds from a problem flock and found several positive samples. These birds were euthanatized and showed lesions in the ovaries, from which *Salmonella* Pullorum was isolated. The owner then requested additional testing and 65 additional birds were selected. Twenty-two tested positive with titers ranging from 1:25 to 1:2000. More importantly, *Salmonella* Pullorum was isolated from 20 of 21 seropositive birds, and in most cases these birds had abnormal ovaries. There was no apparent relation between the titer and the degree of pathology. Jones mentioned that fresh cultures or early passages provided a better antigen than isolates cultured for a longer period. Rettger *et al.* (33) used the Jones agglutination test in an experiment in which young chicks were infected with *Salmonella* Pullorum and survivors were used for other experiments. All 22 seropositive birds out of 88 surviving birds also had typical lesions in the ovaries.

The test developed by Jones was based on a mixture of bacterial isolates and therefore difficult to standardize. Bushnell *et al.* (10) and Bushnell and Hinshaw at the Kansas State Agricultural College (KSAC) (12) compared the different agglutination tests used since the first description by Jones (38). After approximately 6000 comparative tests, it was concluded that serum dilutions of 1:20 and 1:80 are recommended (12). The "standardized" test at KSAC used a mixture of 8 *Salmonella* Pullorum isolates, which were harvested after a 48- to 72-hr incubation. Concentrated bacterial suspensions in 0.85% NaCl with 0.5% phenol were stored in a refrigerator and diluted prior to use to a turbidity slightly less than in tube No. 1 of the McFarland nephelometer (39). After incubation at 37 C for 20 hr, the tubes are removed from the incubator and read after being kept for 2–4 hr at room temperature. Although this was presented as a standardized test, it clearly had a lot of variables. The need to truly develop a more standardized test was expressed by the Poultry Disease Committee of the U.S. Livestock Sanitary Association during their 31st meeting in Chicago (November 30, December 1–2, 1927). The Committee reported the need for a meeting to control "bacillary white diarrhea" with an emphasis on the standardization of testing methods. Drs. Rettger and Hinshaw, with assistance of Dr. Lentz, invited scientists working on pullorum disease from six northeastern states for a meeting on April 24–26, 1928, which was held in the Paige Laboratory of the Massachusetts Agricultural College in Amherst (Massachusetts). The following scientists participated: Dr. L. F. Rettger from the Connecticut Agricultural Experiment Station in New Haven, Professor E. R. Hitchner and Dr. F.L. Russell from the University of Maine in Orono, Dr. E. M. Gildow from New Hampshire, Dr. J. B. Lentz, Dr. W. R. Hinshaw, Dr. E. F. Sanders, Dr. N. J. Pyle, Mr. C. B. Waite, and Miss Miriam K. Clarke from the Massachusetts Agricultural Experiment Station, and Professor A. W. Lohman from the University of Vermont. Rhode Island was represented by Dr. J. C. Weldin of the Rhode Island State College in Kingston, but he was only present during the joint meeting with the New England Livestock Sanitary Officials with the purpose of accreditation of poultry to be free of pullorum.

The specific objectives of the meeting were:

> *To obtain cooperation between the six New England States; to attempt standardization of laboratory methods and equipment, and for the development of better fellowship between the laboratory workers of this section (40).*

The goals for the meeting were to compare test methods for the detection of antibodies against *Salmonella* Pullorum and to develop a standardized agglutination test. It was agreed to standardize the test fluid (antigen) in the future for turbidity, pH value, bacterial isolates, age and purity of the culture, preservative to be used, maximum storage time, and antigenic properties (40). Participants were asked to bring bacterial isolates as well as positive and negative sera. The bacterial isolates from each group were pooled and comparative tests were conducted. Figure 2 shows the different participants at work. The participants were asked to continue the testing using the standardized approach in their own laboratory and report the results the next year. In the early years of this group, much time and effort went into standardization of test procedures, with Rettger and others leading the effort. During many of the early NECAD meetings, researchers compared bacterial isolates, amount of antigen, blood, and temperature conditions, which led to a standardized approach in the northeastern states of the United States. One of the major keys to control the disease was the insistence that all birds in a flock needed to be tested. During the next 25 yr, the results of the development of the standardized test resulted in a tremendous reduction of the incidence of pullorum-positive birds (Table 2) (41).

The other outcome of this meeting was the decision to form a more formal group, which was named the Northeastern Conference of Laboratory Workers in Bacillary White Diarrhea or NCLWBWD. The name of the organization changed over the years and is now known as the Northeastern Conference on Avian Diseases, or NECAD. As mentioned before, the history of NECAD and the importance for the establishment of the American Association of Avian Pathologists and the journal *Avian Diseases* will be discussed in detail elsewhere (Calnek and Schat, manuscript in preparation). The second meeting was held at Yale University with 12 participating groups with the purpose to further standardize the agglutination test. During this meeting, Rettger proposed to

Table 2. Test results for pullorum disease presented at the 1953 NECAD meeting (adapted from the minutes of the 1953 meeting (41)).

State	Historical data			Results reported in 1953		
	Year	No. birds tested	% Positive	Year	No. birds tested	% positive
Connecticut[A]	1925	20,743	2.40	1952	630,018	0.006
Delaware	1925	4300	5.70	1953	546,379	0.021
Massachusetts[A]	1921	24,718	12.50	1953	1,155,359	0.04
Maryland	1927	3725	21.00	1953	815,250	0.18
Maine[A]	1921	2730	22.30	1953	1,365,314	0.027
North Carolina	1932	64,702	4.02	1953	1,668,830	0.056
New Hampshire[A]	1926	35,237	2.50	1953	1,512,219	0.00006
New Jersey	1926	52,611	7.86	1953	1,025,449	0.035
New York	1926	59,576	6.2	1953	810,619	0.0035
Pennsylvania	1924	2077	15.00	1952	1,882,712	0.20
Rhode Island[A]	1925	8175	6.97	1952	61,948	0.00
Virginia	1925	13,000	20.0	1952	1,001,364	0.37
Vermont[A]	1928	8555	7.4	1953	234,282	0.09
West Virginia	1928	9005	6.0	1952	201,968	0.069
Nova Scotia	1929	2041	7.0	1952	81,357	0.0
Ontario	1928	15,000	8.0	1952	1,086,026	0.05

[A] States participating in the first meeting in 1928.

change "Bacillary White Diarrhea" to "Pullorum Disease," which was accepted by unanimous vote of the participants.

ADVANCES IN SEROLOGIC TESTING METHODS

As mentioned before, the tube agglutination test originally developed by Jones (37) at Cornell University in 1913 remained in continual use and proved valuable in control and elimination of the disease. The tube test, referred to as the standard tube test by Graham *et al.* (42), consisted of mixing 0.04 ml of serum with 1 ml of the *Salmonella* Pullorum antigen solution in 0.85% saline with 0.5% phenol and reading the reactions after 42–48 hr at 37 C. The antigen concentration was based on using the McFarland nephelometer standard 1 turbidity. Clearly, this test could not be used in the field, preventing widespread use at the national level. The subsequent development of fast tests had a significant impact on control of the disease. Runnels *et al.* (43) in 1927 reported the first rapid serum agglutination test, but this test was not used extensively, probably because of the need to separate the serum from blood prior to testing. Their test used for the first time a glass plate on which antigen was mixed with serum. Positive serum caused a rapid agglutination and within 5 min results could be read. Placing the glass plate on a box with black walls facilitated reading the results. This approach was based on the serologic test used for the detection of reactions against *Brucella abortus*, the cause of Bang's disease in cattle (44). In 1929, Bunyea *et al.* (45) developed a whole blood test using live culture for an antigen. The use of live culture was obviously a disadvantage for this test. Hence, in 1931 the above workers (46) and Coburn and Stafseth (47) at Michigan State College independently developed a stained antigen whole blood test. Graham and Thorp (48) developed a tube test that could be used in the field, which was also based on a similar test for the detection of Bang's disease. This test used whole blood that could be added in the field to previously prepared tubes containing antigen, but it required a 72-hr incubation before the results could be read. Graham *et al.* (42) evaluated the standard test tube, the field test tube, and the rapid plate methods, with or without stained antigen, using blood samples from different flocks. Based on comparisons using 20,719 samples from breeding birds in Illinois, the authors concluded that the rapid whole blood antigen test agreed at 72%–90% with the standard tube test. Repeated tests of the flocks increased the degree of agreement to 98%. Clearly, the ease of the whole blood test using stained antigen was a major contribution to control and elimination of the disease. It was simple, rapid, accurate, and could be used effectively in the field (Fig. 3). This test is still being used for the verification of the salmonella status of flocks participating in the U.S. National Poultry Improvement Plan (49).

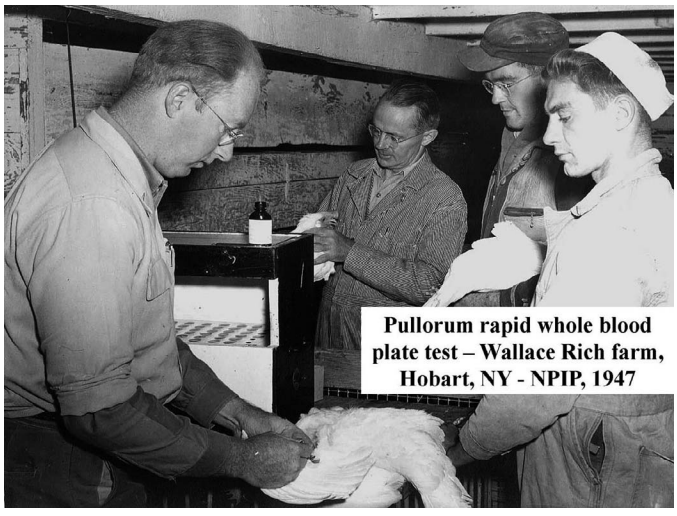

Fig. 3. The pullorum rapid whole blood test using stained antigen at a farm in NY State in 1947. This test is still in use.

IMPACT OF PULLORUM DISEASE ON POULTRY PRODUCTION IN THE EARLY 1900S

After the first isolation of *Salmonella* Pullorum (4), it became clear that BWD was an important problem that was rapidly increasing in North America. Reports of the disease in most parts of the United States and Canada are reviewed by Bushnell *et al.* (10). It is not surprising that the disease became more and more important based on early serologic data. After Jones published his macroagglutination

test to detect seropositive chickens (38), Rettger and associates started a systematic campaign against pullorum disease in 1914 that continued for several years. The campaign was in first instance directed to large-scale testing programs for the presence of what was called "immune bodies" in the hope to find enough negative breeding flocks or to develop negative breeding flocks. In the first year, 107 flocks were tested for a total of 13,831 hens and 786 male birds, with 1413 (10.24%) and 11 (2.9%) positives, respectively (50). Importantly, 2/11 seropositive males were also positive for *Salmonella* Pullorum in the testis, one of which was also positive in the pericardial sac. An additional three males were also positive for *Salmonella* Pullorum in the pericardial sac. This was an important finding, suggesting that males can be transmitting the infection. The distribution of the flock sizes is also of interest in relation to the number of positive birds. In total, 79/107 (73.8%) of the flocks had positive birds, with the following results for the different type of flocks: <25 birds: 13/23 (56.5%), between 25 and 50 birds: 10/17 (58.8%), between 50 and 100 birds: 20/26 (77%), between 100 and 500 birds: 32/36 (88.9%), and >500 birds: 5/6 (80%). Clearly this large percentage of positive adult flocks was contributing considerably to the spread of the disease.

The results for the next period (1915–1916) were only marginally better. Of the 6262 hens and 96 males tested for the first time, 515 (8.2%) hens were positive while all males were negative (51). In 1919, Rettger *et al.* (52) published the following results for the period 1915 and 1916: 21,317 hens and 1037 males were tested with 9.3% and 2.1% positives, respectively. The number of positives in a flock ranged from 0% to 56.3%! Hatchability of 293,580 eggs set was a low 56.7%, and mortality during the first 3 wk was 10.2%. Similar results were obtained in Massachusetts when testing 1251 birds with 21.5% positives from several regions of the State. Kansas started extensive testing in 1921, and over a 4-yr period tested birds from 227 flocks, resulting in 35% positive birds with 8220 birds tested (10). Based on approximately 75% of the flocks testing positive, with an estimated 25% of the birds being carriers of *Salmonella* Pullorum and a total poultry population of 16 million birds, the authors estimated that about three million birds would be carriers in Kansas. Similar incidences in numbers of positive flocks and positive birds within flocks have been reported for several other states (reviewed in 10), clearly indicating the major impact of this disease on the poultry production during the first 25 years of the 20th Century.

DEVELOPMENT OF CONTROL METHODS TO ELIMINATE PULLORUM DISEASE

From the previous sections it is obvious that controlling the disease was of imminent importance for the further development of the poultry industry in the United States and Canada. Yet, until the development of reliable detection methods of carriers, there were very few tools available to control the disease. The disease was rapidly spreading into new areas through shipment of eggs with perhaps only a few of the eggs infected with *Salmonella* Pullorum. Cleaning and disinfecting incubators between hatches was important to reduce the risk of infection but would not prevent spread of the disease if positive eggs would be incubated. The selection of appropriate methods to disinfect incubators and brooders was problematic. In his first paper on pullorum disease, Rettger had examined the effects of common disinfectants on the viability of *Salmonella* Pullorum (4). For example, a 1:220 dilution of carbolic acid (phenol) or 1:60,000 dilution of corrosive sublimate ($HgCl_2$) killed *Salmonella* Pullorum in bouillon cultures kept at 37 C in 2 hr. Jones (15) used a mixture of 1 part phenol, 1 part sulphuric acid (H_2SO_4) in 20 parts of water to spray brooders used in his experimental work with *Salmonella* Pullorum. General advice for disinfection of incubators, brooders, etc., was provided in *Diseases of Poultry* by Pearl, Surface and Curtis published in 1915 (53). Methods included the use of phenol and interesting formaldehyde gassing by using 23 ounces of permanganate with 3 pints of formaldehyde to each 1000 cubic feet of space.

Attempts made by Jones (32) to vaccinate chicks with killed preparations of *Salmonella* Pullorum followed by challenge 24 hr afterward did not result in protection. This result is not surprising, based on our current knowledge of immunology, but it was certainly a worthwhile attempt in 1911–1912. In the same publication, treatment efforts were reported using a mixture of sulfo-carbolates ($C_6H_5NaO_4S$) and creosote, but without success. Apparently, this combination had been reported for treatment of fowl cholera and diarrhea, but Jones did not provide a reference for this statement. The use of sulfa-carbolates was apparently not unusual and is mentioned in the 1915 *Diseases of Poultry* book (53). Additional chemicals tested for control of pullorum disease were potassium permanganate 1:1000 and 1:3000, mercuric chloride (HgCl2) combined with sulfa-carbolates, hydrochloric acid (HCl 1:250), recorcin (one of three isomeric benzenediols, $C_6H_4(OH)_2$), and hypochlorite. These components were given instead of water as the source of liquids! Not surprisingly many of these components were toxic for the chickens, and only hypoclorite with 0.02% chlorine seemed to have some beneficial effect (11).

In 1908, Eli Metchnikoff published his book "The prolongation of life," which was translated by R.C. Mitchell into English (54). In this book Metchnikoff claimed that yoghurt-like milk products containing *Bacillus bulgaricus* (current nomenclature: *Lactobacillus bulgaricus*) was responsible for the long lives of populations using these products and that it would provide protection against diarrhea. Bushnell and Maurer in 1913 (55) decided to determine if feeding newly hatched chicks *L. bulgaricus* would prevent the development of pullorum disease. In their study they indeed claimed a beneficial effect. At the same time, Rettger *et al.* (26) were also influenced in 1912 by the ideas of Metchnikoff. In several experiments using "sour" milk, an apparent benefit was noted in the control of pullorum disease. However, in a subsequent paper published 2 yr later, Rettger *et al.* (33) failed to confirm the earlier studies. Using the recently described agglutination test and pathology, they concluded that there was no benefit in using sour milk for the control of pullorum disease (Table 3). Subsequently, Rettger *et al.* (56) compared the effects of feeding of "sweet" or "sour" milk *vs.* the absence of milk in the feed (controls). Both sweet and sour milk led to increased weight gain compared to the controls, which was independent of the infection status of the chicks. Likewise, overall mortality from all causes was reduced in chicks fed either type of milk, and the effect was not linked to the presence of *Lactobacillus.* Feeding of milk to young chicks continued for a while (e.g., see 13).

Another approach to control the disease was to determine if genetic resistance could be used to reduce the incidence. Roberts and Card (13) tried to develop resistant birds starting with different breeds. Over a 3-yr period they used >3600 chicks representing four different breeds, one of which (white Plymouth Rock) consisted of an inbred and noninbred group. Chicks were challenged with rather high doses of *Salmonella* Pullorum. They concluded that there was

Table 3. Effect of sour milk on the incidence of pullorum disease and comparison of the agglutination test and lesions in the ovary.

Experiment	Exposure		Fed "sour" milk	No positive/tested[A]	
	At age in hours	*Salmonella* Pullorum		Agglutination test	Ovary lesions
1	Very young	+	+	3/14	3/14
	Very young	+	−	1/12	1/12
	Very young	−	+	0/12	0/12
	Very young	−	−	0/12	0/12
2	24–36	+	+	1/16	1/16
	24–36	+	−	4/12	4/12
	48–60	+	+	7/22	7/22
	48–60	+	−	5/12	5/12
	48–60	−	+	1/17	1/17
	48–60	−	−	0/16	0/16

[A]Birds with a positive agglutination test had also typical pullorum lesions in the ovary (33).

an indication that natural resistance to infection could provide a basis to establish a resistant line. However, follow-up publications were not found, and this approach became irrelevant based on the elimination of carrier hens.

After the finding that *Salmonella* Pullorum was spread in part through infected eggs from carrier hens, a potential method to control and eventually eradicate the disease became feasible. In the first instance carrier hens were identified by collecting eggs from individual hens using trap nests. Eggs were incubated preferably for at least 1 wk and examined for the presence of bacteria using the different culture methods (26,57). However, the sometimes infrequent presence of *Salmonella* Pullorum in the eggs made this approach problematic, and it was not until the development of the agglutination test to detect carrier hens that it became feasible to start eradication programs (33,34).

THE NATIONAL POULTRY IMPROVEMENT PLAN (NPIP)

In September 1923, the Washington Hatchery and Breeders Association, later known as the Washington Poultry Improvement Association (WPIA), started the Record of Performance (R.O.P.) program using trap-nesting to improve poultry production (58). This was the first official state-associated trap-nesting program in the United States with, in first instance, a focus on improvement of the breeding results by selecting for increased egg production. In 1925, WPIA started testing for pullorum disease and made it a requirement that all flocks in the R.O.P. program were tested. This was formalized in 1926, requiring that 100% of the breeders and cockerels needed to be tested, although this requirement was somewhat relaxed in 1928 due to the high costs of testing (12 cents/bird), although several members were in favor of testing 100% of the birds. Around that same time (1927), Dr. Jull of the U.S. Department of Agriculture participated in a meeting of the WPIA discussing the nation-wide attempt to adopt and put into operation some national poultry improvement plan. It took eight more years before the NPIP came into existence in 1935 to provide a cooperative industry, state, and federal program through which new diagnostic technologies could be effectively applied to the improvement of poultry and poultry products throughout the country (58). The original NPIP was clearly based on the program initiated by the WPIA, with a major focus on selecting hens producing more than 200 eggs/hen/season, while the average production was around 80 eggs/hen. The second important reason for the NPIP was to develop a national approach for the control of pullorum disease, which could cause up to 80% mortality. A lengthy document was developed to outline the total program, with major revisions discussed and instituted during the Conference on the National Poultry Improvement Plan in 1936 (Chicago, May 25–29) (59). Each state could participate in the program as long as the states would adhere to all regulations of the program, which were quite extensive. For example, "State Pullorum Testers" needed to have good vision, endurance, patience, thoroughness, integrity, and knowledge of poultry husbandry. Specific serology tests were described in detail, with interpretations of tests using pullorum stained antigen, rapid whole blood plate antigen. Relevant for this review are suggested changes specifically related to pullorum control, which were published in 1936 by the U.S. Department of Agriculture (60). The original goal of control of pullorum disease was changed to eradication approaches during the conference. Other important changes adopted in 1936 were the requirement that all pullorum disease control work be done by accredited veterinarians and that changes in pullorum qualifications were badly needed. The original document (which unfortunately has not been preserved) described a confusing array of qualifications such as U.S. Approved Pullorum-tested; U.S. Approved Pullorum-Passed, or U.S. Approved Pullorum-Clean. The term "Approved" was sometimes substituted by "Certified" or "R.O.P." The differences between these categories were not clear in the updated document but apparently left the possibility of using flocks that were not 100% negative. Following van Roekel (40), the term "pullorum-tested" was used for flocks with less than 10% reactors, a qualification that was not acceptable to the NECAD workers. During the period of 1937 to 1940, NECAD adopted several resolutions to propose changes in the pullorum control program of NPIP. It was not until 1943 that it became an official requirement and prerequisite for participation in a breeding phase of the NPIP that the birds had to be officially tested for pullorum disease. When the NPIP started in 1935, only 58.2% of the birds hatched were officially tested for pullorum disease, with 34 states participating. The percentage of birds tested continued to increase over the years, reaching 96.1% in 1942. The number of states adopting control programs for pullorum increased from 2 states in 1920 to 45 in 1942, and the volume of testing increased from 1 million birds in 1920 to 18 million in 1942 and over 30 million in 1948, revealing 1.8% reactors with 47 states participating. In that year, the NPIP also updated the requirements for the pullorum program. The qualification of U.S. Pullorum-Tested, in which all breeder chickens over 5 mo were tested and had between 3% and 8% reactors, would be deleted from the plan after

the 1948–1949 hatching season. The category of U.S. Pullorum-Controlled would allow up to 2% reactors, while U.S. Pullorum-Passed would have no reactors, with the last test made within the testing year preceding the date of sale of hatching eggs or chicks from these flocks. Finally, the U.S. Pullorum-Clean status was given to flocks that had been tested not less than 6 mo apart or in three consecutive tests not less than 30 days apart within the testing year preceding the date of sale of hatching eggs or chicks from these flocks. All tests were done by people authorized under the NPIP guidelines (61). Some of the other highlights of the NPIP and NTIP included recognizing flocks free of *Salmonella* Gallinarum, the cause of fowl typhoid, based on pullorum disease testing. In 1961, 4.5 million turkey breeders were tested with only 0.003% reactors, which resulted in the classification U.S. Pullorum Typhoid (PT) clean (62). In the same year, the percentage of PT reactors in chickens reached a new low of 0.013% (63). A milestone was reached in 1967, when all products handled by all NPIP and commercial chicken hatcheries were 100% U.S. PT clean (64). By using antigens to *Salmonella* Pullorum in the blood tests, the NPIP not only eliminated pullorum disease, but also fowl typhoid because *Salmonella* Pullorum and *Salmonella* Gallinarum are both in the D serogroup of salmonellas, with antigens in common. It is obvious that the NPIP and the National Turkey Improvement Plan (NTIP) have contributed tremendously to the control and elimination of the disease.

Rules for hatchery sanitation were also a part of the NPIP program. Early studies in several states and in Canada illustrated the value of fumigation in preventing the spread of pullorum disease. Bushnell *et al*. (10) reported on the effectiveness of formaldehyde gas in controlling the organism in hatcheries. This seminal work was followed by a substantial amount of work by researchers and incubator manufacturers describing the details of the fumigation process. This was indeed a breakthrough in incubator and hatchery sanitation. The crowning achievement of all these efforts came in 1975 when no PT reactors were detected in the United States.

The pullorum disease control/elimination story serves as a prime example of what can be achieved when scientists, government, and industry cooperate to solve health issues.

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