The Role of *Chlamydia psittaci* in Outbreaks of Blepharo-Conjunctivitis in Chickens and Ostriches: Clinical and Laboratory Findings

Perelman, B.,[1]* Mechani, S.,[2] Perl, S.[3] and Lublin, A.[2]

[1] Kibbutz Beit Kama P.O. Box 38, D.N. Negev 85325, Israel.
[2] Division of Avian and Fish Diseases, Kimron Veterinary Institute Bet Dagan, Israel.
[3] Division of Pathology, Kimron Veterinary Institute Bet Dagan, Israel.

* **Corresponding author:** Dr. Beny Perlman, Kibbutz Beit Kama, P.O. Box 38, D.N., Negev 85325, Israel. E-mail address: benyperelman@gmail.com

ABSTRACT

Several outbreaks of severe purulent blepharo-conjunctivitis were observed sporadically in ostrich (*Struthio camelus)* and chicken flocks in Israel during the years 2008-2011. During these outbreaks of conjunctivitis in the ostriches, 30-70% present in the feed lot was affected. The acute muco-purulent conjunctivitis was followed in most of cases by severe corneal damage and blindness of the affected birds. Due to the extensive ocular pathology and blindness, most of the affected ostriches had to be culled thus causing vast economic losses to the farm. In heavy breeder replacement pullets, conjunctivitis and blepharitis have appeared sporadically in 5 different flocks of 5 to 12 weeks of age. Untreated flocks suffered from economic losses due to the ocular damage and condemnation of affected animals for breeding purposes.

Extensive laboratory work ruled out the presence of respiratory viruses including Infectious Laryngotracheitis (ILT), Newcastle Disease (ND), Avian Influenza (AI), Infectious Bronchitis (IB) and Avian Pneumovirus (APV). Bacteriology revealed only secondary contamination with non-specific bacteria. PCR tests for *Mycoplasma gallisepticum* proved to be negative in all the cases involved. PCR or Real Time PCR (RT-PCR) from direct swabs taken from the conjunctiva of affected birds proved positive for *Chlamydia psittaci.* Only a systemic treatment based on oxytetracycline or doxycycline and eye washing with a solution containing oxytetracycline provided recovery of the affected birds thus supporting the assumption that *Chlamydia* was the main causative agent of the blepharo-conjunctivitis in the ostriches and chicken affected flocks.

Key words: Chlamydia, Conjunctivitis, Blepharitis, Ostriches, Breeders.

INTRODUCTION

Avian Chlamydiosis is caused by the obligate intracellular Gram-negative bacteria *Chlamydia psittaci*. The genus *Chlamydia* includes six species: *C. psittaci, C. abortus, C. felis, C. caviae, C. pneumoniae* and *C. pecorum* (1). *Chlamydia psittaci* includes 9 serovars that have been reported in psittacines, pigeons, ducks, geese, turkeys, snowshoe hares and cattle (1-7). Based on their natural pathogenicity *C. psittaci* can be divided in two categories: highly pathogenic strains and low virulence strains. Highly pathogenic strains (serotype D) may cause a fatal disease characterized by septicemia, vascular congestion and high mortality. Low virulence strains (serotypes B and E) may be found sporadically in clinically healthy animals including pigeons, ducks and wild birds (1).

Chlamydia spp. have been associated with eye infections in different species (1, 2, 3, 4, 5, 6). *Chlamydia trachomatis* causes blepharitis in humans (8) while *C. psittaci, C. pecorum* and *C. felis* have been reported to cause blepharitis and conjunctivitis in birds, guinea pigs and cats, respectively (1, 2, 3, 4, 5). *C. psittaci* has been identified in young ostriches suffering from septicemia (9). Despite the fact that *C. psittaci* can be found sporadically in different species of

domestic and wild birds it is rarely involved in outbreaks of clinical disease.

In this report we describe various cases of outbreaks of Chlamydiosis in chickens and ostriches; in all the cases described here the clinical and pathological picture was characterized by mucopurulent blepharo-conjunctivitis without any signs of septicemia or involvement of internal organs. In all the cases described in this paper the disease occurred in flocks involving thousands of birds (chickens and ostriches). Differential diagnoses included the most common infectious agents causing respiratory and eye infections such as *Mycoplasma gallisepticum* (MG), Newcastle disease (NDV), Infectious Laryngotracheitis (ILT), Infectious Bronchitis (IB) and Avian Pneumovirus (APV-TRT). In this paper we discuss the role of the *C. psittaci* as the primary pathogen involved in cases of blepharo-conjunctivitis in chickens and ostriches.

MATERIALS AND METHODS

Clinical description of outbreaks

Ostriches

Data was collected from 4 severe outbreaks of blepharo-conjunctivitis that appeared in the same ostrich feedlot between the years 2006-2010. The feedlot was located in a semi-desert area in southern Israel where sand storms are common during the summer and autumn. At the time of the outbreaks about 4000 ostriches from 3 to 10 months of age were confined in open lots separated by fences. Water was provided as fresh water from a pipe line and feed was based on a balanced diet of feed formulated specially for ostriches consisting of feed mixed with corn, wheat silage or alfalfa hay (Nir-Oz Feed Mill, Israel).

Ostriches were kept in the feedlot in groups of 150-300 ostriches according to their age. All the ostriches were vaccinated against Newcastle disease, Avian Pox and LPAI H9N2 with commercial vaccines (Abic Ltd., Israel). All three vaccines were applied at 3 and 8 weeks of age before moving the birds to the feedlot. The ostrich chicks were moved to the feedlot at 12 weeks of age and separated in different lots according to their age.

On several occasions and usually after sandstorms, some ostriches started developing blepharo-conjunctivitis that in most cases developed into a severe mucopurulent blepharo-conjunctivitis with secondary corneal keratitis and ulceration. Within a short period of time this clinical syndrome seemed to spread among many ostriches within some lots. All ages were affected but some pens or lots were more affected than others. While in some pens the morbidity was relatively low with a few ostriches affected, others suffered from high numbers of affected ostriches often reaching more than 50% of the birds in a pen.

Chickens

Five sporadic cases of conjunctivitis were observed between 2008 and 2011. The cases were observed in different farms and locations at different times, but the clinical picture in all the affected flocks was almost identical. Four out of the five outbreaks occurred in heavy breeder replacement flocks between the ages of 9 and 18 weeks, and only one case was observed in a broiler flock at the age of about 39 days. In most of the cases one or two houses were affected while the rest of the chickens in the other houses remained healthy. The outbreaks always started with a few birds within the flock showing some degree of conjunctivitis reaching 50%-80% morbidity within 5-10 days. In untreated flocks 10 to 20% of the affected birds developed severe mucopurulent blepharo-conjunctivitis. No significant increase of mortality was observed in the affected flocks. Post mortem examination of dead or culled animals did not reveal any macroscopic pathological changes in the internal organs of affected birds.

From all the described cases, swab samples (sinus, trachea and eyelid) from affected birds at different stages of the conjunctivitis were taken. The swab samples and affected birds were submitted to the Kimron Veterinary Institute, Beit Dagan, Israel, for laboratory examination.

Laboratory tests

Laboratory testing was performed on the swabs and samples taken from the affected ostriches and chickens. The laboratory tests included isolation and identification of pathogenic bacteria, attempts to isolate pathogenic bacteria were carried out in Blood agar plates, Chocolate agar plates and MacConkey agar (Difco, Israel) under aerobic and anaerobic conditions as described (7).

Viral isolation and identification tests (PCR) were carried out for ILT, NDV, IB, and APV (10, 11, 12, 13, 14). *Mycoplasma gallisepticum* presence was based on PCR tests (15), *Chlamydia psittaci* identification was based on regular and RT (Real Time) PCR (16, 17). Due to lack of molecular

tools for serovar definition at our laboratory during the time period of these cases, *Chlamydia* identification was limited to species and not to the serovar.

Direct immunofluorescence (DIF) tests were carried out from swabs taken from the conjunctiva of affected birds: the swabs were smeared with PBS on a slide, fixed with cold acetone and stained with fluorescein-marked monoclonal antibodies against chlamydial lipopolysaccharides (18).

Specific antibodies against *Chlamydia* were detected using the ImmunoComb–test (ImC) for *Chlamydia* (Biogal Galed Labs. Israel). Serological test for detection of chlamydial antibodies in serum was conducted using the ImmunoComb Avian *Chlamydia psittaci* Antibody Test Kit (19).

Histopathology was conducted on eyelid specimens from affected birds. The samples were fixed in 10% formaldehyde solution, stained with haematoxylin and eosin (H&E) and examined under a light microscope.

RESULTS

Ostriches

At first cases of conjunctivitis were presumed to be caused as a result of sand storms resulting in the presence of sand in the eyes of the ostriches and subsequently secondary bacterial infection. The treatment applied to the affected ostriches was based on washing the eyes of the affected animals with a solution of povidone (Polydine, Dr. Fisher, Israel) and the administration of eye drops containing polymixin and steroids (Vi-Polyxine, Abic, Ltd.). Some ostriches were injected with antibiotics such as penicillin–streptomycin (Pen-Strep 20/25, Eurovet, Heusden-Zolder, Belgium) or amoxicillin (Octacillin, Eurovet, Heusden-Zolder, Belgium) or treated with enrofloxacin (Enrabic, Abic, Ltd., Israel) in the drinking water or by direct administration after gastric intubation.

In most of the cases the problem seemed to spread very rapidly among the ostriches in the same pen becoming a serious outbreak within one or two weeks. In some of the most severe outbreaks, as many as 50% of the ostriches present in the pens were affected, with a culling percentage of more than 60% due to further development of corneal perforation and blindness in both eyes.

Swab samples from the eyelids of affected birds at different stages of the conjunctivitis were taken for laboratory examination at the Kimron Veterinary Institute in Beit Dagan Israel for identification of potential pathogens involved.

Clinical and pathologic examination of affected ostriches revealed only severe mucopurulent blepharo-conjunctivitis and corneal keratitis (Figures 1 and 2). Post mortem examination of affected ostriches did not reveal any signs of pathological changes in the internal organs of any of the ostriches examined, in some ostriches signs of secondary dehydration were observed apparently due to the fact that affected ostriches could not reach the sources of water in the pen.

Bacteriological tests from affected eyes revealed only secondary contamination of bacteria including *E. coli* and *Pseudomonas* spp., in some samples *Staphylococcus albus* and *Staphylococcus auricularis were* isolated. The appearance of these bacteria was random and sensitivity tests to these bac-

Figure 1: Six months old ostrich showing the first stages of the blepharitis and conjunctivitis with foamy exudates in the eye.

Figure 2: Six months old ostrich showing a more advanced mucopurulent blepharo-conjunctivitis., at this stage of lesion most of the ostriches suffered from chronic corneal damage and loss of sight.

teria groups showed susceptibility to polymyxin, penicillin-streptomycin and amoxicillin or enrofloxacin.

However, treatment of affected ostriches with these antibiotics either locally or systemically did not cause result in improvement.

Attempts to isolate different avian viruses were carried out by injecting embryonated SPF eggs with the suspensions obtained from the samples taken from the conjunctiva of affected ostriches at different stages, the suspensions were prepared by shacking the swabs thoroughly in a vortex after adding in 3-5 ml of sterile PBS containing penicillin and streptomycin. The suspensions obtained were injected in 10 - 11 days incubated embryos by the Chorioallantoic Membrane (CAM) and the Allantoic Sac inoculation method as described (10). Inoculated embryos were incubated for 6 days after inoculation and examined for any sign of a viral infection. The allantoic fluid was tested for hemagglutinating viruses (NDV, Avian influenza, Paramyxoviruses) and the Chorioallantoic membrane was examined for any pathologic changes suggesting Poxvirus or Laryngotracheitis virus. All attempts to isolate any avian viruses proved negative.

DIF tests of samples taken from the conjunctiva of affected ostriches revealed that more than 60% of the samples were positive to *Chlamydia psittaci*, however PCR tests were negative, About 60% of the serum samples were positive for the presence of specific antibodies to *Chlamydia psittaci* using the ImmunoComb test.

Based on the preliminary laboratory findings, affected ostriches were treated by eye washing with a solution containing oxytetracycline at 1 gram/liter and systemic treatment with doxycycline at 40 mg/Kg body weight which brought a dramatic improvement in those ostriches where the injury was acute or sub-acute.

Therapeutic and prophylactic treatment in the drinking water of all the ostriches in the feedlot with doxycycline at 30 grams/ton body weight for a period of 5-7 days caused a dramatic improvement with complete recovery of the ostriches within 7-10 days.

Appearance of blepharo-conjunctivitis in further lots of ostriches were immediately treated with oxytetracycline or doxycycline in the drinking water for periods between 5-7 days with a very rapid recovery of the affected animals and complete cessation of the spread of the disease among other ostriches.

Chickens

Clinical and post mortem examination of affected animals did not reveal any pathological changes indicative of a systemic disease. The clinical picture was characterized only by different degrees of blepharo-conjunctivitis varying from slightly congested to severe swollen eyelids and conjunctiva and corneal keratitis (Figures 3 and 4).

Bacteriological tests revealed only secondary contamination and isolation of common bacteria such as *E. coli, Pseudomonas* and *Staphylococcus* spp.

All the PCR and Virus isolation attempts proved negative for NDV, ILT, Pox, IB, and APV either by PCR or isolation attempts in SPF chicken embryos. DIF and PCR

Figure 3: Severe conjunctivitis in a 7 weeks old replacement pullet, edema of the eyelid and corneal keratitis can also be observed.

Figure 4: Severe blepharitis and conjunctivitis observed in a 7 weeks replacement pullet. Swollen edematous and hemorrhagic mucosa were the most common clinical finding in this cases.

were positive for *Chlamydia psittaci* in four tested flocks. ImmunoComb test proved positive in the sera of the affected flocks with one of the flocks having 100% positive sera to *Chlamydia psittaci*. The only *Chlamydia psittaci* case observed was in a 5-week-old broiler flock at the time of slaughter presenting unilateral keratitis with foci of white-colored patches within the lens. This flock was found negative for *Chlamydia psittaci* by DIF test and by PCR but positive by the real-time RT-PCR test.

Histological examinations

Specimens for histology were taken from the eyes and conjunctivae of the replacement broiler breeder pullets and those of the broiler flock.

In replacement pullets, multifocal infiltration of heterophils and histiocytes and fibrinoid material were observed around the sclera. Necrosis and prominent edema were observed in the eyelid mucosa, in addition to substantial multifocal infiltration of mononuclear cells and heterophils, the inflammation was limited to the conjunctiva and the outer layers of the eyelid without spreading inward to the globe of the eye (sub-acute conjunctivitis) (Figure 5).

In broilers, diffuse infiltration of neutrophils and histiocytes and areas of necrosis were observed in the eyes, with secondary infection of bacilli that were seen, both in the conjunctiva and in the inner parts of the eye including the lens (conjunctivitis and panophthalmitis) (Figure 6).

DISCUSSION

Chlamydia spp. have been involved in cases of respiratory and systemic disorders in birds and mammals including humans (1, 2, 4, 20, 21). In most of the cases attributed to Chlamydiosis in birds, the clinical picture usually involves

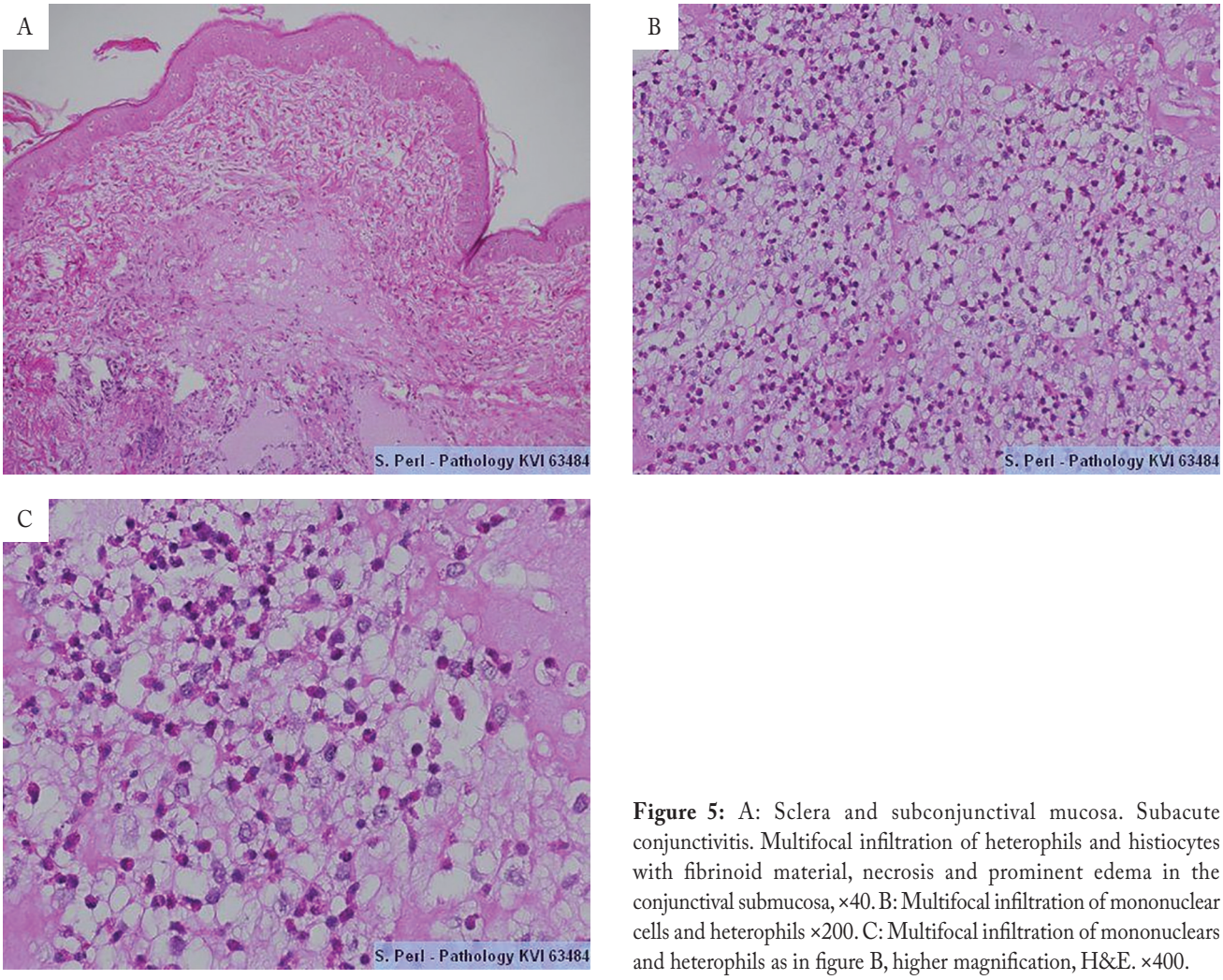

Figure 5: A: Sclera and subconjunctival mucosa. Subacute conjunctivitis. Multifocal infiltration of heterophils and histiocytes with fibrinoid material, necrosis and prominent edema in the conjunctival submucosa, ×40. B: Multifocal infiltration of mononuclear cells and heterophils ×200. C: Multifocal infiltration of mononuclears and heterophils as in figure B, higher magnification, H&E. ×400.

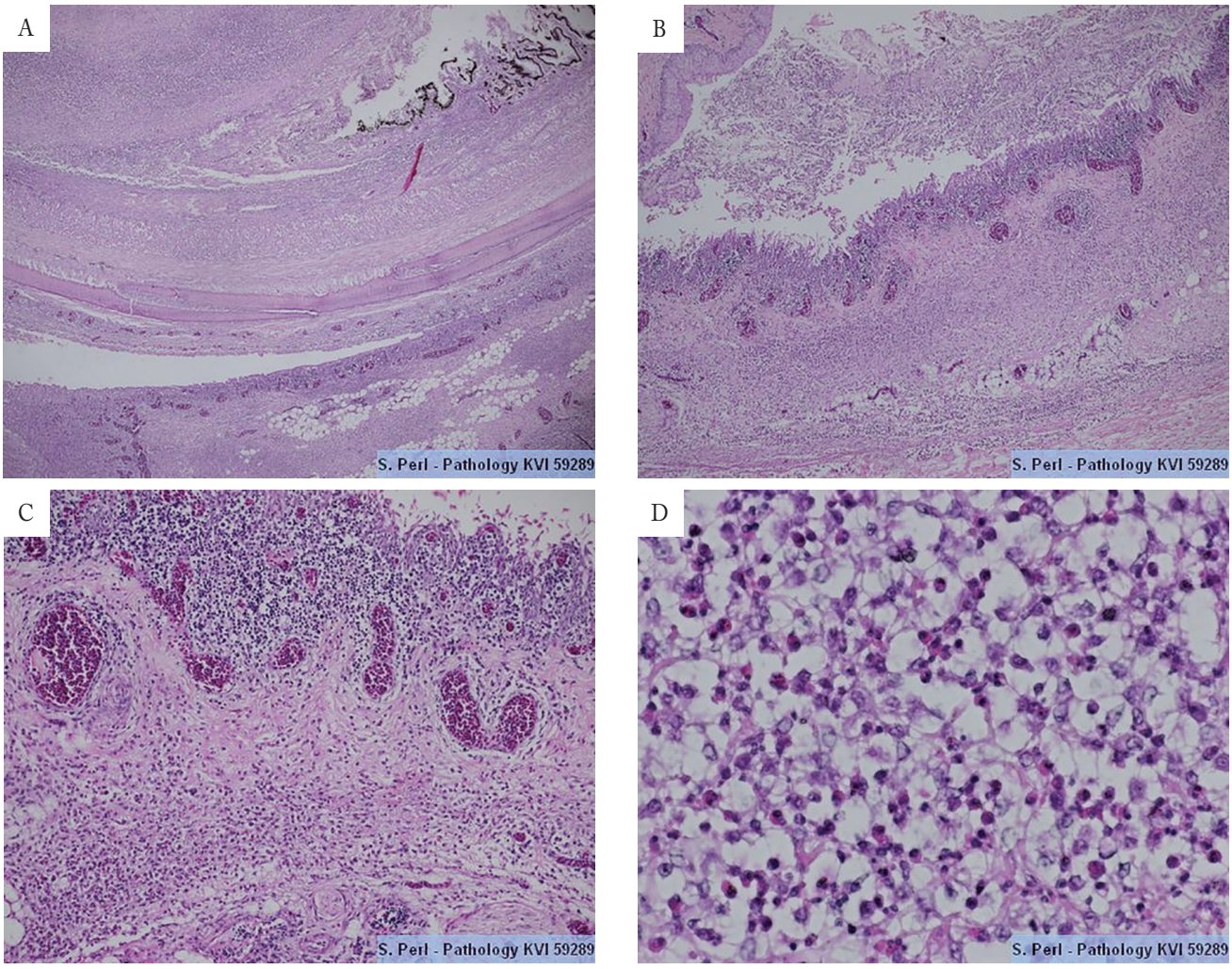

Figure 6: Diffuse infiltration of heterophils and histiocytes and areas of necrosis were observed in the eyes, with secondary infection of bacilli, both in the conjunctiva and in the inner parts of the eye including the lens (conjunctivitis and panophthalmitis). A: Panophthalmitis. Heterophilic and histiocytic infiltrates in the eye, ×20. B: Conjunctivitis. Infiltrates in the squamous epithelium of the mucosa and the submucosa of the eyelid, with normal epithelium on the upper left hand side of the slide. Cell debris with inflammatory cells are also seen, ×40. C: Conjunctivitis. Mucosa and submucosa of the eyelid severely infiltrated with a large number of inflammatory cells with congested blood vessels, ×100. D: Inflammatory infiltrates at a higher magnification, H&E. ×400.

the respiratory system causing a systemic acute or chronic disorders (1). In ratites, *Chlamydia* spp. has been sporadically isolated from young ostriches and Rheas suffering from systemic infections affecting the liver and the spleen, however the isolation of *Chlamydia* from various organs and it's relation to the clinical and pathological picture has not been clarified (6, 22).

Different species of *Chlamydia* such as *Chlamydia psittaci* and *Chlamydia pecorum* may be related to eye infections in birds, lambs, goats, and guinea pigs (1, 3, 4, 20, 23). *Chlamydia felis* (previously *Chlamydia psittaci* var. felis) has been found to cause conjunctivitis in cats (2) Trachoma and inclusion conjunctivitis in humans is caused by *C. trachomatis* whereas *Chlamydia pneumonia* has been documented to be involved in cases of conjunctivitis sicca in humans (21).

It appears that *Chlamydia* may be present in the mucosa of the eyes and respiratory tract of birds and mammals without causing any disease, however under certain conditions probably related to viral infections, stress or other factors the *Chlamydia* may become a serious pathogen.

In the present case no history of Chlamydiosis was observed previously at any age in the breeding flock or the ostrich chicks during the rearing or growing period. In this specific farm ostriches were reared up to 3 months of age and

then moved to a feedlot located about 20 km away. Ocular disorders have been observed in ostriches in that feedlot as a sporadic problem affecting only a few animals every season, however most of the problems observed previously included conjunctivitis that in many cases were apparently considered to be related to sand storms. Washing the eyes of affected ostriches and local treatment with polymyxin and steroids as eye drops usually brought about complete recovery of the affected animals.

During the years 2006 to 2010 the eye problem became more severe with some pens displaying morbidity greater than 50%. Ostriches within those pens were affected by severe blepharo-conjunctivitis and treated by eye washing and polymyxin B, penicillin, and amoxicillin antibiotics without any improvement related to the treatments. More than 60% of the affected ostriches had to be culled due to perforation of the cornea and bilateral blindness.

The differential diagnosis of the mucopurulent blepharo-conjunctivitis included infections including *Mycoplasma* (24), *Staphylococcus aureus*, and *Chlamydia* spp. and respiratory viruses such as avian Pneumoviruses (TRT), Paramyxovirus (NDV), Avian Influenza (AI) viruses, Pox virus, Larygotracheitis virus (ILT) and IB virus (1, 10).

The only pathogen consistently found from the samples taken was *Chlamydia psittaci* that was identified using the techniques of DIF, PCR or RT-PCR and ImC. Although DIF may reflect false positive results, PCR and RT PCR are very sensitive and specific techniques thus strengthening the definitive diagnosis. As *Chlamydia psittaci* was the only consistent potential pathogen found in the samples from the ostriches including a seropositive antibody reaction, a more specific treatment was applied to treat the eye infections including local washing of the eyes with a solution of 0.1% oxytetracycline and a systemic treatment with a solution of doxycycline at 30-40 mg/kg body weight in drinking water or by gastric intubation in the affected animals that could not reach water by themselves. All the ostriches in the feedlot were treated with the doxycycline solution as a prophylactic measure. The response to the local and systemic treatment was good with most of the affected ostriches recovering within 7 days. Animals that lost both eyes were culled, but most of the ostriches in the acute phase of the conjunctivitis recovered completely.

The administration of doxycycline or oxytetracycline in the drinking water as a prophylactic measure gave excellent results and the spread of the disease ceased within a week. Treatment in the drinking water was repeated for 5 days once a month during the growing season in order to prevent a relapse of the problem. Further cases of blepharo-conjunctivitis in the feedlot were immediately treated as described above.

The fact that *Chlamydia psittaci* was the only pathogen consistently identified in samples of affected ostriches and the rapid recovery of the affected animals after treatment with oxytetracycline and doxycycline strongly suggested that *Chlamydia psittaci* was the main cause of the severe blepharo-conjunctivitis outbreaks observed in the ostriches.

The clinico-pathological process that was observed in the five outbreaks of conjunctivitis in chickens, all of them belonging to heavy lines, was very similar to the clinical picture in the ostriches thus making a preliminary diagnosis more straightforward. Four out of the five cases reported, occurred in heavy breeder replacement pullets at different locations and time, however any correlation to a specific parent flock has not been found, The fact that any other bacteria including *Mycoplasma gallisepticum* or viral agents could be identified in any of the affected flocks and the rapid response in all affected flocks to the treatment with doxycycline or oxytetracycline suggests that *Chlamydia psittaci* was the infectious agent related to the eye infection outbreaks.

Chlamydia psittaci is considered a sporadic pathogen in birds. Isolation of *Chlamydia* spp. *per se* is not an indication of a pathologic situation. It is important to consider all the clinical and pathological findings and perform differential diagnostic tests before making a conclusion about the role of the *Chlamydia* as part of the etiology.

The cases described in this paper strongly suggest a direct role of the *Chlamydia psittaci* in the blepharo-conjunctivitis process in the chickens and ostriches. *Chlamydia psittaci* etiology must be taken into account in any case of eye infection and blepharo-conjunctivitis outbreaks in poultry and ostriches. No reports of infection with *Chlamydia* among the people working closely with the affected birds were observed but due to the zoonotic nature of *Chlamydia psittaci* (25, 26) the potential hazard to humans should also be taken into consideration.

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