

Detection of Newcastle disease virus-specific antibodies in ostrich sera by three serological methods

G. Koch, G. Czifra, B. E. Engström

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Sera from 211 ostriches were tested for the presence of Newcastle disease virus (NDV)-specific antibodies by the virus neutralisation test, the haemagglutination inhibition (HI) test and a recently developed avian paramyxovirus serotype 1 (PMV-1) specific monoclonal antibody blocking ELISA (b-ELISA). The virus neutralisation test was used as the reference for the estimation of the sensitivity and specificity of the b-ELISA and HI tests. Of the 211 sera, 140 contained NDV-specific neutralising antibodies, 130 were positive by the HI test and 122 by the b-ELISA. The sensitivity, specificity and predictive accuracy of the HI and b-ELISA tests relative to the virus neutralisation test were similar. The good agreement between the HI and b-ELISA test ($\kappa = 0.85$) suggested that the two methods are interchangeable.

THE breeding and trading of ratites (ostriches, emus and rheas) has expanded considerably all over the world in recent years. They are susceptible to several diseases of domestic fowl, including Newcastle disease (Samberg and others 1989). The characterisation of 14 Newcastle disease virus (NDV) isolates from ostriches in South Africa showed that they carried vaccine strains (like La Sota) and a number of velogenic strains (Manvell and others 1996). Ostriches may therefore carry and spread Newcastle disease and imported birds should be tested serologically, like poultry flocks, and positive results should be confirmed by virus isolation.

The haemagglutination inhibition (HI) test is the accepted method for detecting NDV-specific antibodies in poultry sera (Alexander 1991). However, sera from other species often induce non-specific reactions in the HI test and, in order to avoid these reactions, sera have to be adsorbed with chicken red blood cells (RBCs) before they are tested.

Allwright (1996) reported that the HI test gave poor results when ostrich sera were tested, although chicken RBCs were replaced by ostrich red blood cells. Inactivation and kaolin treatment (King and Hopkins 1983) reduced the number of false positive reactions but false negative results still remained a serious problem. The results obtained with a modified indirect ELISA, using coated plates from a commercial Newcastle disease kit and a biotinylated rabbit anti-ostrich-IgG conjugate, correlated very well with the results of the microneutralisation test. Recently, a PMV-1 specific monoclonal antibody blocking ELISA (b-ELISA) has been described by Czifra and others (1996). The specificity of this test is based on a reaction between a monoclonal antibody (mAb) and its well conserved PMV-1 specific binding site (epitope). The mAb reacted with all strains representing different serogroups within the PMV-1 serotype, but not with any strain belonging to other PMV serotypes. Consequently, sera from any PMV-1 virus-infected or vaccinated animal 'block' the binding of the mAb. It was therefore decided to test ostrich sera with the b-ELISA and collect data on the prevalence of NDV-specific blocking antibodies in ostriches.

This paper describes the results of the examination of 211 ostrich sera by the virus neutralisation (VN) and haemagglutination inhibition (HI) tests, and by the b-ELISA.

Materials and methods

Ostrich sera

The 211 ostrich sera were sent from different Dutch and Swedish ostrich breeder farms for export testing. No clinical signs of disease were observed in any of the birds tested. There was either no information available about vaccinations, or the owners declared that none of the ostriches were vaccinated.

Serological methods

The VN test was carried out as described by Koch and Roozelaar (1994). Briefly, a mixture of 1/10 diluted ostrich sera and 10^7 EID₅₀ of virulent NDV, PMV1/Ch/NI/152608, was incubated for an hour, serially diluted and inoculated on to monolayers of the chicken embryo-related (CER) cell line. The monolayers were checked for a cytopathic effect 48 hours after infection. The neutralisation index (NI) is the difference between the log₁₀ titre of the virus in the absence and the presence of serum. Sera with a NI of 2 or more were considered positive.

The HI test was carried out as described in Council Directive 92/66/EEC (1992), using 8 haemagglutination (HA) units; the sera were tested without pre-adsorption with chicken RBCs. Titres of 1/8 or more were regarded as positive.

The b-ELISA was carried out as described by Czifra and others (1996), using the commercially available NDV antibody test (SVANOVA; Biotech). Briefly, NDV-coated ELISA plates were first incubated with serum samples diluted in phosphate-buffered saline containing 0.05 per cent Tween-20 (PBS-T) for 30 minutes. After washing, the NDV-specific horseradish peroxidase-labelled mAb was added to the wells, and incubated for a further 30 minutes. The binding of the conjugated mAb was visualised with a substrate solution containing tetramethylbenzidine hydrochloride and hydrogen peroxide. The test was performed at room temperature. The wells were washed between the incubation steps three times with PBS-T.

Optical density (OD) values were determined with a microtitre plate photometer (Multiscan Plus; Labsystems OY). The OD values of the serum samples were compared to the NDV-negative reference serum which had an OD value of 0.6 to 0.8. The percentage inhibition (PI) was calculated from the formula:

$$\text{PI (per cent)} = 100 \times \frac{\text{negative reference OD} - \text{sample OD}}{\text{negative reference OD}}$$

The starting dilution of the sera was 1/10, and all sera showing a PI of 50 per cent or more were considered positive. The b-ELISA titres were expressed as the highest two-fold dilution still giving a PI greater than 50 per cent.

Statistical analysis

Sensitivity was defined as the proportion of VN-positive samples that were correctly identified by the HI or b-ELISA, and the specificity was defined as the proportion of correctly identified VN-negative samples. They are expressed in percentages.

The positive predictive value was defined as the proportion of sera with positive b-ELISA (or HI) and VN results relative to the total number of b-ELISA (or HI)-positive sera. The negative predictive value was defined as the proportion of sera with negative b-ELISA (or HI) and VN results relative to the total number of b-ELISA (or HI) negative sera. Their maximum value is 1. The higher the value, the higher the probability that examination of the same sample by the reference method would give similar results.

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TABLE 1: Comparative results of the examination of 211 ostrich sera by virus neutralisation, haemagglutination inhibition and b-ELISA

	VN		HI	
	positive	negative	positive	negative
b-ELISA				
positive	116	6	119	3
negative	24	65	11	78
HI				
positive	121	9		
negative	19	62		

b-ELISA Blocking enzyme linked immunosorbent assay, HI Haemagglutination inhibition, VN Virus neutralisation

The Chi-squared test was used to compare the sensitivity and the specificity of the HI and b-ELISA tests. Correlation coefficients (r) were calculated in order to measure the strength of the associations between the VN index, the HI titre and the b-ELISA titre. Kappa (κ) was calculated to measure the strength of the agreement between two methods (Martin and others 1987). Statistix (version 4.0) was used for the calculations.

Results

Of the 211 ostrich sera, 140 contained NDV-specific neutralising antibodies as shown by the VN test, whereas 130 sera had HI titres greater than 1/8 and 122 sera were positive by b-ELISA at a 10-fold dilution. The relationships between the three tests are shown in Table 1.

There was no significant difference between the sensitivity, specificity and predictive accuracy of the HI and the b-ELISA tests when they were compared with the VN test (Table 2). When the b-ELISA was compared to the HI test, using the latter as a reference test, its sensitivity was 91.5 per cent and its specificity was 96 per cent. The probability that any of the b-ELISA-positive samples would also be HI-positive was 0.97 (positive predictive value), and the probability of a concordant negative result was 0.87 (negative predictive value).

A close correlation ($r=0.71$) was found between the b-ELISA titres and the VN indices of the same sera (Fig 1). Similar correlations ($r=0.72$) were obtained when either the b-ELISA and HI titres, or the VN indices and the HI titres were compared (data not shown).

There was a good agreement between the HI test and the b-ELISA ($\kappa=0.85$), and the VN and HI tests, and the VN tests and the b-ELISA also showed good agreements ($\kappa=0.70$ in both cases).

Discussion

Koch and van Roozelaar (1994) examined 147 ostrich sera for antibodies to NDV by serum neutralisation and the HI test. The number of negative sera was too low to estimate the sensitivity and specificity of the HI test. It was stressed that some ostrich sera

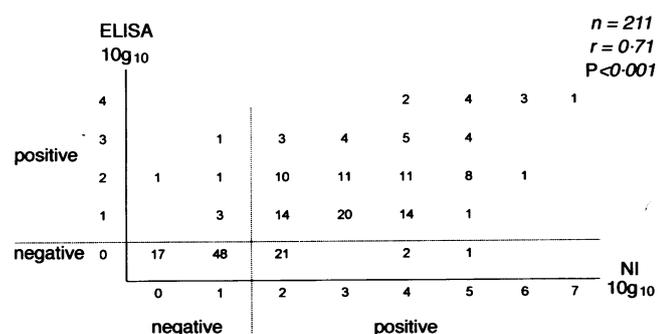


FIG 1: Correlation between b-ELISA titres and virus neutralisation indices (NI). The numbers are the numbers of sera having identical VN and b-ELISA results. b-ELISA titres ≥ 2 and VN indices >1 were considered positive

TABLE 2: Relative sensitivity, specificity and predictive accuracy of the HI and b-ELISA tests

	HI compared to VN	b-ELISA compared to VN	b-ELISA compared to HI
Sensitivity (%)	86	83	91
Specificity (%)	87	91	96
Positive predictive value	0.93	0.95	0.97
Negative predictive value	0.76	0.73	0.87

with relatively high neutralisation indices were negative by the HI test. Nevertheless, the predictive value of the HI test for detecting NDV antibodies at the flock level was high. It was suggested that HI-negative ostriches from a flock in which there were some HI-positive birds should only be moved, traded or exported if they remained negative two to three weeks after they had been isolated from the positive birds.

One hundred and forty-one sera from the same panel were retested with the b-ELISA, and 70 additional ostrich sera originating from Swedish farms were examined by all three methods. The VN test was used as the reference test for the estimation of the sensitivity and specificity of the b-ELISA and HI tests.

The sensitivity of the HI test relative to the VN test was 86 per cent, and its specificity was 87 per cent. The sera were not inactivated before the test because none of them showed non-specific reactions.

The sensitivity of the b-ELISA relative to the VN test was 82 per cent, and its specificity was 91 per cent. The ostrich sera were examined in 10-fold dilutions instead of the two-fold dilutions defined for poultry sera (Czifra and others 1996). Ten of the 24 b-ELISA-negative but VN-positive sera (Table 1, Fig 1) were retested at lower dilutions and five became positive. Sixty VN-negative sera retested at lower dilutions remained b-ELISA negative. Thus, the use of lower dilutions of ostrich sera increased the sensitivity of the b-ELISA without reducing its specificity.

There was no evidence of the presence of NDV in the birds tested apart from the results obtained by serological methods. However, the concordant results and the close relationship between the results of the HI and b-ELISA ($\kappa=0.85$) suggest that the two methods are interchangeable and that both are measuring the amount of NDV-specific antibodies.

The presence of NDV-specific antibodies in traded ostriches must be further examined. Systematic serological studies are needed to study the seroconversion and the persistence of antibodies in ostriches after vaccination or after infection with apathogenic and pathogenic PMV-1 viruses. There is also a need for a standardised, reliable serological method which is relatively easy to perform with sera from ratites.

The VN test would be the most accurate and most reliable test for this purpose but it is very laborious and needs special skills. The serological examination of ostrich sera by the HI test may also be difficult. Attempts to eliminate non-specific reactions decrease the sensitivity of the HI test and the number of false negative results increases (Allwright 1996). The effect of inactivation was not studied systematically in this study. However, non-specific reactions have been observed with ostrich sera in both laboratories and the authors are aware that these treatments may significantly influence the result obtained by the HI test.

The b-ELISA had a similar sensitivity and specificity to the HI test in this study. It could be the method of choice, particularly when the sera from exotic birds must be tested serologically.

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Conservative and surgical treatment of tibial fractures in cattle

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Ninety-five cattle with tibial fractures, all but one with a unilateral fracture, were examined between 1990 and 1994. The feasibility of repairing the fracture was assessed radiographically, also taking into account the bodyweight and value of the animal. Twenty-two cattle were slaughtered. Conservative treatment with stall confinement and/or a splint or cast was applied in 18 cases, with satisfactory results in eight of them (44 per cent). They were fattened to normal bodyweight, but they all had a severe deformity of the affected leg. In 55 animals, the fractures were fixed externally with Steinmann pins and methylmethacrylate bridges under image-intensified fluoroscopy. Ten of them could not bear weight on the affected leg and were slaughtered before the pins were removed. In the remaining 45 animals the pins were removed after a mean (sd) period of 71 (14) days. Four animals re-fractured the affected leg shortly after the pins were removed and six others were slaughtered prematurely because of inadequate weight bearing. The results were successful in 35 cases (64 per cent). Slight deviations of the affected leg and/or the contralateral leg were often observed. The overall survival rate of the cattle with tibial fractures was 45 per cent.

TIBIAL fractures are commonly encountered in every age group of breeding and fattening cattle. These fractures are often comminuted and can involve all parts of the tibia (Tulleners 1986). Their treatment can be a real challenge, especially in heavy and double muscled animals. Tibial fractures in cattle have been reported to heal satisfactorily with stall confinement and/or a modified Thomas splint or cast (Gish 1941, Beckenhauer 1958, Adams and Fessler 1983, Adams 1985). Different types of hanging pin casts have also been applied successfully (Kendrick 1951, Kumar and others 1973). The fracture can be reduced surgically by using bone plates, Rush pins, Kuntscher nails, cross-pinning and transfixation pinning (Verschooten and others 1972, Rao and Rao 1973, Hamilton and Tulleners 1980, Kumar and others 1981, Vijaykumar and others 1982). Transfixation pinning is accomplished by placing two or more pins transversely through the proximal and distal bone fragments, and fixing the protruding ends with external bars. The site and configuration of the fracture are important factors in determining which treatment to apply.

This paper reviews the treatment of 95 cattle with tibial fractures.

Materials and methods

Ninety-five cattle with tibial fractures were referred to the Large Animal Surgical Clinic between 1990 and 1994. The animals' history, and the results of the physical and radiographic examinations and management were obtained from the medical records. The bodyweight of the animals could often not be determined. Follow-up information about the animals discharged was obtained by questionnaire or by telephone.

The feasibility of fracture repair by external transfixation was assessed radiographically, taking into account the site and configuration of the fracture, and the bodyweight and value of the animal. Conservative treatment with stall confinement and/or a splint cast was used only for animals that had not already reached slaughter weight, or when external transfixation was technically impossible, or when the owner was unwilling to pay the costs of surgery.

Surgically treated animals were fasted for one day. The animals were sedated with xylazine hydrochloride intramuscularly (0.1 to 0.2 mg/kg) (Rompun 2 per cent; Bayer). Anterior epidural analgesia with lidocaine (Xylocaine 2 per cent; Astra) or mepivacaine (Sandicaine 2 per cent; Astra) or bupivacaine (Marcaine 0.5 per cent; Astra) was used in the sternal recumbent animal to obtain complete paralysis of the hindlimbs. The effect of the epidural analgesia was evaluated by assessing the responses of the animal to stimulation of the coronary band of the claw with forceps; analgesia was considered sufficient for the surgical intervention when no external reactions were observed. Ketamine hydrochloride (1 mg/kg) (Imalgène 1000; Rhône Mérieux) was administered intravenously during surgery when the reactions of the animal indicated that analgesia was inadequate. One 14-month-old bull was treated surgically under standard inhalation anaesthesia (halothane in oxygen).

The affected leg was surgically prepared before the animal was placed dorsally in a V-shaped rack. Soft foam rubber pillows were used to prevent possible myopathies. The affected leg was pulled up by means of a tackle. A medial approach was used and the surgical procedure was done under image-intensified fluoroscopy. At each pin site, a stab incision was made in the skin and the holes were pre-drilled (diameter 6 mm). Afterwards, 6 mm Steinmann pins were drilled through both cortices and the skin on the opposite side. Whenever possible, three pins were inserted in the proximal bone fragment and three in the distal fragment. These pins were placed in a mediolateral direction, parallel, in one vertical line and if possible 3 cm apart from each other. In distal epiphyseal fractures, the lower pins were placed through the metatarsal bone, incorporating the hock into the transfixation device. In heavier animals, two series of pins were inserted (Fig 1a). The first row was inserted in a mediodorsal to lateroplantar direction, and a second row was inserted in a medioplantar to laterodorsal direction, more or less perpendicular to the first row of pins. After fracture reduction, plastic tubes (3.5 to 5 cm diameter) were placed over the pins

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