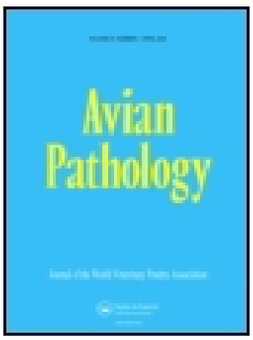

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ABSENCE OF NEUTRALISING ANTIBODIES TO DUCK PLAGUE VIRUS IN THE COMMERCIAL DUCK AND GOOSE POPULATIONS IN WEST GERMANY (1980 – 1985)

H. TORO[1] and E.F. KALETA

Institut für Geflügelkrankheiten, Justus Liebig Universität, Frankfurter Strasse 87, D-6300 Giessen, West Germany

SUMMARY

Commercial duck and goose serum samples were examined in a viral neutralisation test in chicken embryo fibroblast cultures for the presence of specific antibodies against duck plague virus. All 2256 serum samples tested, which originated from different locations in West Germany from 1980 to Spring 1985, showed neutralising titres of less than 1.0 ($\log_2$) and were considered to be negative.

INTRODUCTION

Duck plague (DP), also known as eendenpest (Dutch), peste du canard (French), Entenpest (German), or duck virus enteritis, is an acute contagious herpesvirus infection of *Anatidae* (ducks, geese and swans). The disease is characterised by vascular damage with tissue haemorrhages, enanthematous digestive mucosal lesions, lesions of lymphoid organs, and retrograde changes of the parenchymatous tissues (Jansen and Wemmenhove, 1960; Leibovitz and Hwang, 1968). Cross-neutralisation tests have demonstrated that the aetiologic agent is not antigenically related to other avian herpesviruses (Kaleta *et al.*, 1980).

Since the first reports of DP in the Netherlands (Baudet, 1923; De Zeeuw, 1930; Bos, 1942), the disease has also been diagnosed in many other countries in Europe, Asia, and North America (Gough, 1984). Duck plague virus (DPV) has been isolated from extensively maintained waterfowl showing high mortality in West Germany (Kaleta *et al.*, 1983). So far, there have been no reported cases in commercial duck or goose farms in this country.

This study was initiated to gather information about the incidence of neutralising antibodies against DPV in some duck and goose farms in West Germany. Some serum samples were taken in 1984 from the Schleswig-Holstein locality, in which a

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[1] Visiting Scientist from the Facultad de Ciencias Veterinarias, Universidad de Chile, Casilla 49, Correo 15, Santiago, Chile.

previous outbreak of DP in extensively maintained waterfowl was diagnosed (Kaleta *et al.*, 1983).

MATERIALS AND METHODS

Serum samples

Blood samples were collected from different commercial duck and goose farms located in Schleswig-Holstein, Hamburg, Niedersachsen, Nordrhein-Westfalen, and Baden-Württemberg from Spring 1980 to Spring 1985 (Table 1). All sera (938 geese and 1318 duck samples) were inactivated by heating at 56°C for 30 min and then tested individually in a viral neutralisation test (VNT) against DPV in chicken embryo fibroblast (CEF) cultures.

Table 1. Neutralisation titres of duck and goose serum samples against duck plague herpesvirus in chicken embryo fibroblast microplate cultures.

Source of sample	No. of serum samples tested	Neutralising titre
Schleswig Holstein		
Domestic geese (*Anser domesticus*)	180	<1.0[a]
Hamburg		
Muscovy ducks (*Cairina moschata*)	60	<1.0
Domestic geese	40	<1.0
Niedersachsen		
Muscovy ducks	106	<1.0
Pekin ducks (*Anas platyrhynchos*)	379	<1.0
Domestic geese	311	<1.0
Nordrhein-Westfalen		
Muscovy ducks	641	<1.0
Domestic geese	217	<1.0
Baden Württemberg		
Domestic geese	68	<1.0
Uncertain origin		
Muscovy ducks	132	<1.0
Domestic geese	122	<1.0
Control ducks (Pekin ducks) positive		5.7
negative		<1.0

[a] Serum neutralising titres expressed as a $\log_2$ of the reciprocal of the highest dilution of serum causing neutralisation of infectivity

Duck plague immune serum

Positive control serum was employed for confirmation of each test. Immune serum was prepared by inoculation of an avirulent virus (VR 684, ATCC) in susceptible mature Pekin ducks. Following a booster inoculation, serum samples were obtained and tested for neutralising antibodies against DPV in CEF and in Pekin duck embryo fibroblast (DEF) cultures.

Virus strain

A prototype of DPV of the Holland strain (American Type Culture Collection, VR 684, Rockville, Maryland, USA) was used in all assays. Two passages were made in CEF before using this strain.

Cell culture

The VNTs were performed in CEF cultures. DEF cultures were also used, as control systems of the virus infectivity. Ten-day-old chicken eggs from SPF hens and Pekin duck eggs were incubated and used for preparing CEF and DEF cultures according to standard procedures (Purchase, 1980). Cells were grown in MEM Earle's BSS (Serva, Heidelberg, BRD) containing 5% foetal bovine serum, either in 96-well flat bottomed tissue culture plates (Falcon 3072, Becton Dickinson Labware, Oxnard, USA) for the VNTs, or in tissue culture flasks for viral multiplication. Cultures were incubated at 37°C.

Virus neutralisation test (VNT)

The constant virus-diluted serum VNT was carried out in 96-well flat bottomed plates (Wolf *et al.*, 1974). Twofold dilutions of serum were prepared in MEM. An equal volume of medium containing 100 $TCID_{50}$ of the Holland virus was then added to the serum. The cultures were infected with the serum-virus mixture and then incubated at 37°C for 6 days. Cells were observed daily for the appearance of cytopathic effect (CPE). On the 6th day of incubation, cells were fixed and stained by dipping the plates in a formaldehyde-crystalviolet solution for subsequent macroscopic evaluation. Titres were calculated as described by Reed and Muench (1938), and the neutralisation titres were determined from the serum dilution (log_2) capable of inhibiting the appearance of CPE.

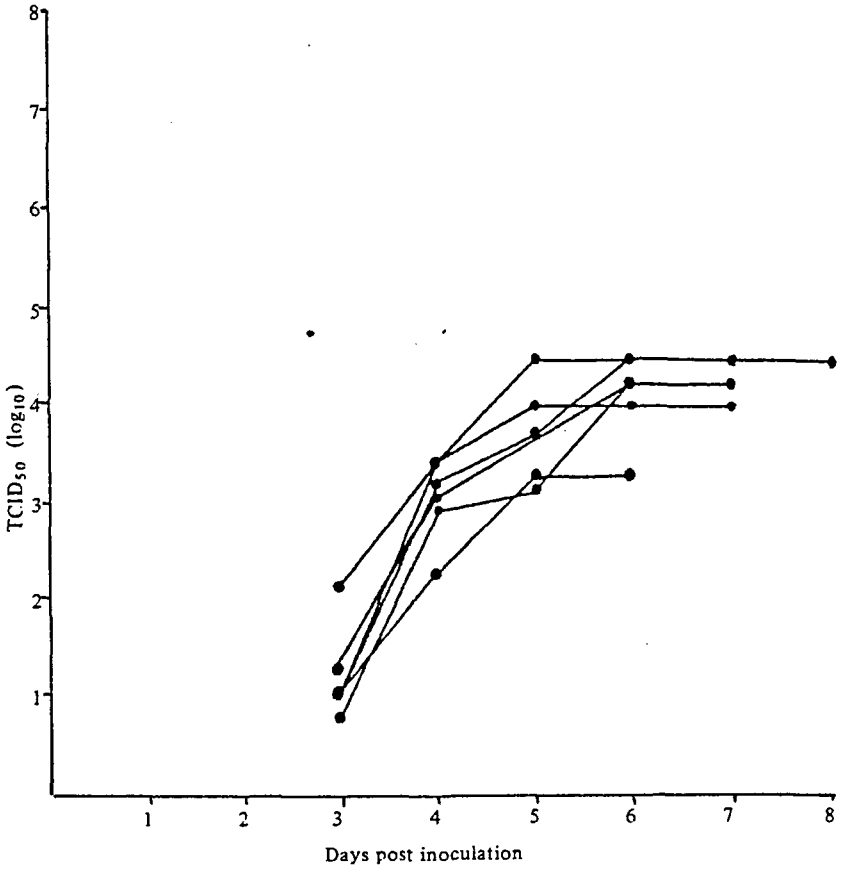
RESULTS

All serum samples submitted to this laboratory for DPV neutralisation tests, showed neutralising titres of less than 1.0 (log_2) (Table 1). Positive control serum confirmed the results obtained in each test with a neutralising titre of 5.7 (log_2) in CEF cultures. The neutralising titre obtained with the same control serum in DEF, was 3.2 (log_2). The infected cell cultures (CEF and DEF) showed a CPE in the form of syncytial formations, beginning with low titres on the 3rd day post-inoculation. The maximal expression of the virus titre in CEF was observed 5 to 6 days post-inoculation (Text-fig. 1). The maximal expression of the virus titre in DEF cultures was observed already on the 4th day post-inoculation.

DISCUSSION

Duck plague has been diagnosed in many European countries (Bos, 1942; Devos *et al.*, 1964; Gaudry *et al.*, 1970; Hall and Simmons, 1972; Bergmann *et al.*, 1979; Prip *et al.*, 1983), including West Germany (Kaleta *et al.*, 1983). In addition, it has been suggested that the migration of wild waterfowl during winter and early spring, is linked with DP outbreaks (Gough, 1984). The neutralisation titres obtained (of less than 1.0 log_2) with all serum samples tested, were considered to be negative. The differences observed between CEF and DEF cultures in relation to the neutralising titres obtained with the control serum and the differences in time to achieve the maximal titre expression, are probably due to differences in the susceptibility between the cell systems to this virus infection. The absence of

Text-fig. 1. Measurement of the duck plague virus titre in CEF cultures on different times post-inoculation



neutralising antibodies in the commercial duck and goose population from 1980 to 1985 suggests that DP does not play an important role in duck pathology in this country. Nevertheless, the susceptibility of the duck and goose populations must be emphasised. Preventive and control measures must be considered in order to prevent outbreaks of the disease in commercial farms and free-living native waterfowl.

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RESUME

L'absence d'anticorps neutralisants contre le virus de la peste du canard dans la population des canards et des oies commerciales en Allemagne Fédéral (1980-1985)

Des échantillons de canards et d'oies commerciales ont été examinés sérologiquement pour prouver la présence d'anticorps spécifiques contre la peste du canard. Ceci a été effectué par le test de neutralisation viral avec des fibroblastes d'embryons de poule. Les titres de tous les 2256 échantillons examinés provenant d'exploitations dans différentes régions de l'Allemagne Fédérale, de 1980 jusqu' au printemps de 1985, étaient en dessous de 1.0 ($\log_2$) et ont été considérés négatifs.

ZUSAMMENFASSUNG

Abwesenheit von Neutralisierenden Antikörpern gegen das Entenpestvirus in kommerziell gehaltenen Enten-und Gänse populationen in der Bundesrepublick Deutschland (1980-1985)

Serumproben kommerziell gehaltener Enten und Gänse wurden im Virusneutralisationstest in Hühnerembryofibroblastenkulturen auf spezifische Antikörper gegen Entenpestvirus untersucht. Die Neutralisationstiter aller 2256 untersuchten Proben, die von 1980 bis Frühling 1985 aus verschiedenen Betrieben der Bundesrepublick Deutschland stammten, lagen unter 1.0 ($\log_2$), und wurden als negativ beurteilt.

RESUMEN

Ausencia de anticuerpos neutralizantes contra el virus de la peste de los patos en la poblacion comercial de patos y gansos en Alemania Federal (1980-1985)

Sueros provenientes de patos y gansos comerciales fueron examinados en pruebas de neutralización viral en cultivos de fibroblastos de embrión de pollo con el objeto de constatar la presencia de anticuerpos contra el virus de la peste de los patos. Los títulos neutralizantes de las 2256 muestras serológicas procedentes de explotaciones de diversa ubicación en Alemania Federal, tomadas desde 1980 hasta la primavera 1985, demostraron valores inferiores a 1.0 ($\log_2$) y fueron consideradas negativas.