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ORIGINAL ARTICLE

A preliminary study of the role of ducks in the transmission of Newcastle disease virus to in-contact rural free-range chickens

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Abstract The role of ducks in the transmission of Newcastle disease virus (NDV) to free-range village chicken was investigated experimentally. Newcastle disease (ND) seronegative ducklings reared in a pen were infected oronasally with velogenic NDV of intracerebral pathogenicity index (ICPI) 1.8 isolated from outbreaks in village chickens in Uganda. A first group of 3-week-old ND seronegative chicks was mixed with the ducks and they were kept together for 7 days. Both ducks and chicks were observed for ND clinical signs and any mortality, and they were bled and their sera were tested for ND antibodies by haemagglutination inhibition (HI) test. The chicks were removed, euthanized and examined for any ND lesions, while the ducks were transferred to a fresh pen and a second group of chicks was introduced and observed and treated as above. The ducks and the chicks tested positive for ND antibodies 7 days post infection and contact, respectively, but showed no clinical signs, post-mortem lesions or mortality. The mean ND antibody titre of the second group of chicks was lower than for the first group. This study has shown that although ducks can be infected with velogenic NDV, they do not show clinical signs but are able to transmit NDV to in-contact chicks. Further investigations are needed of the lack of clinical signs in the in-contact chicks and how long the ducks remain infective.

Keywords Chickens · Ducks · Excretion · Newcastle disease virus · Transmission · Velogenic

Abbreviations

GM	geometric mean
HI	haemagglutination inhibition
ICPI	intracerebral pathogenicity index
ND	Newcastle disease
NDV	Newcastle disease virus
p.i.	post infection

Introduction

Newcastle disease, a worldwide highly contagious avian viral disease caused by Newcastle disease virus (NDV) of the family *Paramyxoviridae* in the order *Mononegavirales* is the most devastating disease of rural chickens (Murphy *et al*., 1995). The infection was first reported in Newcastle-upon-Tyne in 1926, and the disease is now recognized worldwide (Alexander, 1997). Although its epidemiology is well understood in industrial poultry production systems, little has been published on its behaviour in village poultry populations (Awan *et al*., 1994). Seroepidemiological

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and isolation studies have shown that velogenic NDV is endemic in rural poultry populations (Spradbrow, 1993/4; Otim *et al*., 2004, 2006). The major mode of transmission appears to be by the faecal–oral route, with the respiratory route playing a role where there are close bird-to-bird associations (Alexander, 1988). Besides domestic avian species, natural or experimental infection with NDV has been demonstrated in numerous species; the most disease-resistant species appear to be aquatic birds, while gregarious birds forming temporary or permanent flocks are the most susceptible (Kaleta and Baldauf, 1988). Psittacines are known to be capable of habouring and spreading velogenic NDV (Spalatin and Hanson, 1975; Olabode *et al*., 1992). A study of risk factors of ND found salvage sales of chickens during suspected ND outbreaks, purchase of restocking chickens from the markets and the presence of wild birds to be important determinants of ND outbreaks in rural chickens in Uganda (Otim *et al*., 2006). Other factors that have been reported to be important in the maintenance of NDV infection of NDV in village environment include the presence of carrier chickens, frequent introduction of susceptible birds, other poultry species, and heterogeneity of NDV (Martin, 1992). However, the role of other birds as carriers in initiating outbreaks in villages is not well documented (Spradbrow, 2000). In Uganda, ducks are one of the poultry species often reared together with chickens under village conditions (Otim *et al*., 2006). They may be infected by NDV and show few or no clinical signs even with strains lethal to chickens but are capable of spreading the virus (Higgins, 1971; Spradbrow, 2000; Alexander, 2001). Other species of poultry are also affected by ND but, owing to their small numbers relative to chickens, they have received little attention (Awan *et al*., 1994).

A previous study did not provide any direct evidence that the presence of ducks, pigeons and turkeys increases the risk of ND outbreaks in indigenous free-range chickens (Otim *et al*., 2006). However, this lack of association was attributed to the small sample size of the study population with these poultry species. In Vietnam, strains of NDV virulent for chickens were also isolated from ducks (Spradbrow, 2000). In Tanzania, it was observed that ND was a greater problem in villages where ducks are also kept (IAEA/FAO, 1999). Certain strains of NDV have become adapted to pigeons and cause disease in both pigeons and chickens (Spradbrow, 2000). Earlier reports indicated that NDV persisted a long time in a flock of ducks in a village situation in Indonesia (Kingston and Dharsana, 1979).

Despite several indications of the role of ducks in the transmission and maintenance of NDV in the rural poultry production system, no proper investigations into the dynamics of transmission of NDV from ducks to chickens have been conducted. This study was intended to to investigate whether ducks transmit velogenic NDV strains to in-contact chickens and to investigate how long they remain infective for chickens.

Materials and methods

Ducklings

Day-old ducklings that were seronegative for ND antibodies were raised in isolation until the age of 8 weeks. Water and food were provided *ad libitum*.

Infection material

A virulent NDV strain with intracerebral pathogenicity index (ICPI) 1.8, F gene cleavage site motif ^{112}RRQKRF^{117} and GenBank accession number AY367559 isolated from Uganda in an earlier study (Otim *et al*., 2004a) was used to inoculate ducklings.

Chicks

Chicks hatched from local free-range hens that had been determined to be negative for ND by haemagglutination inhibition test were reared on-station in isolation until the age of 3 weeks.

Challenge

At 8 weeks of age, 10 ducks were bled and sera from them were tested by haemagglutination inhibition (HI) to reconfirm freedom from ND antibodies (Meulemans *et al*., 1987; Alexander *et al*., 1997), performed according to European Community directive 92/66/EC (CEC, 1992). A modified protocol for the determination of ICPI test was adopted for the inoculation of the birds (Alexander, 1989). The birds were inoculated oronasally with tenfold dilutions of 0.2 ml of infective allantoic fluid. Three days post infection (p.i.), six 3-week-old chicks were also bled and confirmed by HI to be ND antibody negative, and subsequently

introduced into the duck pen on the same day and kept together with the ducks for 7 days.

To ensure the infectivity of the inoculum, one chick was separately and directly infected oronasally with the same dose of the virus and kept in isolation.

Clinical observations

The directly infected chick was observed for any clinical signs. In addition, the duck and the in-contact chicks were observed twice daily for any clinical signs of ND. On the 7th day, both the ducks and chicks were bled and sera from them were tested by HI to detect any seroconversion due to NDV inoculation and infection, respectively. Ducks were transferred to a fresh pen and a second group of five chicks confirmed by HI to be ND seronegative were introduced into the same pen with the ducks and observed as previously. The first group of in-contact chickens was killed and subjected *lege artis* to postmortem examinations. The second group of in-contact chickens were also bled on the 7th day of contact and tested for ND antibodies by HI test and subsequently treated as the first group.

Results

The chicks that were in contact with the ND-inoculated ducks did not show any clinical signs or postmortem lesions and no mortality was observed. The directly inoculated chicken, however, became anorexic, stopped drinking water 48 h p.i., and finally developed a whitish diarrhoea. Laboured respiration was noticed after 72 h and terminal prostration and death were observed 96 h p.i., before a second serum sample could be obtained for HI analysis. Postmortem findings included haemorrhagic lesions in the small intestine and trachea and congestion.

All the ducks and chicks tested HI negative for ND antibody on the day of inoculation and first contact, respectively. However, both the ducks and chicks seroconverted after 7 days (Table 1). There was a relative increase in the geometric mean (GM) HI antibody of ducks from week 1 (10 ± 9.3) to week 3 (11). The first group of chicks produced more antibodies (GM HI titre 10.85 ± 8.8) than the second group whose GM HI titre was 9.09 ± 9.8.

Table 1 Geometric mean (GM) HI antibody titres (±SD) for the inoculated ducks and chicks kept in contact with the ducks for 1 and 2 weeks post infection ($n = 6$)

	GM HI titre			
	Week 0	Week 1	Week 2	Week 3
Ducks	0	10 ± 9.3	10.51 ± 9.8	11
Group 1 chicks	0	10.85 ± 8.8	–	
Group 2 chicks*	0	–	9.09 ± 9.8	

[a]Group 2 chicks were introduced after the ducks had been transferred into a new pen two weeks post infection

Discussion

The clinical signs and the postmortem lesions observed in the directly inoculated chick were consistent with those previously observed in field outbreaks due to velogenic NDV among free-range village chickens from which the NDV strain used in this study was isolated (Otim *et al*., 2004). These observations and the subsequent death of the inoculated chick showed that the virus strain used in this study was indeed infective, and might have been velogenic as earlier reported (Otim *et al*., 2004).

The positive HI test results obtained from the duck 7 days p.i. documented seroconversion and proved that the ducks were infected with ND.

The lack of clinical signs in the ducks after NDV infection agrees with earlier reports that NDV strains infect ducks but that these tend to show no or few clinical signs even when infected with the most virulent strains of NDV for chickens (Alexander, 2001).

A study on the risk factors of ND in rural free-range chickens in Uganda did not show the presence of ducks as a risk to ND outbreak in poultry. This lack of association between the presence of ducks in a poultry flock and ND outbreak was, however, attributed to the relatively small sample size of the study population that had ducks (Otim *et al*., 2006).

The first group of in-contact tracer chick tested positive for ND antibodies 7 days p.i., indicating that the ducks transmitted NDV to them.

The second group of in-contact chicks also tested positive for ND antibodies, indicating that the ducks still excreted NDV and were still able to transmit NDV 2 weeks p.i. This supports earlier observations by Kingston and Dharsana (1979), who reported that

the NDV persisted for a long time in a flock of ducks in a village situation in Indonesia and that they were capable of spreading the virus (Spradbrow, 2000). However, it could not be excluded that transmission was a result of persistence of the virus in the environment. In our study, this factor was eliminated since the ducks were transferred to a new, uncontaminated pen. Comparison of the relative HI titres of the two groups of chicks showed that the first group of chicks had higher antibody HI titres. This could be due to the fact that, in the first week, the ducks may have been shedding a heavy virus load in their faeces. Ingestion of the virus by the chicks led to strong antibody production as seen from the GM HI titres and a low standard deviation in the first group of chicks that was in contact with the ducks. The lower antibody titre and a higher standard deviation in the second group chicks may be attributed to the fact the ducks were excreting less virus in their faeces during the second week. The rising GM HI antibody titre in the ducks observed during this period may have neutralized the virus, thereby decreasing the virus load in the duck faeces ingested by the in-contact chicks in group 2.

However, based on our previous knowledge of the virulence of the virus strain used in this study (Otim *et al*., 2004) clinical signs of ND and mortality were expected in chickens infected with this NDV strain. Surprisingly, neither clinical signs nor mortality were observed. It could be speculated that the passage of the NDV in the ducks may have led to attenuation of the virus.

In conclusion, our preliminary investigations confirmed that ducks can transmit NDV to chickens. Owing to lack of access to a confined and uncontaminated environment, we were unable to continue our contact exposure experiment. Further studies including detection of the presence of the infective NDV material in the inoculated duck faeces and how long the ducks remain infective to the chickens are recommended. In addition, investigations on the lack of clinical signs and mortality among contact-exposed chicks that are infected with the NDV from the ducks are suggested.

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References

Alexander, D.J., 1988. Newcastle disease diagnosis: methods of spread. In: D.J. Alexander (ed.), *Newcastle Disease*, (Kluwer Academic, Boston), 256–272

Alexander, D.J., 1989. Newcastle disease. In: S.B. Hitchner, C.H. Domermuth, H.G. Purchase and J.E. Williams (eds), *Isolation and Identification of Avian Pathogens*, (American Association of Avian Pathologists, Kennet Square, PA), 114–120

Alexander, D.J., 1997. Newcastle disease and other paramyxovirus infections. In: B.W. Calnek (ed), *Diseases of Poultry* 10th edn, (Wolfe Publishing, London), 541–569

Alexander, D.J., 2001. Newcastle disease (APMV-1). In: F. Jordan, M. Pattison, D.J. Alexander and T. Faragher (eds), *Poultry Diseases* 5th edn, (Harcourt Publishers, London), 259–268

Alexander, D.J., Manvell, R.J., Lowings, J.P., Frost, K.M., Collins, M.S., Russell, P.H. and Smith, J.E., 1997. Antigenic diversity and similarities detected in avian paramyxovirus type 1 (Newcastle disease virus) isolates using monoclonal antibodies. *Avian Pathology*, **26**, 399–418

Awan, M.A., Otte, M.J. and James, A.D., 1994. The epidemiology of Newcastle disease in rural poultry: a review. *Avian Pathology*, **23**, 405–425

CEC, 1992. Council Directive 92/66/EC of 14 July 1992 introducing community measures for the control of Newcastle disease. *Official Journal of the European Communities*, L260, 1–20

Higgins, D.A., 1971. Nine disease outbreaks associated with myxoviruses among ducks in Hong Kong. *Tropical Animal Health and Production*, **3**, 232–240

IAEA/FAO, 1999. Improvement of health and management of family poultry production in Africa. In: R.H. Dwinger and M.J. Martin (eds), *Proceeding of the IAEA/FAO Research Coordination Meeting no. 1*, February 1999, Rabat, Morocco

Kaleta, E.F. and Baldauf, C., 1988. Newcastle disease in free living and pet birds. In: D.J. Alexander (ed.), *Newcastle Disease*, (Kluwer Academic, Boston), 197–246

Kingston, D.J. and Dharsana, R., 1979. Newcastle disease virus infection in Indonesian ducks. *Philippines Journal of Veterinary Medicine*, **18**, 125–130

Martin, P.A.J., 1992. The epidemiology of Newcastle disease in village chickens. In: P.B. Spadbrow (ed.), *Newcastle Disease in Village Chickens, Control with Oral Thermostable Vaccines*, Proceedings No. 39, international workshop held in Kuala Lumpur, Malaysia, 6–10 October 1991, (Australian Centre for International Agricultural research, ACIAR, Canberra), 40–45

Meulemans, G., Gonze, M., Carlier, M. C., Petit, P., Burny, A. and Le Long., 1987. Evaluation of the use of monoclonal antibodies to haemagglutination and fusion glycoproteins of Newcastle disease virus for virus identification and strain differentiation purposes. *Archives of Virology*, **92**, 55–62

Murphy, F.A., Fauquet, C.M., Bishop, D.H.L., Ghabrial, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A. and Summers, M.D. (eds), 1995. Virus Taxonomy, Classification and Nomenclature of Viruses, Sixth report of the International Committee on Taxonomy of Viruses. *Archives of Virology* (Supplement), **10**, 268–274

Olabode, A.O., Shidali, N.N. and Chukuwuendo, A.A., 1992. Village chickens and Newcastle disease in Nigeria. In: P.B. Spradbrow (ed), *Newcastle Disease in Village Chickens Control with Thermostable Oral Vaccines*, Proceeding No. 39, international workshop held in Kuala Lumpur, Malaysia, 6–10 October 1991, (Australian Centre for International Agricultural research, ACIAR, Canberra), 159–160

Otim, M.O., Christensen, H., Jørgensen, P.H., Kurt, J.H. and Bisgaard, M., 2004. Molecular characterization and phylogenetic study of Newcastle disease virus isolates from recent outbreaks in Eastern Uganda. *Journal of Clinical Microbiology*, **42**, 2802–2805

Otim, M.O., Kabagambe, E.K., Mukiibi-Muka G., Christensen, H. and Bisgaard, M., 2006. A prospective study of risk factors for Newcastle disease in rural free-range chickens. *Preventive Veterinary Medicine* (in press)

Spalatin, J. and Hanson, R.P., 1975. Epizootiology of Newcastle disease in waterfowl. *Avian Diseases*, **19**, 573–582

Spradbrow, P.B., 1993/94. Newcastle disease in village chickens. *Poultry Science Reviews*, **5**, 57–96

Spadbarrow, P.B., 1999. Epidemiology of Newcastle disease and economics of its control. In: Dolberg, F., Peterson, P.H. (eds.), *Poultry as a Tool in Poverty Eradication and Promotion of Gender Equality*. Proceedings Workshop, March 22–26, 1999, Tune, Denmark, pp. 165–173

Étude préliminaire sur le rôle des canards dans la transmission du virus de la maladie de Newcastle à des poulets fermiers ruraux en contact avec celle-ci

Résumé – Une étude expérimentale a été entreprise sur le rôle des canards dans la transmission du virus de la maladie de Newcastle (NDV) à des poulets fermiers de villages. Des canetons séronégatifs à la maladie de Newcastle (ND) élevés dans des enclos ont été infectés oro-nasalement avec du NDV vélogénique d'indice de pathogénicité intracérébrale (ICPI) 1.8 isolé d'épidémies affectant des poulets de villages en Ouganda. Un premier groupe de poussins âgés de trois semaines séronégatifs à la ND a été mêlé à des canards et ils ont été gardés ensemble pendant sept jours. À la fois les canards et les poussins ont été observés pour détecter les signes cliniques de ND et toute mortalité éventuelle et ils ont été saignés et leurs séra testés pour dépister les anticorps à la ND par un test d'inhibition de l'hémagglutination. Les poussins ont été séparés du groupe, sacrifiés et examinés pour le dépistage de lésions éventuelles induites par la ND tandis que les canards ont été transférés à un nouvel enclos et un deuxième groupe de poussins a été introduit et observé et traité de la même façon que ci-dessus. Les canards et les poussins ont été testés positifs pour les anticorps à la ND sept jours après l'infection et un contact respectivement mais ne présentaient aucun signe clinique, lésions post-mortem ou mortalité. Le titre moyen des anticorps à la ND du deuxième groupe de poussins a été plus bas que celui du premier groupe. Cette étude a mis en évidence que bien que les canards puissent être infectés par un NDV vélogénique, ils ne présentent pas de signes cliniques mais peuvent transmettre le NDV aux poussins se trouvant en contact avec eux. Il est nécessaire d'entreprendre des études plus poussées sur l'absence de signes cliniques chez les poussins en contact et sur la durée de l'infectiosité des canards.

Estudio preliminar sobre el papel de los patos en la transmisión del virus de la enfermedad de Newcastle a pollos rurales sin restricción de movimiento con los que están en contacto

Resumen – Se investigó experimentalmente el papel de los patos en la transmisión del Virus de la Enfermedad de Newcastle (NDV) a pollos rurales sin enjaular. Se infectaron oronasalmente a patos pequeños seronegativos para la enfermedad de Newcastle (EN) criados en un corral con virus velogénico de la enfermedad de Newcastle de índice de patogenicidad intracerebral (IPIC) 1.8 aislado de brotes ocurridos en pollos rurales de Uganda. Un primer grupo de pollos seronegativos a la EN de tres semanas de edad se mezclaron con los patos y se mantuvieron juntos durante siete días. Se observaron ambos, patos y pollos, buscando síntomas clínicos de la EN y posibles casos de mortalidad, y se les extrajo sangre y analizó su suero para ver posibles anticuerpos de la EN mediante la prueba de inhibición de la hemaglutinación (IH). Se quitaron los pollos, se sacrificaron y se examinaron por posibles lesiones de EN, mientras que los patos fueron transferidos a un corral nuevo, en el que se introdujo un segundo grupo de pollitos, y se observaron y trataron como se hizo anteriormente. Los patos y los pollitos dieron positivo para anticuerpos de EN a los siete días después de la infección y el contacto, pero no mostraron síntomas clínicos, ni lesiones postmortem, ni mortalidad. El título medio de anticuerpos de EN del segundo grupo de pollitos fue más bajo que en el primer grupo. Este estudio ha mostrado que aunque los patos pueden ser infectados con virus velogénico de la EN, no muestran síntomas clínicos, aunque son capaces de transmitir el virus de la EN a polluelos con los que están en contacto. Se necesitan investigaciones posteriores en cuanto a la carencia de síntomas clínicos en los pollos en contacto, y también para ver cuánto tiempo permanecen los patos infecciosos.