Serological and Virological Studies of Newcastle Disease and Avian Influenza in Slaughter-Age Ostriches (Struthio camelus) in Japan

Kouji SAKAI1), Kaori YADA1), Genki SAKABE1), Orie TANI1), Kazuki MIYAJI1), Masayuki NAKAMURA1) and Kazuaki TAKEHARA1)

1)Laboratory of Zoonoses, School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada, Aomori 034–8628, Japan

NOTE Avian Pathology

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ABSTRACT. Serum samples from 191 ostriches (Struthio camelus) in Japan were tested for antibodies to Newcastle disease virus (NDV) and avian influenza virus (AIV). Twenty-two (12%) contained NDV-specific neutralizing antibodies by a virus-neutralization (VN) test without vaccination. Antibodies to AIV were not detected in the any sera by an agar gel precipitation test. Seven serum samples that had vaccinated with live NDV by eye drop were all positive by the VN test at 1 month post vaccination. A haemagglutination inhibition (HI) test for NDV seemed not to be suitable for ostriches because of non-specific agglutination of chicken red blood cells. No haemagglutinating viruses were isolated. This is the first report on detection of antibodies against NDV in ostriches in Japan.

KEY WORDS: Newcastle disease, ostrich, serological survey.

Newcastle disease (ND) and avian influenza (AI) are two of the most devastating diseases of poultry and domestic birds throughout the world [2]. Newcastle disease virus (NDV) causing ND is classified with the other eight avian paramyxoviruses in the genus Avulavirus, sub-family Paramyxovirinae, family Paramyxoviridae, order Mononegavirales [17]. AI is caused by various subtypes of influenza A virus of the family Orthomyxoviridae [2]. ND and AI cause not only drop in production but also mortality in poultry flocks. Additionally, movement restrictions are placed on disease-stricken districts during active outbreaks and countries where the diseases are endemic encounter trade embargoes. This can lead to devastating effects on the ostrich and poultry industries as well as on other avian enterprises.


AI virus (AIV) isolations from natural infections of ratite have been reported just for several cases. In 1991, AIV was first isolated from South Africa in 1-month-old ostriches showing 80% mortality [4]. The isolate was a H7N1 subtype, which was classified as low pathogenic AI (LPAI) virus for chicken. In Denmark, the isolations were associated with a 146/506 death rate within 23 days of importation, while the virus was of low virulence for chicken [13]. Capua et al. reported natural infection of ostriches with H7N1 highly pathogenic AI (HPAI) virus [7, 8].

Since commercial based ostriches were introduced to Japan in 1988, the number of ostriches increased suddenly and exceeded 9,000 birds by 2002. Because ostriches are kept mainly in a free-range situation, they are easy to come in contact with wild birds and animals that may be infected with pathogens including bacteria, viruses, and parasites. In such conditions, there is an increased concern of ostrich producers in Japan to establish preventive medicine practices such as disease monitoring and surveillance. However, little information is available on infectious diseases of ostriches in Japan. Hence, the objective of this study was to provide virological surveillance information and to determine the prevalence of antibodies to NDV and AIV in commercially raised slaughter-age ostriches. This is the first report on monitoring for NDV and AIV in ostriches in Japan.

At a processing plant in Yamagata prefecture, sera were taken from 181 slaughter-age ostriches (approximately 14-month-old) originating from ostrich farms in many parts of Japan. Ten ostrich sera were also sent to our laboratory from livestock hygiene service centers in 2 prefectures (A and B). The sera were frozen at –20°C until tested. The 102 specimens of tracheal swabs and intestinal contents for virus isolation were collected at the processing plant in Yamagata prefecture. Each specimen was suspended to a concentration of 30% in phosphate-buffered saline containing penicillin at 10,000 units per ml, streptomycin at 10 mg per ml, amphotericin B at 50 µg per ml, and gentamicin at 5 mg per ml. The suspension was centrifuged at 1,100 × g for 10 min, then the supernatant was harvested and frozen at –50°C until tested.

One tenth ml of the supernatant was inoculated into the allantoic cavities of two 10-day-old embryonated chicken eggs. The eggs were incubated at 37°C for 3 days unless death of the embryos were detected. At the end of the incubation period or upon embryo death, the allantoic fluids were harvested, centrifuged, and tested for haemagglutinating activity. At least 2 serial blind passages in eggs were made before the sample was determined to be negative.

Chicken embryo fibroblasts (CEF) were prepared from 10-day-old chicken embryos as described [21, 22]. A virus-
neutralization (VN) test for NDV was performed by the plaque-reduction method with a constant amount of virus and varying serum dilution. Sample sera and antiserum against NDV strain Ishii [24] were diluted in a serial four-fold, and mixed with equal volumes of NDV strain Sato. The neutralizing antibody titer was calculated at 50% plaque reduction point by Behrens-Kaerber’s method [16].

The micro-beta haemagglutination inhibition (HI) test was performed essentially as described for NDV [3]. Each test serum sample was treated at 56°C for 30 min and then incubated with kaolin at 37°C for 30 min to extract nonspecific inhibitors [18, 23]. Subsequently, the treated sera were incubated with 10% chicken red blood cells at room temperature for 10 min [5]. A twofold dilution series of each test serum was made, and titers were expressed as log2 values of the highest reciprocal of the dilution which showed hemagglutination inhibition. Titers equal to or greater than log2 3 were considered positive results.

An agar gel precipitation (AGP) test for AIV was carried out essentially as described by Yamaguchi et al. [25].

The HI and VN titers were analyzed to determine relative sensitivity and specificity, predictive values, and accuracy. The sensitivity was defined as the proportion of VN-positive samples that were correctly identified by HI and specificity was defined as the proportion of correctly identified VN-negative samples. The accuracy was defined as the proportion of 2 tests, both positive and negative, which were correct. The positive predictive value was defined as the proportion of sera with positive HI and VN results relative to the total number of HI positive serum. The negative predictive value was defined as the proportion of sera with negative HI and VN results relative to the total number of HI negative sera. They were expressed in percentages. VN values were linearly regressed on HI titers and the correlation coefficient (Pearson’s r) was obtained.

The relationship between the VN and HI titer for NDV was shown in Fig. 1. Of 38 serums tested, 10 (26.3%) were positive and 10 (26.3%) were negative with both the VN and the HI tests. Fifteen serums (39.5%) were positive only with the HI test, whereas three (7.9%) were positive only with the VN test (Table 1). The relative sensitivity of the VN test was 76.9%, the specificity was 40.0%, and the accuracy between the two tests was 52.6%. The positive predictive value (40.0%) and the negative predictive value (77.0%) were shown in Table 1. There was no strong correlation (r=0.363) between the VN and HI tests (Fig. 1). There are no detailed data reported for antibody response on the VN test in ostriches, as far as we could determine. The VN test would be the most accurate and most reliable test but it is very laborious and needs special skills. In the present study, the VN test was used for the detection of anti-NDV antibodies in ostrich sera.

A total of 181 slaughter-age ostrich serum samples were collected in Japan between 2002 and 2004. Twenty-two of the 181 (12.2%) were positive by the VN test. As shown in Table 2, eleven of the positive samples were low level (VN titers 4–10), 6 were middle level (10–100) and 5 were high level (more than 100). All of these VN positive ostriches were unvaccinated, whereas 17 of negative ostriches were administered with live ND vaccine by the drinking water at 3- to 5-month-old. Three ostrich serum samples from a livestock hygiene service center in prefecture A where ND outbreaks had occurred in chickens were positive with high level VN titer (171, 250, and 1990, respectively).

Seven serum samples of approximately 2-month-old ostriches that were vaccinated with live ND B1 vaccine by eye drop at a livestock hygiene service center in the prefecture B were all positive by the VN test (VN titer; 4–37) at 1
Antibodies to NDV were found in 22 of 181 slaughter-age ostriches. Antibodies to AIV were not detected in any of the sera by the AGP test.

The tracheal swabs and intestinal contents from 102 ostriches were collected in Japan between 2002 and 2003. After the second passage in embryonated eggs, no haemagglutinating viruses were isolated. Some samples had NDV-specific neutralizing antibodies by the VN test without vaccination.

In this study, the heat, kaolin and chicken red blood cell treatment of serum samples reduced the number of false positive reaction in the HI test, but there was still no correlation between the HI test and the VN test (r=0.363). The value of the specificity and the positive predictive value were very low, because of false positive reaction. The higher the predictive value, the higher the probability that examination of the same sample by the reference method would give similar results. In this study, we considered the HI test to lack specificity. Williams et al. [23] and Cadman et al. [6] developed an indirect enzyme-linked immunosorbent assay (I-ELISA) for detecting antibodies in ostrich sera. Williams et al. [23] concluded that the HI test gave a high incidence of false positive results and felt that the heat and kaolin treatment used to remove non-specific agglutination reactions reduced the sensitivity of the HI test. These authors also considered the HI test to lack sensitivity. In contrast, Koch et al. [14] used a blocking enzyme-linked immunosorbent assay (B-ELISA) [9], the VN test, and the HI test without pre-treatment of serum. The sensitivity, specificity, and predictive accuracy of the B-ELISA and the HI test relative to the VN test were similar. Moro et al. [18] reported that a liquid phase B-ELISA and HI test with heat and kaolin treatment showed good agreement. False positive reaction in the HI test might arise from ostrich species.

Antibodies to NDV were found in 22 of 181 slaughter-age ostriches without vaccination in this study. Deaths due to NDV have been reported in the ostriches in many foreign countries [11, 12, 20]. It has also been reported that apparently normal ostriches had antibodies to NDV [6, 14, 15]. No clinical signs of disease were observed in any of the birds tested in this study. Of 181 samples, 159 did not have antibodies to NDV and even in the same farms, there were serum positive and negative ostriches. Three serum samples from the prefecture A showing 171, 250, and 1990 VN titers, respectively, suggested that these ostriches were infected with virulent NDV. These data indicate that NDV has infiltrated into ostrich farms in Japan. Establishment of vaccine programs for ND in ostriches is important to protect not only the ostrich industry but also the commercial poultry industries.

Ostrich samples tested in this study were serologically negative for influenza. The AGP test for detecting antibodies to type A influenza viruses may not be sufficiently sensitive to detect low levels of viral antibody [10]. Otsuki et al. [19] considered that antibodies of only very high titers against influenza virus in chicken sera in the HI test were detected in their AGP test. Abraham et al. [1] reported that the I-ELISA detected a positive antibody response in vaccinated turkeys earlier than the HI test or the AGP test and also detected more antibody-positive turkeys than the HI test or the AGP test. The competitive enzyme-linked immunosorbent assay (C-ELISA) was more sensitive and more specific than the AGP test and as sensitive and as specific as the HI test [26]. It seems to be nice to use more sensitive and more specific screening methods such as C-ELISA to detect lower level antibodies to influenza A virus in ostrich sera to confirm the negative results.

In the attempt of virus isolation from the tracheal swabs and intestinal contents, all tested birds were adult and apparently healthy. Samberg et al. [20] reported that 28% mortality in 5- to 9-month-old ostriches following nervous disease signs while no mortality occurred in older birds. They also reported that in the natural infections NDV could only be isolated from the brains of a juvenile ostrich, however, the attempts to isolate the virus from the liver, spleen, heart and kidney were unsuccessful [20]. In experimental infection of five, 3-month-old ostriches with the virulent “Israel-67” strain of NDV, the virus could be reisolated from different organs (liver, spleen, brain, pancreas and intestine), from all sick or dead ostriches [20]. As far as we know, there are no detailed data in experimental infection of juvenile and older ostriches with lentogenic strain of NDV. In the present virological research, some samples had NDV-specific neutralizing antibodies by the VN test without vaccination, but no haemagglutinating viruses were isolated. It seems to be difficult to isolate NDV from adult and healthy ostriches, especially low virulence virus.

In conclusion, ND infection was demonstrated by the NDV neutralization test in ostriches in Japan. No haemagglutinating virus was isolated in the present study. As ND and AI outbreaks have occurred in Japan, further surveillance of NDV and AIV in ostriches will be important.
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REFERENCES