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EFFECTS OF VARIOUS APPLICATION ROUTES OF NEWCASTLE DISEASE VACCINE ON SPECIFIC ANTIBODY TITRES IN OSTRICHES

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Newcastle disease (ND) is one of the most important diseases of poultry and other avian species. The usual mean to control ND is specific immunoprophylaxis. Although chickens are routinely vaccinated against ND, vaccination of ostriches is less well understood. We investigated the effect of vaccination against Newcastle disease via different routes on specific antibody titer in 24 adult ostriches, divided into three experimental and one control group. The vaccine was administered in drinking water to the first, by spraying to the second, and oculo-nasally to the third group. The results have indicated antibody production with titers sufficient for humoral immunity in all experimental groups. The strongest immune response was determined in the group vaccinated by spraying.

Key words: Newcastle disease, vaccine, application route, ostrich

INTRODUCTION

Newcastle disease (ND) is among the most important diseases of poultry and other birds (Bolte *et al.*, 1999; Verwoerd, 2000). Since the 1950s when ND was first reported in ostriches, immunoprophylaxis has been actively developed and remained the basic prevention procedure for specific immunoprophylaxis (Senne *et al.*, 2004). Much effort has been directed toward the determination of the dosage of the vaccine, considering the size of the bird (Blignaut *et al.*, 2000).

In spite of many methods used for prevention of ND, its eradication has not been successful. Therefore, the disease persists in various parts of the world (Capua *et al.*, 2000; Wakamatsu *et al.*, 2006; Aldous *et al.*, 2007).

Different strains of Newcastle disease virus (NDV) have different tropisms, which often correlates with their virulence (Al-Garib *et al.*, 2003, Wambura *et al.*, 2006). The preference for the respiratory, digestive or nervous system, as well as the severity of the disease, depends on the viral hemagglutinin-neuraminidase protein (Huang *et al.*, 2004). Taking into account variations among epizootic conditions between specific parts of the world, vaccines obtained from NDV

strains of various pathogenicity and tropism have been applied. It is generally recommended to use those strains with similar tropism to the pathogenic strains that have caused the disease in that particular area.

Commercial ND vaccines are already well established, but their effects on ostriches still need to be fully understood. Pantropic vaccine viruses can be applied using different routes: by spraying, oculo-nasally or via drinking water. Although ostrich vaccination against ND has been discussed by other authors (Bolte *et al.*, 1999; Blignaut *et al.*, 2000), we consider it to be necessary to address the practical question of which is the best administration route in a manner adapted to the ostrich anatomy.

Hemagglutination inhibition assay is an established method widely used for the detection of avian pathogens including NDV (Tsai and Lee, 2006). Using this assay, we assessed variations in antibody titer values over 42 days after the administration of the vaccine against ND via drinking water, by spraying or oculo-nasally.

MATERIALS AND METHODS

Ostriches

The investigations were performed in 24 adult ostriches, hybrids between blue-neck and African black ostriches, weighing approximately 120 kg each. The ostriches were divided into four groups, three study groups and a control group. Each group comprised six ostriches divided into two families, consisted of one male and two female birds. One family was kept in an enclosure with 800 square meters of open space and 12 square meters of sheltered space. The ostriches were fed once a day with a feed mixture adapted to the winter period when the investigation was conducted. The animals had free access to drinking water, except before vaccination, when they were deprived of water for one hour. Selected ostriches were clinically healthy and had not been previously vaccinated against ND. Nevertheless, previous contact with NDV could not be excluded, since the birds were imported from different parts of the world where they might have been exposed to certain strains of NDV.

Vaccination

A live vaccine against ND, produced from La Sota virus strain (*PESTIKAL® LA SOTA SPF, Veterina Ltd.*, Zagreb, Croatia), was used in the study. To the birds from group 1, the vaccine was administered via drinking water as a suspension in five liters of distilled water per family. The vaccine was administered oculo-nasally to the birds in group 2, as a suspension in 1.05 mL of distilled water per bird. Of this total quantity, five drops were dropped into the bird's nose and two drops into the eye. In group 3, the vaccine was administered by spraying. The required amount of the vaccine was suspended in three milliliters of distilled water and sprayed using an atomizer.

Each ostrich in all three study groups received approximately $20x10^{6.0}EID_{50}$ of the vaccine virus. Birds in group 4 were not vaccinated and served as controls.

All birds were monitored daily, so that any possible reaction to the given vaccine against ND could be noted.

Hemagglutination inhibition assay

Blood samples for analyses were collected before vaccination at 7, 14, 21 and 42 days after the vaccination. Blood samples were taken from the wing vein (*v. cutanea ulnaris*). The collected sera were inactivated in a water bath at 56°C for 30 minutes and investigated using hemagglutination inhibition (HI) assay method (Allan and Gough, 1974) for the confirmation of specific HI antibodies to the administered vaccine.

Statistics

Differences between individual parameters and their significances in repeated measurements were assessed using the general linear model procedure of repeated measurements. Linear correlation was used to assess interrelations between individual groups. Data processing was performed using the *SAS statistical programme*, version 8 (SAS Institute Inc., Cary, N. C., USA) and *Statistics 5.0* (SPSS Inc., Chicago, USA).

RESULTS

The obtained HI antibody titers for NDV are presented in Figure 1. A small amount of specific antibodies (titer 1:5 to 1:6) was present in all groups before vaccination, indicating that the birds had a previous contact with some of the

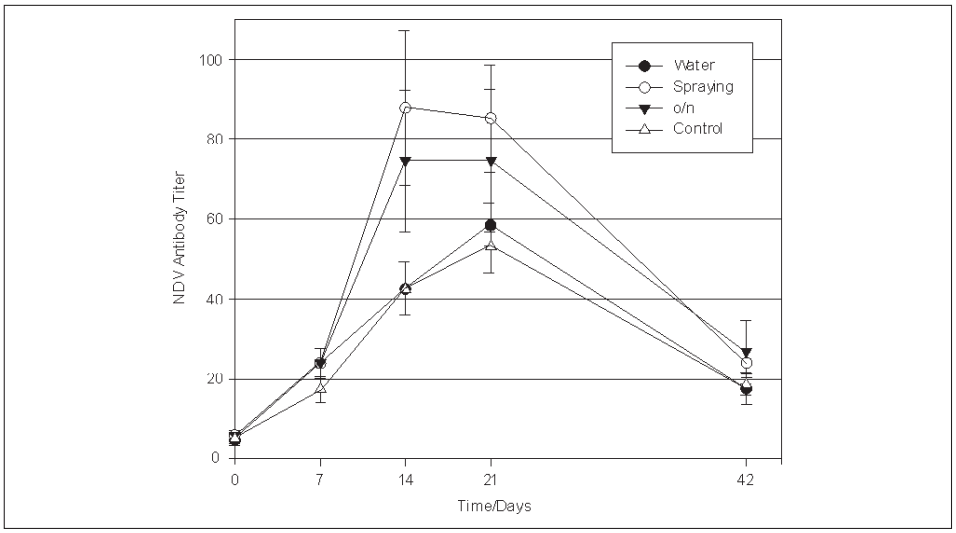

Figure 1. HI titer of antibodies against NDV within six weeks after vaccination. Different vaccine administration routes are indicated by symbols (o/n stands for oculo-nasal)

strains of NDV. This is further corroborated by the relatively steep increase in antibody production in the first week after vaccination observed in all groups (titer 1:17 to 1:24). A significant difference in the NDV antibody titer has been observed three and four weeks after vaccination. In groups which received the vaccine by spraying and oculo-nasally, antibody titers 1:88 and 1:75, respectively, have been measured after three weeks; values remained similar (1:85 and 1:75) in the fourth week. This represents a statistically significant increase when compared to the group which received the vaccine via drinking water (titer 1:43 in the third and 1:59 in the fourth week after vaccination) or the control group (titer 1:43 after three and 1:53 after four weeks). Antibody production declined after six weeks, with all four groups attaining similar low levels corresponding to the titer in the range from 1:18 to 1:27. Therefore, administration of the vaccine against NDV by spraying is the method of choice, closely followed by the oculo-nasal application. Both methods are superior to vaccination via drinking water.

DISCUSSION

The common feature shared between many of the published protocols for vaccination against ND is the live vaccine containing the La Sota NDV strain. It is used practically universally in ostriches and is also applied in other poultry species (Madeiros, 1997; Alexander, 2000). The La Sota vaccine PESTIKAL® SPF (Veterina Ltd., Zagreb, Croatia) was administered to the ostriches in approximately 20 times higher doses than prescribed for other poultry. This was done in accordance to the doses used by other investigators (Madeiros, 1997).

Regardless of the route of administration, the vaccine did not induce any harmful effects. The death of one ostrich during the experiment could not be associated with either the vaccine or the route of administration. This was confirmed by macroscopic and microscopic pathomorphological investigations, as well as bacteriological and virological tests.

Regardless of the selected route of administration, each one induced a rapid increase in HI antibody titers; this is understandable taking into account the age of the ostriches, as well as the fact that the birds had already come into contact with the virus and were carriers of specific antibodies to NDV. The HI antibody titer assessed on the day of vaccination in the blood serum ranged from 1:5 to 1:6, which is a further indicator of a previous contact with NDV.

Antibody titer increase could be observed in the non-vaccinated ostrich group as well. Although the antibody level was lower than in the vaccinated groups, the increase is statistically significant and this observation cannot be simply ignored. It points to the possibility of a low-level contamination of the control group by the vaccine. However, due to the measures undertaken to isolate the groups from each other, as well as the slow spread of ND through affected ostrich flocks (Alexander, 2000), we felt it was reasonable to continue the experiment and evaluate the data for the other groups.

The attained HI serum titers for NDV in all the ostrich groups were protective, i.e. higher than 1:16, so it could be presumed that, analogous to chickens,

ostriches would remain clinically healthy in the case of exposure to pathogenic NDV (Allan and Gough, 1974; Kapczynski and King, 2005).

The swift onset of specific immunity after spraying of the vaccine reported in chickens (Gough and Alexander, 1973) was also observed in ostriches. The different and better action of the vaccine when administered into the respiratory system and oculo-nasally compared to the application via drinking water could be to a great extent ascribed to the contact of the virus with the bronchi-associated lymphoid tissue (BALT) system. This system comprises lymphoid tissues of the anterior part of the respiratory system, as well as the Harderian gland. In contrast, when the La Sota ND vaccine is administered via drinking water, the virus first comes into contact with the cells of the digestive system. Since the La Sota strain has a much higher affinity for the cells of the respiratory system, this incompatibility between the administration route and virus specificity might be the underlying cause for the weaker immune response to vaccination via drinking water, leading to the observed slower increase and lower peak of the HI antibody titer.

Relatively low peak HI antibody titer in all the investigated ostrich groups (up to 1:88) and its rapid decrease within six weeks after vaccination in all groups, were similar to those observed in young poultry (chickens and turkeys), but this was not expected in adult ostriches, since their immune system is fully developed (Blignaut *et al.*, 2000).

Finally, it may be concluded that in ostriches, La Sota vaccine against ND administered either via drinking water, oculo-nasally or by spraying, induces the development of specific HI antibodies. Regardless of the administration route, no adverse reactions to the vaccine had been determined. In comparison to the vaccine administration via drinking water, oculo-nasal administration and spraying induced a better humoral response manifested by higher HI specific antibody titers in the course of the third and the fourth week after vaccination. Spraying has the additional advantage of easy application. Different responses to the different vaccine administration routes can be accounted for by diverse parts of the immune system which primarily come into contact with the immunogen. The attained HI antibody titers protected from possible ND infection, although, considering the age of the ostriches at vaccination, their attained values were relatively low, and the duration of immunity short.

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UČINCI RAZLIČITIH NAČINA PRIMJENE CJEPIVA PROTIV NEW CASTLE BOLESTI NA SPECIFIČNI TITAR ANTITIJELA U NOJA

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SADRŽAJ

New Castle bolest je jedna od najznačajnijih bolesti peradi i drugih ptica. Najčešće sredstvo kojim se ta bolest suzbija je specifična imunoprofilaksa. Iako cijepljenje pilića predstavlja rutinski postupak, reakcija noja na cjepivo do sada

nije bila istražena. U radu smo istražili učinak različitih načina primjene cjepiva protiv New Castle bolesti na specifični titar antitijela u 24 odrasla noja, podijeljena u tri eksperimentalne i jednu kontrolnu skupinu. Prvoj skupini cjepivo smo dali u vodi za piće, drugoj raspršivanjem a trećoj okulo-nazalno. Rezultati su ukazali da je titar stvorenih specifičnih antitijela bio dovoljan za humoralnu imunost u sve tri eksperimentalne skupine, a najbolji rezultat je bio zabilježen u skupini cijepljenoj raspršivanjem.