



Experimental infection of vaccinated slaughter ostriches with virulent Newcastle disease virus

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ABSTRACT

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A virulent Newcastle disease virus (NDV) isolate from an outbreak in commercial poultry, with virulence indices of MDT = 47–48 h; IVPI = 2,17 and ICPI = 1,8; was used to inoculate 10x vaccinated (standard poultry vaccines) as well as 10x unvaccinated slaughter ostriches via intratracheal, ocular and nasal routes, in a controlled environment. All unvaccinated ostriches developed clinical signs (mainly respiratory); two of them died while the other eight recovered. No vaccinated ostriches developed any clinical signs. All remaining (18) ostriches were slaughtered 14 d after the last mortality. Virulent NDV could be re-isolated from the dead birds, but not from organs, muscle (fresh), muscle (24 h chilled), gastro-intestinal tract, bone-marrow or respiratory system taken from the slaughtered 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 the world.

Keywords: Experimental infection, Newcastle disease, vaccinated ostriches, virulent

INTRODUCTION

Newcastle disease virus (NDV) is the most important infectious agent influencing poultry production throughout the world, both in the commercial sector (Alexander 1991, Alexander 1995, Capua, Sacchia Toscani & Caporale 1993, McFerran & McNulty 1993) as well as on subsistence-farmer ("village chickens") level. (Awan, Otte & James 1994; Bell & Moulodi 1988, Spradbrow 1993/94). Most strains are spread on farms either by aerosol (intensively housed poultry) or by the faecal-oral route (free-range chickens), while feed trucks, personnel, etc. can act as mechanical carriers spreading the virus to other areas. (Jordan 1990; Awan *et al.* 1994; Alexander 1995; Beard & Hanson 1984; Alexander 1991).

Although the concept of vertical transmission is controversial (Alexander 1991) velogenic NDV as well as live vaccine strains have been isolated from embryonated eggs, ovaries and oviducts of clinically affected or recently vaccinated hens (Capua *et al.* 1993; Lancaster 1966).

Southern Africa experienced a particularly severe NDV epidemic in 1993/94 with high mortalities in poultry as well as ostriches and other avian species. (Verwoerd 1995a, 1995b), Huchzermeyer & Gerdes 1993). The isolate of NDV has previously been recorded in commercial ostriches in Israel (Samberg, Hadash, Perelman & Meroz 1989) as well as in European zoos (Huchzermeyer 1994). Isolated NDV outbreaks occur sporadically in commercial ostriches in South Africa, in most cases related to concurrent poultry (commercial and/or informal) outbreaks (unpublished observations since 1993: author & W. Burger in South Africa, Y. Hemberger & F. Klein in Mariental, Namibia; C. Foggin in Zimbabwe). Ostrich meat is exported fresh, vacuum packed, from southern African countries as well as Israel (NDV endemic

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countries) to several parts of Europe (non-endemic NDV status in most of these). South African export slaughter ostriches are vaccinated against NDV with both live and inactivated vaccine at least 30 d and no longer than 6 months before slaughter under strict control of the Directorate of Animal Health. The spread of NDV through a flock of ostriches is extremely slow compared with that in commercial poultry (Verwoerd 1995b; Huchzermeyer 1994; W. Burger, personal communication) probably owing to the extensive nature of ostrich farming in South Africa. It was nevertheless considered imperative to investigate the possibility that vaccinated slaughter ostriches could be exposed to virulent NDV, not show any clinical signs and be slaughtered while harbouring the virus, and thus spread it via the meat trade.

MATERIALS AND METHODS

Materials

NDV strain

Field isolate (Labno. M142/94) from an outbreak in commercial poultry, virulence indices of: MDT = 47–48 h; IVPI-2,17; ICPI = 1,8 (for detail on virulence indices in poultry see Jordan (1990).

Birds

Twenty slaughter ostriches (by definition weighing a minimum of 90 kg live mass, and older than 10 months) were divided into two groups:

- *Unvaccinated*, i.e. known history of no exposure to either live or inactivated NDV vaccines
- *Vaccinated*, i.e. live vaccine (La Sota) used during chick rearing at ages 6 weeks and 10 weeks, as well as vaccination with inactivated (La Sota) vaccine in an aluminium hydroxide carrier (Trade name: Lomovac TAD), 3 ml s/c (dorsal neck) per bird, done by first author on the farm exactly 4 weeks before the challenge date.

Experimental procedure

Both groups of ostriches were transported from their respective farms to the Onderstepoort Veterinary Institute (OVI) 2 d before the challenge date to allow for evaluation of possible transport-related injuries. At the OVI they were housed in a building with positive air pressure, shower-in access control and inside feed storage. They were fed a commercial finisher ration at 2 kg per bird per day (Volos-Meadow Feeds) and had access to water *ad lib* for the entire experimental period. The cement floors were washed daily with the run-off collected in a closed tank which forms part of the building. On the date of challenge/infection each bird was caught, a blood sample (5 ml)

TABLE 1 Newcastle disease ELISA OD values obtained from NCD-challenge experiment; prechallenge and 14 d after last mortality

Animal l/d	25/10/1996	19/11/1996
1	0,448	1,385
2	0,895	1,102
3	0,275	1,336
4	0,506	1,294
5	0,687	1,162
6	0,109	1,266
7	1,056	1,352
8	0,954	1,398
9	0,176	1,316
10	0,583	1,356
11	0,067	1,128
12	0,071	1,195
13	0,097	1,153
14	0,141	1,218
15	0,876	1,286
16	0,124	1,247
17	0,111	1,230
18	0,097	1,242
19	0,405	Dead
20	0,086	Dead

Nos 1–10 Vaccinated

Nos 11–20 Unvaccinated

For comparison of the HI-results; sensitivity and specificity evaluations, see original article (Williams *et al.* 1996)

taken from the jugular vein for pre-challenge serology, and the virulent NDV inoculum 10^6 ELD₅₀; 5 ml/ostrich) was given to each bird by intratracheal, ocular, and nasal routes. See Table 1 for ELISA results (pre-challenge as well as 14 d after the last mortality). Re-isolation from the inoculum had an HA = 10 log₂ dilution; $10^{4,25}$ virus/ml. All ostriches were observed three times per day until the end of the experimental period, to evaluate the development of clinical signs (cf. Table 2). The following samples were taken from all mortalities and survivors (by definition alive 14 d after the last mortality) when they were slaughtered. Slaughter of survivors followed normal abattoir procedures, i.e. electrical stunning, bleeding, skinning, evisceration, but was conducted inside the facility on day 22 PI.

Samples

- Liver, spleen, kidney as an organ pool
- Muscle (fresh)
- Muscle (after 24 h of chilling)
- Bone-marrow
- Trachea and lung
- Several sections of the gastro-intestinal tract as a GIT-pool

Choanal and cloacal swabs were also taken from clinically severely affected birds on day 5 PI to evaluate their use as a diagnostic technique. Histopathology was done on samples from the mortalities.

Virus-isolation technique

Allantoic-sac route inoculations of 9–11-day-old embryonated SPF hens' eggs, five eggs per sample, minimum of three passages of 7 d each were used to reach a negative result. Allantoic fluid of all embryonated deaths was harvested, and bacterial culture and haemagglutinating activity were tested. Positive HA activity was further tested against reference sera for haemagglutination inhibition (HI) for NDV confirmation. Negative results on this material would result in another passage in SPF eggs.

RESULTS (See also Table 2)

Mortalities (x 2)

Macroscopic post mortem

- General congestion, petechial haemorrhages on epicardial surface and in lungs (on cut surface). Severe mucus discharge from sinuses via choanal to oral cavity. Suspected enteritis in duodenum and jejunum.

Histopathology

- Moderate congestion of brain with perivascular cuffing
- No significant lesions in any organs, trachea, gastro-intestinal tract

Virology

- No virus isolated from choanal/cloacal swabs
- Cause of death confirmed as velogenic NDV by successful re-isolation from bone-marrow and muscle

TABLE 2 Progression of clinical signs in ten unvaccinated slaughter ostriches after experimental infection with velogenic NDV

Days post infection	Clinical signs	Number
3	Conjunctivitis, lacrimation, scratching of head with foot	2
4	Same as above; severe coughing in one bird	10
5	Productive cough; lie down often (choanal and cloacal swabs from two most severely affected)	
7	Remainder same as previous day Dead. Remainder show clinical improvement	3 1
8	Dead. Remainder almost normal.	1
22	Slaughter all survivors and take samples for virology	8

- No virus could be isolated from any of the samples taken from slaughtered ostriches from either of the two groups

Serology

The newly developed indirect ELISA with an ostrich conjugate, (Williams, Verwoerd, Schoeman, Van Wyk, Gerdes, Roos, & Boshoff 1996) was used to evaluate the serological response of both groups after challenge.

Note: At no stage did any of the ten vaccinated slaughter ostriches in the pen next to the above group develop any clinical signs whatsoever.

DISCUSSION

Several field investigations of NDV outbreaks in southern Africa by the first author, other ostrich veterinarians (W. Burger in the Klein Karoo, South Africa, C. Foggin in Zimbabwe; Y. Hemberger in Mariental, Namibia as well as reports in the literature (Huchzermeyer & Gerdes 1993; Samberg *et al.* 1989, Verwoerd 1995b) have suggested the following important differences from outbreaks in commercial poultry by the same NDV strain:

- There is a significant individual variation in susceptibility to challenge by virulent NDV in ostriches.
- Susceptible birds are usually younger, are poor performers, are in poor physical condition, have concurrent infections, e.g. airsaccuillitis, or are on an unbalanced diet.
- Virulent NDV spreads very slowly within a group, and in practice almost never to adjoining groups even if they are separated only by a fence/poles.
- Vaccination with standard poultry vaccines (both live and dead) in most cases provides protection against the development of clinical signs even if the challenge is by velogenic NDV.
- There are no pathognomonic clinical signs nor macroscopic or histopathological lesions seen in NDV in ostriches.
- Choanal and/or cloacal swabs for direct virological isolation are not an accurate diagnostic method for antemortem diagnosis of NDV.

The results of this experiment supported all of these assumptions even in the confined space and high stress levels associated with the facility used compared with the usual extensive farming method employed in ostrich farming in South Africa. Additionally, no virus could be isolated from potential carriers (both vaccinated as well as unvaccinated) that had recovered from a very high challenge level of virulent NDV, that in some cases resulted in clinical

symptoms. It is therefore extremely unlikely that the international trade in fresh ostrich meat could be the way in which virulent NDV could spread from NDV endemic areas to NDV-nonendemic areas.

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REFERENCES

- ALEXANDER, D.J. 1991. Newcastle disease and other paramyxovirus infections, in *Diseases of poultry*, 9th ed., edited by B.W. Calnek, H.J. Barnes, C.W. Beard, M.W. Reid, & H.W. Yoder. Iowa: Iowa State University Press. USA: 496–519.
- ALEXANDER, D.J. 1995. Epidemiology and control of avian influenza and Newcastle disease. *Journal of Comparative Pathology*, 112:105–126.
- AWAN, M.A., OTTE, M.J. & JAMES, A.D. 1994. The epidemiology of Newcastle disease in rural poultry: A review. *Avian Pathology*, 23:405–423.
- BEARD, C.W. & HANSON, R.P. 1984. Newcastle disease, in *Diseases of Poultry*. 8th ed., edited by M.S. Hofstad, H.J. Barnes, B.W. Calnek, W.M. Reid & H.W. Yoder. Ames, Iowa: Iowa State University Press: 452–470.
- BELL, J.G. & MOULODI, S. 1988. A reservoir of virulent Newcastle disease virus in village chicken flock. *Preventative Veterinary Medicine*, 6:37–42.
- CAPUA, I., SACCHIA, M., TOSCANI, M. & CAPORALE, V. 1933. Unexpected isolation of virulent Newcastle disease virus from commercial embryonated fowls eggs. *Journal of Veterinary Medicine*, B40: 609–612.
- HUCHZERMAYER, F.W. & GERDES, G.H. 1993. Newcastle disease virus isolated from ostriches in South Africa. *Journal of South African Veterinarian Association*, 64:14.
- HUCHZERMAYER, F.W. 1994. *Ostrich diseases*. Pretoria: Agricultural Research Council, Onderstepoort Veterinary Institute.
- JORDAN, F.T.W. 1990. Paramyxoviridae (Newcastle disease and others), in *Poultry diseases*, 3rd ed., edited by P. Bailliere. Tindall: 121–136.
- LANCASTER, J.E. 1966. Newcastle disease—modes of spread. *Veterinary Bulletin*, 33:221–226.
- McFERRAN, J.B. & McNULTY, M.S. 1933. *Virus infections of birds*. Amsterdam: Elsevier.
- SAMBERG, Y., HADASH, D.U., PERELMAN, B. & MEROZ, M. 1989. Newcastle disease in ostriches (*struthio camelus*) field case and experiment infection. *Avian Pathology*, 18: 221–226
- SPRADBROW, P.B. 1933/1994. Newcastle disease in village chickens. *Poultry Reviews*, 5:57–96.
- VERWOERD, D.J. 1995a. Newcastle disease and poultry production in Africa. *Proceedings of the Second All Africa Conference on Animal Agriculture: Food Security in Africa*, 1–4 April 1996, South Africa, edited by H.H. Meissner.
- VERWOERD, D.J. 1995b. Velogenic Newcastle disease epidemic in South Africa II. Ostriches, waterfowl, exotic bird collections and wild birds. *South African Vetmed*, 8:44–9.
- WILLIAMS, R., VERWOERD, D., SCHOEMAN, M., VAN WYK, A., GERDES, G.H., ROOS, K. & BOSHOFF, C.H. 1996. Detection of antibodies to Newcastle disease virus in ostriches (*Struthio camelus*) by an indirect ELISA. *Avian Diseases* (in press).