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Serosurvey Of Selected Avian Pathogens In Brazilian Commercial Rheas (*Rhea americana*) And Ostriches (*Struthio camelus*)

■ **Author(s)**

Freitas Neto OC de[1]
Carrasco AOT[2]
Raso TF[3]
Sousa RLM[4]
Berchieri Júnior A[1]
Pinto AA[1]

[1] Department of Veterinary Pathology, Universidade Estadual Paulista, Jaboticabal, São Paulo, Brazil
[2] Department of Veterinary Medicine, Universidade Estadual do Centro-Oeste, Guarapuava, Paraná, Brazil
[3] Department of Pathology, Universidade de São Paulo, São Paulo, São Paulo, Brazil
[4] Department of Basics Sciences, Universidade de São Paulo, Piracicaba, São Paulo, Brazil

■ **Mail Address**

Adriano Oliveira Torres Carrasco
Departamento de Medicina Veterinária
Universidade Estadual do Centro-Oeste
85.040-08. Guarapuava, Paraná, Brazil
Telefone: +55 42 36298216

E-mail: adriano.carrasco@gmail.com

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ABSTRACT

Ratite farming of has expanded worldwide. Due to the intensive farming methods used by ratite producers, preventive medicine practices should be established. In this context, the surveillance and control of some avian pathogens are essential for the success of the ratite industry; however, little is known on the health status of ratites in Brazil. Therefore, the prevalence of antibodies against Newcastle Disease virus, *Chlamydophila psittaci*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and *Salmonella* Pullorum were evaluated in 100 serum samples collected from commercial ostriches and in 80 serum samples from commercial rheas reared in Brazil. All sampled animals were clinically healthy. The results showed that all ostriches and rheas were serologically negative to Newcastle disease virus, *Chlamydophila psittaci*, *Mycoplasma gallisepticum*, and *Mycoplasma synoviae*. Positive antibody responses against *Salmonella* Pullorum antigen were not detected in ostrich sera, but were detected in two rhea serum samples. These results can be considered as a warning as to the presence of *Salmonella* spp. in ratite farms. Therefore, the implementation of good health management and surveillance programs in ratite farms may contribute to improve not only animal production, but also public health conditions.

INTRODUCTION

The farming of ratites (ostriches, emus, and rheas) has expanded considerably all over the world in recent years. In Brazil, the commercial breeding of ostriches (*Struthio camelus*) and rheas (*Rhea americana*) has also increased. Due to intensive farming methods used by ratite producers, preventive medicine practices, such as disease monitoring and surveillance, should be established. Thus, health surveillance is important to the ratite industry, and may also influence the trade of other types of poultry at the international level (Ley *et al*., 2000).

Newcastle disease is an acute and highly contagious viral disease of birds, which can cause high (up to 100%) mortality in chickens, the most important natural host of the disease; however, it can also affect a wide variety of avian species, also causing severe disease (Alexander, 1997). Newcastle disease is endemic in many countries, and it is caused by an avian Paramyxovirus type 1 (APMV-1), which is a member of the genus Avulavirus of the Paramyxoviridae family (Mayo, 2002). As demonstrated by intensive surveys, nearly 236 species from 27 of the 50 orders of birds were reported to be susceptible either to natural or experimental infection with Newcastle virus (Kaleta & Baldauf, 1988; Wan *et al*., 2004, Carrasco *et al*., 2008) and, on several occasions, Newcastle disease virus (NDV) was isolated from some free-living birds (Alexander & Parsons, 1986). Ratites are susceptible to many diseases of domestic fowl, including Newcastle disease (Samberg *et al*., 1989;

Koch *et al*., 1998). Efforts to control and prevent this disease through efficient vaccination programs and corresponding serological monitoring in ratites have been adopted (Sousa *et al*., 2000).

Chlamydiosis is an infectious disease caused by *Chlamydophila psittaci* (*C. psittaci*). The list of *Chlamydophila*-positive birds includes six major domestic species (chicken, turkey, Peking duck, Muscovy duck, goose, and pigeon), three minor domestic species (Japanese quail, bobwhite quail, and peafowl), as well as 460 free-living or pet bird species belonging to 30 orders (Kaleta & Taday, 2003). Clinical signs of chlamydiosis include anorexia, dyspnea, dehydration, diarrhea with yellowish-green urates, weight loss, conjunctivitis, rhinitis, and sinusitis. Many of the affected birds become chronically infected, but only present clinical signs when stressed. Most carriers may intermittently shed *C. psittaci*, and are a significant source of infection both for humans and other birds (Fudge, 1996). This microorganism is responsible for an occupational zoonosis called psittacosis, which is a health hazard for pet owners, veterinarians, and workers of poultry abattoirs and processing plants. However, there are few studies in this disease in ratites (Camus *et al*., 1994; Andersen *et al*., 1998; Uhart *et al*., 2006), and little is known on the pathogenesis and the significance of the humoral antibody response in Brazilian ratites flocks.

Mycoplasma spp. has been implicated in economic losses in the poultry industry. It causes reduced egg production, worse feed efficiency, increase in mortality rates, as well as carcass condemnations, in addition to increased medication costs (Nascimento *et al*., 2005). Mycoplasma spp. in ratites is usually associated with respiratory disease, causing inflammation of upper respiratory tract (Huchzermeyer, 1994). Poultry mycoplasms (*Mycoplasma gallisepticum*, *Mycoplasma synoviae*) have long been thought to be responsible for these infections (Verwoerd, 2000), and the search for antibodies against these microorganisms has been considered a useful tool to identify infected birds and to prevent the maintenance of mycoplasmosis in ratite farms (Cadman *et al*., 1994; Ley *et al*., 2000).

The genus *Salmonella* is divided in two species: *Salmonella bongori* and *S. enterica,* which is divided in six subspecies with several serovars (Popoff & Minor 2001). More than 2,500 serovars have been described, but about ninety of them are more frequently involved in human and animal infections (Porwollik *et al*., 2004; CDC, 2007).There is little information on the occurrence of *Salmonella* spp. in ratites. According to literature, *Salmonella* spp causes clinical signs and mortality in ratite chicks (Shivaprasad, 1993; Stewart, 1994; More, 1996). However, immunossupressed adults can shed *Salmonella* spp contributing to contamination of carcasses (Huchzermeyer, 1994; Gopo & Banda, 1997; Ley *et al*., 2001; Gaedirelwe & Sebunya, 2008), and, consequently, posing a risk to public health.The control and prevention of *Salmonella* spp in ratite farming are currently based on general hygiene and disinfection practices associated with bacteriological and serological exams (Tully & Shane, 1996; Cooper, 2000; Ley *et al*., 2000; Black, 2001).

Little is known on the status of those diseases in ratites in Brazil. In this context, the objective of this study was to evaluate antibody responses to Newcastle Disease virus, *Chlamydophila psittaci*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and *Salmonella* Pullorum in serum samples collected from commercial ostriches (*Struthio camelus*) and commercial rheas (*Rhea americana*) reared in Brazil.

MATERIAL AND METHODS

Sample collection

One hundred blood samples collected from commercially raised slaughter-age ostriches and eighty blood samples from commercially raised slaughter-age rheas were assessed. Birds were reared in two dedicated ratite farms located in the southeast of Brazil. Birds were clinically healthy and had not been vaccinated against any avian pathogen.

Birds were manually restrained and samples were collected by jugular puncture with sterile needles and syringes. The blood was centrifuged at 2,000 xg for 10 minutes. The supernatant was then harvested and frozen in sterile plastic microtubes at -20ºC until tested. The ostriches sera was collected between 2006-2007, while the sera of rheas were collected between 1998-1999.

Serologic test procedures

Serodiagnostic of NDV was performed by hemagglutination inhibition (HI) test. Serum samples were treated with 25% (w/v) kaolin solution (Carrasco *et al*., 2008). The HI test was performed in microtitration plate using four hemagglutination units (UHA) of NDV LaSota vaccine strain propagated in the allantoic cavity of 9- to 10-day-old embryonated specific pathogen free (SPF) eggs. Results were recorded as log_2 X values of the highest reciprocal of the dilution presenting hemagglutination inhibition.

Birds with HI titers equal to or higher than 4 log_2 were considered positive for NDV (O.I.E., 2004).

Serodiagnostic of chlamydiosis was carried out by complement fixation test (CFT), according to the method described by Bier *et al*. (1968), and adapted to microplates by Raso *et al*. (2006). Briefly, 25 µL of the test sera and 25 µL of the chlamydial antigen, followed by 50 µL of complement, were added to each well and incubated overnight at 4°C. Subsequently, 25 µL of sensitized sheep red blood cells were added to each well and the plates were incubated for 30 min at 37°C. Controls for serum and antigen were included in every run, as well as for the complement and the hemolytic system. Finally, plates were centrifuged at 800 x g for 5 min and the degree of lysis was visually assessed. Samples showing more than 50% lysis at serum dilutions of 1:16 or higher in the presence of 2 units of complement were considered positive.

Serum samples were also tested for *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and *Salmonella* Pullorum by serum plate agglutination using commercial antigens (Myco-Galli Teste®, Synovitest® and Pulor-teste®, Biovet, São Paulo, Brazil). The tests were conducted by mixing a drop (50 µL) of serum samples with a drop (50 µL) of antigen on a clean glass plate at room temperature (25°C). Serum samples and antigen were mixed until homogenous distribution was achieved. The glass plate was rotated for five seconds, left resting for one minute, and then read. Samples presenting agglutination, asshown by the formation of clots, were considered positive. Positive and negative serum controls were used.

RESULTS

All serum samples from ostriches and rheas were negative for NDV in the HI test, for *Chlamydophila psittaci* in CFT, as well as for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* antigens in serum plate agglutination tests. Additionally, all ostrich serum samples were negative for *Salmonella* Pullorum in serum plate agglutination test. However, two serum samples (2.5%) were positive for the commercial *Salmonella* Pullorum antigen (Table 1).

DISCUSSION AND CONCLUSIONS

All sampled birds were apparently healthy, with no evidence of disease, and all were serologically negative for the Newcastle disease virus, *Chlamydophila psittaci*, *Mycoplasma gallisepticum*, and *Mycoplasma synoviae*.

Our results indicated that the population of ratites in this study was not previously exposure to NDV. However, in an earlier investigation with Brazilian *Rhea americana*, antibodies against NDV were detected in 56% of samples by HI test (Sousa *et al*., 2000). Deaths due to NDV have been reported in ratites in many foreign countries (Huchzermeyer, 1994; Williams *et al*., 1997; Verwoerd, 2000).

It is important to highlight that the hemagglutination inhibition technique tends to yield false positive results in serum samples of some species, including ostriches, due to the presence of nonspecific hemagglutination inhibitors (Willians *et al*., 1997; Alexander, 1997; Koch *et al*., 1998; Sousa *et al*., 2000). According to the OIE (2004), serum samples can be incubated with 10% chicken red blood cell (RBC) suspension at room temperature for 10 min in order to eliminate those agents. Additionally, other serum treatments based on kaolin (used in the present study), periodate, heparin-manganese, and acetone have been also used to eliminate these inhibitors (Hovi, 1978).

Although *C. psittaci* appears to be widespread among Brazilian psittacine birds, evidence of exposure or disease has not been reported in ratites to date in Brazil (Raso, 2006). In the present study, all birds were *Chlamydophila*-negative. Few reports of the disease in ratites have been published in the world. Cases of chlamydiosis in commercial rheas were reported in the United States of America, when birds were found dead without exhibiting any signs of the disease (Camus *et al*., 1994). Eleven isolates from a number of ratites (10 from rheas and one from ostrich) were serotyped and characterized by diagnostic laboratories in Texas and California (Andersen *et al*., 1998). All isolates were determined to be serovar E, although the origin of the natural host of this serovar is not known. Moreover, antibodies against *Chlamydophila* were found in 92.6%

Table 1 - Results of the serological survey of antibodies against selected avian pathogens in ostriches and rheas reared in the southeast of Brazil.

Avian pathogen	Test	Positive in test	Ostriches	Rheas
Newcastle Disease Virus	HI	Titer: ≥16	0/100	0/80
Chlamydophila psittaci	CFT	Titer: ≥16	0/100	0/80
Mycoplasma gallisepticum	Agglutination test	Agglutination	0/100	0/80
Mycoplasma synoviae	Agglutination test	Agglutination	0/100	0/80
Salmonella Pullorum	Agglutination test	Agglutination	0/100	2/80

(25/27) of free-ranging adults and semicaptive juvenile rheas tested in Argentina (Uhart *et al*., 2006).

Antibody responses to poultry mycoplasmas have been described in ratites (Cadman *et al*., 1994; Peccati *et al*., 1995). However, in the present study, no positive serological responses to *Mycoplasma gallisepticum* or *Mycoplasma synoviae* were found neither in rheas nor in ostriches. These results are consistent with the findings of other authors (Shane & Tully, 1996; Ley *et al*., 2000), who reported no positive serological responses to *Mycoplasma gallisepticum* or *Mycoplasma synoviae* in any of common ratite species. According to literature, *Mycoplasma* spp. in ostriches would be associated with respiratory disease, causing inflammation of the upper respiratory tract (Huchzermeyer, 1994; Verwoerd, 2000). On the other hand, in our study, none of examined birds presented any clinical signs of the disease.

Some authors have suggested that ostriches may harbor unique species-specific mycoplasmas different from *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, Mycoplasma meleagridis, or Mycoplasma iowae, which are typically associated with respiratory infections in poultry (Shivaprasad, 1993; Shane, 1998; Botes *et al*., 2005). In this case, commercial *Mycoplasma gallisepticum* and *Mycoplasma synoviae* antigens would not be suitable to test ratite sera. Therefore, this needs to be further investigated.

The serum plate agglutination test with *Salmonella* Pullorum antigen is one of available methods to evaluate antibody responses in poultry. It allows identifying birds that are or were infected with serovars belonging to serogroup "D" (Davies & Wray, 1994). However, some studies have shown no antibody responses to *Salmonella* Pullorum antigen in the sera of ostriches or rheas (Pereira, 2007; Ley *et al*., 2000). In the present study, positive antibody responses to *Salmonella* Pullorum antigen were not observed in ostrich sera, but were detected in two rhea serum samples. These results suggest that the tested ostriches were not infected with serogroup "D" *Salmonella* serovars, and that the two positive rheas could have been infected with some serovar of serogroup "D" (e.g. *Salmonella* Enteritidis, *Salmonella* Pullorum and *Salmonella* Gallinarum).

Ratite farming system is based on rearing birds on pastures in paddocks surrounded by fences (Tully & Shane, 1996; Cooper, 2000; Black, 2001). This practice could favor contact with wild animals (birds, rodents, and insects) capable of transmitting *Salmonella* spp. (Tully & Shane, 1996; Connolly *et al*., 2006; Pennycott *et al*., 2006). In addition, feed may be an important source of *Salmonella* spp. in ratite production. Feed may be contaminated during the storage process or even during manufacturing, and, as a result, it could play an important role in the introduction or maintenance of *Salmonella* spp. in ratite farms (Gopo & Banda, 1997; Higgins *et al*., 1997; Freitas Neto *et al*., 2009). In the present study, it was not possible to determine how rheas were infected because an epidemiological investigation was not performed; however, this result should be considered as a warning as to presence of *Salmonella* spp in ratite farms. Therefore, the implementation of good health management should not be overlooked.

Finally, serological tests are tools used for surveillance in ratite farming; however, the use of these methods in ratites needs to be further investigated. Therefore, other tests are also required, as false negative or positive results may provide an incorrect interpretation of the flock health status or determine an inadequate management strategy in the farm.

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