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SOME INVESTIGATIONS FOR NEWCASTLE DISEASE IN THE EGYPTIAN OSTRICHES (STRUTHIO CAMELUS)

(With 3 Tables and One Figure)

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(Received at 28/9/2005)

إستبيان عن مرض النيوكاسل في النعام المصري

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خلال خريف وشتاء عامى ٢٠٠٤ -٢٠٠٥، تم تجميع عينات من ٥٤ نعامة (حى ونافق) بأربع مزارع بمناطق مختلفة فى نطاق الجمهورية لفحصهم، لإكتشاف وتحديد نسبة حدوث المرض والاعراض والصفات التشريحية والباثولوجية فى النعام المصرى. وقد تم عزل عترتين افيروس مرض النيوكاسل وتنميتهم على أجنة بيض الدجاج، وقد تم فحص العترتين بالميكروسكوب الالكترونى وعمل اختبار الضراوة لهم. وقد إستعرضت هذة الدراسة إستخدام إختبار مانع تلازن الدم، لتبيان وتقدير الاجسام المناعية للنيوكاسل فى النعام بين السلب والإيجاب.

SUMMARY

During late autumn and winter, the period of December 2004- February 2005, 4 ostrich farms around Egypt were surveyed and samples from 54 clinically diseased and fresh dead birds were collected and tested for detection and determination of the incidence, course, clinical signs, PM, histopathology and the pathogenicity of NDV among ostriches (Struthio camelus). Two NDV isolates were detected, propagated in chicken embryos, pathotyped and examined by EM. The present retrospective study was prompted by the finding of HI was adopted for the detection and quantification of antibodies to NDV. *Abbreviations:* CIAF = clarified infected allantoic fluid; CRBCs= chicken red blood cells; ECEs = embryo chicken eggs; EID50 = median embryo infective dose; ELISA = enzyme linked immunosorbent assay HA = haemagglutination; HI = haemagglutination inhibition; IAF= infectious allantoic fluid; ICPI

=.intracerebral pathogenicity index; IVPI = intravenous pathogenicity index; LPB-ELISA = liquid phase blocking enzyme-linked immunosorbent assay, MDT = Mean death time; ND = Newcastle disease; and NDV = Newcastle disease virus.

Key words: *Newcastle, ostriches.*

INTRODUCTION

Ostrich (Struthio camelus) farming as a source for meat, skin and feather has been introduced into Egypt, and other developed countries in recent years. Where the ostrich industry in Egypt started since 1997.since 2000 ostrich chicks and layers were reared in 55 farms with a total population of 4000 birds Kamel *et al.* (2000).

Relatively little is known not only about the infectious diseases that occur in farmed ostriches but also about the microorganisms that may be present in healthy birds. They are susceptible to several diseases of domestic fowl, including ND, Samberg *et al.* (1989).

Newcastle disease (ND) is caused by specified viruses of the avian paramyxovirus type I (APMV-I) serotype of the genus *Avulavirus* belonging to the subfamily *Paramyxovirinae,* family *Paramyxoviridae*. The paramyxoviruses isolated from avian species have been classified by serological testing into nine serotypes designated APMV-1 to APMV-9; ND virus has been designated APMV-1, Alexander D.J. (1997).

Ricardo *et al.* (2000) reported that LPB-ELISA titers regressed significantly ($P<0.0001$) on the HI test titers with a high correlation coefficient ($r=0.875$). The two tests showed good agreement (=0.82; $P<0.0001$), relative sensitivity (90.91%) and specificity (91.18%), and accuracy (91.02%), suggesting that they are interchangeable.

During the last decades there have been many reports on viral disease in ostriches. These reports lack evidence about the nature of the diseases, characterization and the pathogenicity of the isolated viruses for ostriches compared to chickens and other avian species. In this paper will be discussed only the ND which is the first economically important viral disease in ostrich industry.

MATERIALS and METHODS

Birds:

54 ostriches were examined during this study collected from 4 flocks of ostriches, aged from 1 months to 4 months, all of them

vaccinated against ND with commercial available Hichner and La Sota NDV vaccines, suffering from one or more of these clinical signs (respiratory and or nervous). These were located in different localities of Ismailia and Alexandria Governorates. (Table 1).

Sera:

34 serum samples were collected of these birds, distributed as in Table (2).

Virus antigen:

The NDV live vaccine strain LaSota was propagated in the allantoic cavities of 9 to 11 day-old embryonated chicken eggs by inoculation with 0.1 ml of allantoic fluid containing $10^{7.2}$ EID_{50}. IAF was harvested and clarified by centrifugation at 3,000xg for 20 minutes at 4°C according to Ricardo *et al.* (2000). The reciprocal of HA titers of the stock NDV harvested was 1024. The clarified IAF was used as antigen in the HI test.

HI test:

The micro-beta HI test was performed using 8 HA units of the LaSota vaccine strain of NDV CIAF and 0.75% washed CRBCs, Elizabeth *et al;* (2000). Each test serum sample were pretreated at 56°C for 30 min. and then incubated with kaolin at 37°C for 30 min. to extract nonspecific inhibitors, Williams *et al.* (1997). According to Ricardo *et al.* (2000) HI titers equal to or greater than 3 log_2 were considered positive results in ostriches.

Samples:

Blood was collected from the neck vessels. The serum was harvested. The following samples were taken for the detection of virus from each of the dead ostriches: brain; liver; spleen and kidney as an organ pool; trachea and lung as a respiratory system pool; several sections of the gastrointestinal tract as a gastrointestinal tract pool; muscle (after minimum 24 h of chilling); and bone marrow. Swabs were taken to evaluate as a diagnostic technique in ostriches.

Virus isolation technique:

Samples from were submitted to standard virus isolation technique for NDV. Alexander (1997) via allantoic sac route inoculation of 9 to11day-old embryonated hens' eggs. Positive HA activity was further tested against reference sera for HI for NDV confirmation. A negative result on this material resulted in another passage in eggs.

Pathotyping tests

Performed on NDV-isolates according to Alexander and Allan (1974).

Electron micrograph (EM):

According to Woolcock *et al.* (1996).

Pathology:

According to Bancroft *et al.* (1996).

REUSLTS

Seasonal variation and diseases

Table (1) showed that during late autumn and winter, the period of December - February, the time of year when outbreaks in ostriches typically started to occur and considered the most critical period for rearing of ostriches.

Table 1: Results of monthly distribution of NDV in ostriches during the time of this study.

Month	No of cases Examined	% of cases Examined	No of + ve NDV /No of dead ostrich	%of + ve NDV
October 2004	——	0%	0	0 %
November 2004	——	0%	0	0 %
December 2004	10	18.5%	0	0 %
January 2005	36	66.7%	2 / 1	50%
February 2005	8	14.8 %	1 / 1	100%
March 2005	——	0%	0	0 %
April 2005	——	0%	0	0 %

Signs:

The disease spread was limited to isolated groups of ostriches, are characterized by an increase in the mortality rate, decreased in appetite and green faecal colour; include usually neurological and respiratory signs.

Neurological signs (There are subacute to chronic nervous disease, usually limited to a small number of birds): include reluctance to walk and stand, and signs of brain damage such as a slight tilting of the head, frequent scratching of the head, cervical muscular tics, the

typical Incoordination, torticolis and neck paralysis. A common condition secondary to the neck paralysis is swollen head and the lower part of the neck due to the edema in the subcutis, limp necks, loss of balance, recumbency, paralysis, latter torticollis, uncontrolled head movements, and finally inability to light the head off the ground.

The respiratory signs includes: productive cough, few birds showed mild tachypnoea and sometimes Dyspnoea, but improved there after undefiled conditions.

The viremic period in infected ostriches is between nine and eleven days and there is no indications of a carrier state or presence of the virus in the meat or any tissues after this period with peak immunoglobulin response reached on day fourteen post infection, where sharp increase in the levels of HI titers can be observed in flock after exposure to the virus HI titres after 5 days exceeded $\log_{12}$.

3 ostriches died over three weeks. Ostriches which have died from ND do not show any typical pathological lesions, except there are petechiae on the heart. Pneumonia and enlarged liver. Or histopathological lesions, except in some cases small foci of gliosis may be seen in the brain steam. While Perivascular cuffing is absent.

Results of virus isolation and E.M:

NDV was isolated from only one dead bird (100%) in flock No (1) and also from only one bird of two dead birds (50%) in flocks No (4) from the brain of the dead birds in chick embryos. while trials to isolate NDV from the second bird of flock No (4) were failed. Attempts to isolate NDV from the spleen, kidney, liver and heart were unsuccessful. Also swabs were taken was shown to be an ineffective diagnostic technique in ostriches. Embryo mortality occurs on first passage, these isolate was identified by haemagglutination of chicken RBCs, a HA titre of 64 was detected in the allantoic fluid at 7 days post inoculation. In the second passage the harvested allantoic fluid has an HA titre of 1024 inhibited by specific antiserum to NDV, and reproduction of the disease in experimental chicks, and was shown to contain NDV particles by negative stain EM. Generally, negative contrast electron microscopy of NDV reveals very pleomorphic virus particles, they are rounded and 100 - 150 nm in diameter (Fig1).

Table 2: Results of history of ostriches under investigations and samples collected.

No of flock	Location	Age/ Month	Vaccination / day	No of Serum/ ostriches examined	Morbidity %	Mortality %	Signs
1	Ismailia	2	10,23	11/17	3/17 =17.6%	1/17 =5.9%	Respiratory, Nervous
2	Alexandria	4	5,17.35.64	9/12	1/12 =8.3%	0	Respiratory,
3	Alexandria	2	8, 15,47	7/10	1/10 =10%	0	Respiratory,
4	Ismailia	1	11,24	7/15	4/15 =26.7%	2/15 =13.3%	Nervous Respiratory,

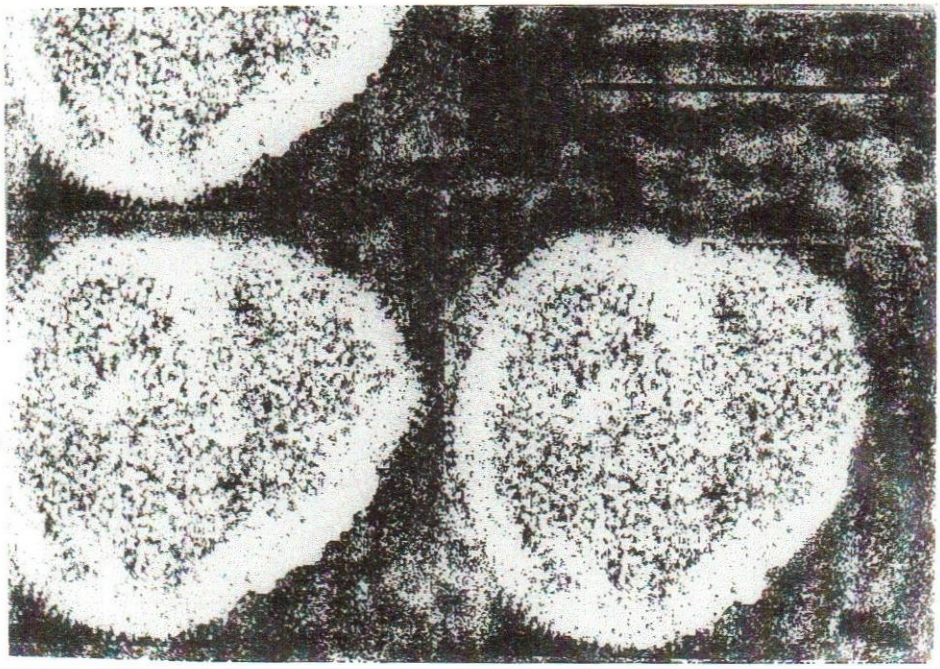

Fig. 1: Electron microscope of negatively stained NDV particles (Bar = 100 nm).

Pathotyping:

Isolated NDV with virulent indices of MDI = 48 h, IVPI = 2.17 and ICPI =1.8.

Serological identification of serum samples by HI test:

Table (3) shows rising antibody concentrations until day 14. Where the cumulative proportion of HI-seropositive (HI titer ≥ 3 $\log_2$) ratites was 16/34 = 47.1% with different ranges, means and CV of HI titers of 4 groups.

Table 3: Results of serological responses of NDV by HI test of examined ostriches.

Flock No	No of sera tested	HI range	HI Mean ±SE	CV %	No of +ve HI titer	% of +ve HI titer
1	11	2 - 14	13.3 ±1.08	27 1%	6	54.6%
2	9	0 - 11	8.5 ±0.53	18.8%	3	33.3%
3	7	0 - 8	7.7 ±0.46	15.6%	3	42.9%
4	7	1 -13	12.7 ± 1.61	33.1%	4	57.1%

DISCUSSION

Limited information is available about disease agents present in ratite (ostrich) population. ND is found a disease of concern.

About seasonal variation Table (1) showed that during late autumn and winter, the period of December - February, the time of year when outbreaks in ostriches typically started to occur and considered the most critical period for rearing of ostriches.

Table (2) showed that 9 ostriches developed clinical signs (mainly respiratory and nervous); 3/9 (33.3%) of them died while the other 6/9(66.7%) recovered. The recorded signs in our study were agreed with that reported by Samberg *et al.* (1989). All of the dead birds had age less than 3 months; it is therefore concluded that the mortality was highest in young birds.

Our results tend to confirm that, as signs of clinical disease in ostriches vary widely and diagnosis may be complicated further by the different responses to infection by different hosts, clinical signs alone do not present a reliable basis for diagnosis of ND. However, the characteristic signs and lesions associated with the virulent pathotypes will give rise to strong suspicion of the disease. In our outbreak of ND

ostriches died over three weeks, ie, The spread of NDV through a flock of ostriches is extremely slow compared with that in commercial poultry, indicating that course of ND in ostrich is differed that that of chickens in other words, disease spread was limited to isolated groups of ostriches, agreed with that reported by Verwoerd *et al.* (1997), as well as reports from Israel, Samberg *et al.* (1989) suggest crucial differences in the epidemiology and pathogenesis between outbreaks of the same NDV strain in ostriches and commercial poultry.

The recorded PM lesions in ostriches which have died from ND in this study agreed with Samberg *et al.* (1989).

Ostriches which have died from ND do not show any typical histopathological lesions, perivascular cuffing is absent, agreed with that reported by Allwright (1996) differed than that reported by Huchzermeyer (1996) who stated that perivascular cuffing is rare.

Distribution of NDV isolated from ostrich in flocks number --- where the farms in these localities are near to poultry farms which facilitate the spreading of NDV in these areas.

Isolation of NDVs in farms No 1 and 4 although the ostrich of these farms vaccinated by eye drop with La Sota strain (Table 2) can be explained by Blignaut *et al.* (2000) they reported that, 5-week-old ostriches were found not to produce serum antibody i.e, did not elicit a humoral immune response after eye drop vaccination with live LaSota vaccine but did produce serum antibodies after vaccination with inactivated LaSota vaccine. In other species such as chickens, live LaSota vaccine, vaccinated by eye drop, has been shown to confer mucosal immunity. In the future, we intend to investigate the mucosal immune response of ostrich chicks to vaccination with live LaSota vaccine by assessing IgA levels in mucous secretions in the upper respiratory tract, in this way we hope to establish whether eye drop vaccination of ostriches with live La Sota vaccine is capable of inducing protective mucosal immunity.i.e., the best results can be obtained by the vaccination of ostrich with live and inactivated ND vaccines.

Viruses were isolated from the brain affected birds (Table 2). Attempts to isolate virus from the liver, spleen, heart and kidneys were unsuccessful agreed with that reported by Samberg *et al.* (1989). Cloacal and choanal swabs used for direct virological screening in clinically infected cases in our study could not detect.NDV,.ie. Swabs were taken was shown to be an ineffective diagnostic technique in ostriches agreed with reported by Allwright (1996) and Verwoerd (2000).

NDV could be re-isolated form the dead birds during the viremic stage up to 12 days, while 14 days after the last mortality, NDV could not isolated from organs, muscles (fresh), muscle (24 h chilled), gastrointestinal tract, bone marrow or respiratory system taken from the diseased ostriches. It is suggested that it would be extremely unlikely that the international trade in ostrich meat could act as a mechanism for spreading virulent NDV from endemic to non endemic parts of Egypt, agreed with Verwoerd *et al.* (1997). The low percentage of isolation may be due to the rapid disappearance of the virus in the tissues of the host, these irregularities also encountered by Brandly *et al.* (1946) in chickens.

Our results showed that ICPI was 1.8, according to the OIE definition for reporting an outbreak of ND is: Newcastle disease is defined as an infection of birds caused by a virus of avian paramyxovirus serotype 1 (APMV-1) that meets the following criteria for virulence: The virus has an ICPI in day-old chicks (Gallus gallus) of 0.7 or greater. So we recorded that ostriches under examination were infected with NDV.

The results showed that both ICPI and IVPI values increased after passage through chickens or embryonated chicken eggs. This suggests that isolates from birds other than poultry may not show their potential virulence for chickens in conventional pathogenicity tests this agreed with work by Alexander and Parsons (1986) on NDV (APMV-1) isolates from pigeons.

Another interesting feature in our study of ND in ostriches is the fact that NDV does not haemagglutinate ostrich red blood cells, so that HI test can be performed using chicken-RBC and not ostrich-RBC., agreed with Elizabeth *et al.* (2000).

To exclude possible nonspecific reactions to allantoic fluid components or reactivity between the sera tested and the antigen, we conduct the further purification of purified NDV by red-cell adsorption elution to enhance the effectiveness of antigen, agreed with that reported by Sheffield *et al.* (1954).

The working test serum dilution of 1:4 was chosen as being the minimum serum dilution at which no gelation was observed during the test, it continued even the respective sera were submitted to clarification by centrifugation (10,000xg for 10 min. at 4°C), probably this phenomenon is related to high fat or low protein diets offered to the ostriches surveyed, Mushi *et al.* (1998).

Serological monitoring of ND in ostriches using the HI may sometimes be confusing; especially when titres are low (early stage of infection with NDV) and false negative are common. Furthermore, the use of serial treated serum dilution, as described in our test system is less practical, because it increases the preparation time and the number of micro titer plates required

Antibodies reactive with ND in naturally exposed ostriches from Ismailia and Alexandria were also detected by HI test 16 of 34; (47.1 %) agreed with that reported by Cadman *et al.* (1994), The lower sensitivity of HI titre in our study may be due to genetic background of the host (ostriches) or due to that discussed by Yachida and Iritani (1974) in chickens. They reported that the sensitivity of the HI test increased significantly when a homologous antigen (prepared from allantoic fluid of 10-day-old chick embryos infected with the same strain) used, or may be due to the time of development and persistence of HI in ostriches was different that reported by Doll *et al.* (1950) in chickens. This difference was determined in part by the strain of virus, the route of infection, and mainly by the host infected and genetic background of ostriches.

Table (3) support the fact that specific HI antibodies to NDV found in the sera of clinically ostriches does not mean that NDV is related to the disease symptoms.

Because of the large variation in titres within the groups, the mean was not a good measure of central tendency, where the CV were 15.6% -33.1 %, hence the median was used to determine the serological response by the HI test. However, because of the large number of 0 titres obtained, Table (3), the median and the mode could not be used as accurate measures of central tendency, and therefore the mean was used in this case to show the serological response curve. It must be pointed out, however, that the response curve for the HI test are not accurate because of the large standard error and standard deviation obtained, for this data and therefore only give an idea and trends.

The development of no detectable HI antibody in about 18/34 (52.9%) of the examined ostriches can be partially be attributed to differences in the response of the individual birds or ostrich sera not respond in the same way as sera from other avian species Perelman, (1999), and it should always be taken into consideration that ostrich antibodies differed than that fowl or turkey antibodies, Cadman *et al.* (1994) indicating that when using serology as a diagnostic tool in ostriches, it is important to recognize that ostriches may have a different immunological response to vaccination or infectious agents compared to

other species, and the techniques used in other animals may not be adequate to detect specific antibodies in ostrich sera.

Our results tend to confirm that although the HI test is still the most widely used conventional serological method for meaning anti-NDV antibody levels in poultry sera and it is considered the standard laboratory test for this disease in chickens. However, sera from other species including ostrich tend to give a high incidence of false-positive results. And although the number of non specific agglutination reactions can be reduced by pretreatment with heat and koalin, our results showed that these procedures decrease the sensitivity of this test.

The results showed that the HI test has provided a useful qualitative but not quantitative data of ND, so the test has found to be a little value in the acute phase of the ND and unreliable as a routine monitoring method these results agreed with that reported by Valadao (1955) in chickens.

According to the literatures reported by Williams *et al.* (1997) that the ELISA was at least 10 times more sensitive than the HI in detecting low levels of ostrich antibodies to NDV. Efforts must be exerted for carrying out ELISA test to evaluate the health status of the Egyptian ostriches using anti-ostrich IgG.

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