

ADAPTATION OF HEMAGGLUTINATION INHIBITION TECHNIQUE (HI) FOR THE DIAGNOSIS OF NEWCASTLE DISEASE IN OSTRICHES (*Struthio camellus*)

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ABSTRACT

The breeding of ratites (ostriches, emus and rheas) has expanded considerably all over the world in recent years. The hemagglutination inhibition (HI) test is considered to be the standard serology test, for detection of antibodies against Newcastle Disease Virus (NDV). However, HI test tends to give false positive results in serum samples of some species, including ostriches. In this study, kaolin was used to eliminate the nonspecific hemagglutination inhibitors in ostrich serum samples. Twenty-seven non-kaolin-treated ostrich samples reacted to the test and the titres ranged from 2 to 64. However, these samples did not react when they were kaolin-treated. The controls did not show any variance on antibodies titres, regardless of treatment with kaolin. The use of kaolin in the routine serodiagnosis of ND not only relieves adsorption of serum samples with avian erythrocytes, but also contributes to the reduction in the maintenance of red blood cells donor birds.

Newcastle Disease (ND) is one of the most important infectious diseases in birds throughout the world. ND is endemic in many countries and is caused by an avian Paramyxovirus type 1 (APMV 1), which is a member of the genus *Avulavirus* of the family *Paramyxoviridae* (Mayo 2002).

The breeding of ratites (ostriches, emus and rheas) has expanded considerably all over the world in recent years (Sousa et al. 2000). In the 1990s, a worldwide renaissance has been observed in ostrich production arising from an increased demand for leather and

fresh meat (Willians et al. 1997). In Brazil, commercial ostriches were introduced between 1993 and 1995. The number of birds increased rapidly, until the occurrence of an outbreak of ND in 1997 (Brasil 1997). Since ostriches are kept mainly in free-range, they easily get in contact with wild birds that can be infected with pathogens, including the Newcastle Disease Virus (NDV) (Sakai et al. 2006a). Because ostriches can carry and spread NDV, and the occurrence of a NDV outbreak greatly impacts on poultry production and trade (Verwoerd et al. 1999), all imported birds should be serologically tested (Koch et al. 1998).

The hemagglutination inhibition (HI) test is used most widely in NDV serology, and it is considered to be the standard serology test, according to OIE protocols (OIE 2004). However, HI test tends to give 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 cells (RBCs) suspension at room temperature, for 10 min, to eliminate these agents. Additionally, other serum treatments based on kaolin, periodate, heparin-manganese and acetone have been performed to eliminate these inhibitors, as well (Hovi 1978).

In this study, kaolin was used to eliminate the nonspecific hemagglutination inhibitors in ostrich serum samples, aiming to reduce the number of false positive results in the serodiagnosis of NDV for this species.

A hundred blood samples from commercially raised slaughter-age ostriches were used. The samples were collected by jugular puncture with sterile needles and syringes. The blood was centrifuged at $2,000 \times g$ for 10 minutes. Then, the supernatant was harvested and frozen in sterile plastic microtubes at -20°C until tested.

Sera from the following species were used as controls: 10 chickens vaccinated against NDV, vaccine strain LaSota (New Vac-LS – Fort Dodge Saúde Animal Ltda.[®]); 10 chickens experimentally infected with the São João do Meriti strain, a pathogenic strain of NDV (Lima et al. 2004, Carrasco 2005); 10 pigeons vaccinated against NDV, vaccine strain LaSota (New Vac-LS – Fort Dodge Saúde Animal Ltda.[®]) (Carrasco 2005); and 10 pigeons experimentally infected with the São João do Meriti strain (Carrasco 2005). Chicken and pigeon samples were from a sera bank and remained stored at -20°C until tested.

Each test serum sample was divided into two aliquots of 100 µL. The first aliquot was subjected to heat (56°C/15 min) and kaolin treatment, while the second one was subjected only to heat (56°C/15 min).

The treatment kaolin-based for each aliquot consisted of the addition of 300 µL of borate-buffered saline (BBS), pH 9.0, followed by the addition of 400 µL of a kaolin suspension (25% wt/vol in BBS). The mixture was kept at room temperature (25°C) for 20 minutes, with occasional vortex. Then, it was centrifuged at 3,000 × *g*, for 20 minutes, and the supernatant was harvested for testing.

The micro-beta HI test was done using 4 HAU of LaSota vaccine strain of NDV, propagated in allantoic cavity of 9 to 10-day-old embryonated specific pathogen free (SPF) chicken eggs. HI titres were recorded as Log₂ values of the highest reciprocal of the dilution which showed hemagglutination inhibition. A specific cut-off value was not adopted, aiming to investigate kaolin effects even on lower dilutions.

Table 1 depicts the means of the titers obtained from HI test for both treated and not treated serum samples, considering the different groups of birds. Twenty-seven ostrich

samples that were not treated with kaolin reacted to the test and the titers ranged from 2 to 64. However, when these samples were treated, they did not react. The others ostriches samples (73) were negative even when they were not treated with kaolin. The controls did not show any variance on antibodies titers, regardless of treatment with kaolin.

Table 1. Means of the titres obtained from HI test for both treated and non-treated serum samples.

Experimental Group	Mean of the titres at HI reaction (Log₂) <u>without</u> kaolin treatment	Mean of the titres at HI reaction (Log₂) <u>with</u> kaolin treatment
Ostrich	4,26	1*
Vaccinated chicken	7,1	7,1
Infected chicken	8,5	8,5
Vaccinated pigeon	6,7	6,7
Infected pigeon	9	9

* Log₂0 = 1; thus, this value indicates an absolute lack of haemagglutination inhibition activity.

The present study describes the use of kaolin to remove from the HI test nonspecific hemagglutination inhibitors, which are responsible for the occurrence of false positive results in the serodiagnosis of Newcastle Disease Virus in ostriches.

The inhibitors are classified into different categories. In bovine, equine and swine sera, lectins associated with mannose and alpha-2-macroglobulins act as inhibitors of viral receptors that present sialic acid residues (Anders et al. 1990, Pritchett & Paulson 1989, Ryan-Poirier & Kawaoka 1993). In serum of birds, sialoglycoproteins containing sialic acid linked to galactose by the 2,3-linkage play similar roles (Zaitsev et al. 2004).

Although some authors report that the use of treatments to eliminate inhibitors can provoke a reduction in the sensitivity of the HI test (Koch et al. 1998, Williams et al. 1997), what would cause direct influence on the serodiagnosis of NDV in ostriches (Koch et al. 1998), our results do not corroborate with these findings. The controls, which were previously known to be positive for NDV, did not show any variance on antibody titres, even after treatment (Table 1, Figure 1). Similar results had already been achieved for sera of vaccinated ostriches. When tested by HI assay or ELISA, the antibodies titers after treatment with kaolin did not vary either (Sousa et al. 2000).

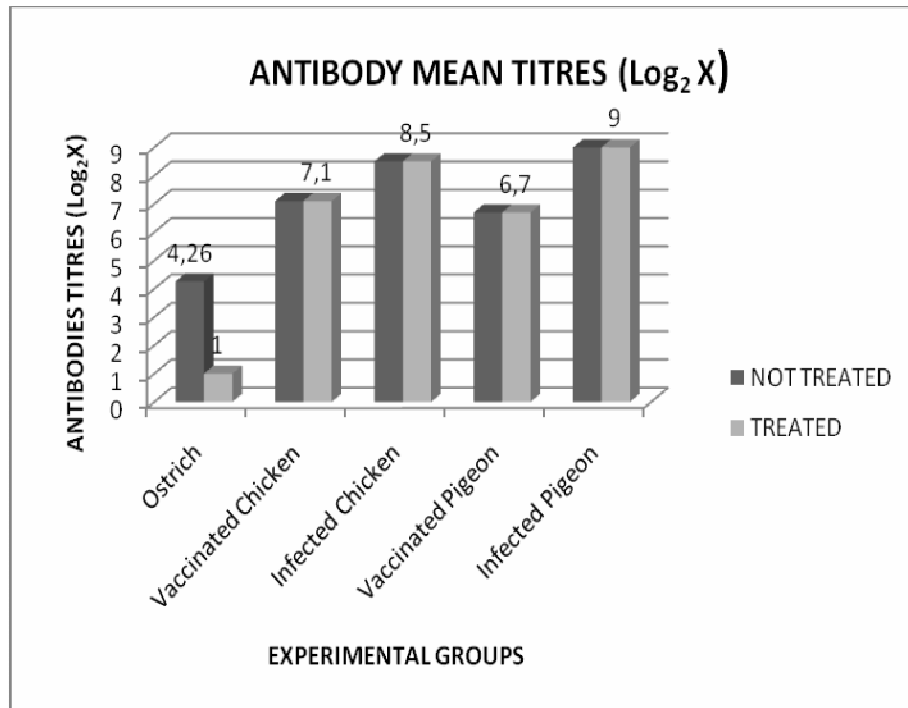


Figure 1. Means of the titres obtained from HI test for both treated and non-treated serum samples.

According to OIE specifications (OIE 2004), the recommended technique to remove nonspecific hemagglutination agents is adsorption of serum with chicken RBCs. This method has been used in treatment of ostriches serum samples (Koch et al. 1998). In what concern to kaolin's use to remove the inhibitors from ostrich's sera, the present study corroborates with results previously reported by other authors, contributing to an increase in repeatability of results obtained by different laboratories (Williams et al. 1997, Sakai et al. 2006a, Sakai et al. 2006b). Kaolin has also been used in the treatment of turkey's sera submitted to ND serodiagnosis, and in the diagnosis of *Clamydophila psittaci* by ELISA (Van Look et al. 2005), and to remove the nonspecific inhibitors in serodiagnosis of many mammal's viral diseases (Ábero 1997). In addition, the use of kaolin in the routine

serodiagnosis of ND not only relieves the adsorption of serum samples with RBCs but also contributes to the reduction in the maintenance of red blood cells donor birds.

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REFERENCES

Ábero AA 1997. 50f. Desenvolvimento e padronização de um ensaio imunoenzimático para o diagnóstico sorológico da infecção por parvovirus suíno. Dissertação de Mestrado. Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul. Porto Alegre.

Alexander DJ 1997. Newcastle disease and other avian Paramyxoviridae infections. In: BW Calnek, HJ Barnes, CW Beard, LR McDougald (eds.). *Diseases of Poultry*, 10th ed. Iowa State University Press, Ames, Iowa, p. 541-569.

Anders E M, Hartley C A, Jackson DC 1990. Bovine and mouse serum beta inhibitors of influenza A viruses are mannose-binding lectins. *Proc. Natl. Acad. Sci. USA* 87: 4485–4489.

Brasil. Ministério da Agricultura e Abastecimento, Portaria N° 144 de 23 de Dezembro de 1997.

Carrasco AOT 2005. 84f. Infecção experimental de pombos com estirpes do vírus da doença de Newcastle de baixa e alta patogenicidade. Dissertação de Mestrado, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal.

Hovi T 1978. Nonspecific inhibitors of coronavirus OC43 haemagglutination in human sera. *Med. Microbiol. Immunol.* 166: 173-176.

Koch G, Czifra G, Engström BE 1998. Detection o Newcastle Disease Virus-specific antibodies in ostrich sera by three serological methods. *Vet. Rec.* 143: 10-12.

Lima FS, Santin E, Paulillo AC, Doretto Junior L 2004. Evaluation of different programs of Newcastle disease vaccination in japanese quail (*Coturnix coturnix japonica*). *Int. J. Poult. Sci.* 3: 354-356.

Mayo MA 2002. Virus Taxonomy. *Arch. Virol.* 147: 1071-1076.

OIE 2004. Newcastle Disease. In: *Office International Des Epizooties - Manual of standards for diagnostic tests and vaccines*, 5th edition, Paris.

Pritchett TJ, Paulson JC 1989. Basis for the potent inhibition of influenza virus infection by equine and guinea pig alpha 2- macroglobulin. *J. Biol. Chem.* 264: 9850–9858.

Ryan-Poirier KA, Kawaoka Y 1993. Alpha 2-macroglobulin is the major neutralizing inhibitor of influenza A virus in pig serum. *Virology* 193: 974–976.

Sakai K, Sakabe G, Tani O, Nakamura M, Takehara K 2006a. Antibody responses in ostriches (*Struthio camellus*) vaccinated with commercial live and killed newcastle disease vaccines. *J. Vet. Med. Sci.* 68: 627-629.

Sakai K, Yada K, Sakabe G, Tani O, Miaji K, Nakamura M, Takehara K 2006b. Serological and virological studies of Newcastle Disease and Avian Influenza in slaughter-age ostriches (*Struthio camellus*) in Japan. *J. Vet. Med. Sci.* 68: 491-494.

Sousa RLM, Montassier HJ, Pinto AA 2000. Detection and quantification of antibodies to Newcastle Disease Virus in ostrich and rhea sera using a liquid phase blocking enzyme-linked immunosorbent assay. *Clin. Diagn. Lab. Immunol.* 7: 940-944.

Van Loock M, Geens T, De Smit L, Nauwynck H, Van Empel P, Naylor C, Hafez HM, Goddeeris BM, Vanrompay D 2005. Key role of *Chlamydophila psittaci* on Belgian turkey farms in association with other respiratory pathogens. *Vet. Microbiol.* 107: 91–101.

Verwoerd DJ, Olivier A, Gummow B, Gerdes GH, Willians R 1999. Experimental infection of vaccinated slaughter ostriches in a natural, open-air feedlot facility with virulent Newcastle Disease Virus. *Avian Dis.*43: 442-452.

Willians R, Boshoff CH, Vervoerd D, Schoemen M, Van Wyk A, Gerdes TH, Roos K 1997. Detection of antibodies no Newcastle Disease Virus in ostriches (*Strutio camelus*) by an indirect ELISA. *Avian Dis.* 41: 864-869.

Zaitsev V, Von Itzstein M, Groves D, Kiefel M, Takimoto T, Portner A, Taylor G 2004. Second sialic acid binding site in Newcastle disease virus hemagglutinin-neuraminidase: implications for fusion. *J. Virol.* 78: 3733-3741.