

CASE REPORT

Mortality in Muscovy ducks (*Cairina moschata*) and domestic geese (*Anser anser var. domestica*) associated with natural infection with a highly pathogenic avian influenza virus of H7N1 subtype

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Among the 413 outbreaks of highly pathogenic avian influenza (HPAI) caused by a virus of the H7N1 subtype, which occurred in Italy during 1999 and 2000, an outbreak diagnosed in a backyard flock was characterized by mortality and nervous signs in ducks and geese. Dead geese (*Anser anser var. domestica*) and Muscovy ducks (*Cairina moschata*) were submitted to the laboratory for bacteriological, virological, histological and immunohistochemical investigations. Routine bacteriological tests resulted negative, while a HPAI virus of the H7N1 subtype was isolated from the geese. Pancreatic damage was observed in both the geese and the ducks, and the pancreas was also positive by immunohistochemistry for avian influenza in the geese. Histopathological lesions were observed in the central nervous system of both species, and this result was supported by positive immunohistochemical findings for the presence of the virus.

Introduction

Highly pathogenic avian influenza (HPAI) is a devastating disease of poultry that is known to cause mortality rates approaching 100% in the majority of domestic birds. However, not all birds are fully susceptible to the clinical disease. Experimental and field evidence obtained from ostriches (*Struthio camelus*) indicates that these birds are resistant (Manvell *et al.*, 1998) or only partially susceptible (Capua *et al.*, 2000a) to HPAI. Waterfowl are known to be resistant to the disease and act as reservoirs for avian influenza viruses (Alexander, 1998). The reason waterfowl are refractory to the clinical disease induced by HPAI viruses remains unclear. Cleavage of the precursor haemagglutinin molecule is essential to confer infectivity on virus particles. A possible explanation that has been put forward is that waterfowl lack a ubiquitous protease(s) capable of bringing about the cleavage necessary for viral activation. It is well known that the clinical disease

HPAI is caused by some viruses of the H5 and H7 subtypes that contain multiple basic amino acids at the deduced sequence of the cleavage site of the precursor of the haemagglutinin molecule. In birds susceptible to HPAI, this feature allows the precursor haemagglutinin to be cleaved by ubiquitous proteases, and therefore viral replication is able to occur in vital organs, resulting in the death of the bird (Rott, 1992). The lack of a ubiquitous protease(s) able to bring about cleavage in the presence of multiple basic amino acids would therefore not permit systemic infection and disease.

It has been suggested that HPAI viruses infecting waterfowl behave like low pathogenicity avian influenza (LPAI) viruses in other species. LPAI viruses do not contain multiple basic amino acids in the cleavage site sequence and this limits the cleavage of the precursor haemagglutinin molecule to trypsin-like enzymes, which are present in the intestine and in the respiratory tract; therefore, viral replication is restricted to these areas.

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However, it should be stated that replication in vital organs of ducks infected experimentally with HPAI viruses has been reported by Wood *et al.* (1995), even though the birds remained clinically healthy. Therefore, the explanation of absence of clinical disease in ducks is probably more complex than the mere lack of a ubiquitous protease(s).

Among the total of 413 outbreaks of HPAI that occurred in Italy during 1999 and 2000 (Capua *et al.*, 2001), a total of 25 backyard flocks was affected. Generally, these flocks consisted of mixed avian species including chickens, turkeys, guinea fowl, peafowl, ducks and geese. During veterinary investigations of the outbreaks, it was noted, in the vast majority of cases, that high or total mortality occurred in all types of birds present, except waterfowl. However, in one outbreak, located in the Padova province, mortality was also observed in two domestic geese (*Anser anser var. domestica*) and two Muscovy ducks (*Cairina moschata*). In the present paper, we report the virological, bacteriological and histopathological investigations performed on the dead domestic waterfowl.

Materials and Methods

Dead birds were necropsied and selected organs were processed for bacteriology by routine methods and virological investigations. Virus isolation was performed as indicated by Council Directive 92/40/EEC (CEC, 1992). Haemagglutinating isolates were identified as described previously (Alexander & Spackman, 1981) and the virulence of the isolates was assessed by sequencing the region encoding for the precursor of the haemagglutinin molecule as described by Wood *et al.* (1994).

Brain, liver, spleen, kidney, adrenal glands, bursa of Fabricius, caecal tonsils, lung, trachea, bone marrow and pancreas were collected for histological and immunohistochemical investigations.

Briefly, selected organs were sampled and immediately fixed in 10% phosphate-buffered formalin. Tissues were embedded in paraffin, sectioned at 3 µm and stained with haematoxylin and eosin. Unstained paraffin-embedded sections were immunohistochemically processed to demonstrate influenza A nucleoprotein. The primary antibody was a monoclonal antibody against type A influenza virus nucleoprotein (kindly supplied by Dr D.E. Swayne, USDA, ARS, Athens, GA, USA). Briefly, an antigen retrieval step was performed by pressure-cooking for 25 min in citrate buffer (pH 6). The primary antibody was used at a dilution of 1:2000, using the En Vision AP (DAKO K1396, Carpinteria, CA) detection system and Nuclear Fast Red (DAKO K1396) as chromogen.

Results

Following veterinary investigation of the outbreak, it was reported that, of the 35 birds originally present on the farm, 15 (chickens, turkeys, peafowl and ducks) had died in the previous week. At the time of the investigation, two geese and one Muscovy duck were found dead. Another Muscovy duck that exhibited nervous signs such as incoordination and tremors was killed humanely and submitted for investigations.

On postmortem examination, both geese exhibited pancreatic lesions. In particular, in one of them,

the pancreas appeared enlarged, hardened and yellowish in colour. Its surface exhibited a foamy appearance with small rounded greyish vesicles (Figure 1). The duodenum appeared congested and, on opening, was found to contain haemorrhagic material. The spleen appeared reduced in size and an inflammation of the proventriculus was also present. The heart appeared congested and enlarged. No other lesions were detected in other organs. No gross lesions were detected in the two Muscovy ducks.

Bacteriological investigations from selected organs (liver and heart) gave negative results.

A haemagglutinating agent was isolated in embryonating specific pathogen free fowl eggs from pooled viscera of the two geese. The isolate was further characterized as an influenza A virus of the H7N1 subtype. Nucleotide sequencing indicated that the deduced sequence of the cleavage site of the precursor of the haemagglutinin molecule of the isolate contained multiple basic amino acids, with a deduced motif of . . . PEIPKGSRVRR*GLF . . ., i.e. identical to the cleavage site sequence of the HPAI virus that had been responsible for the HPAI epidemic in Italy during 1999 and 2000. No virus was isolated from the viscera of the two Muscovy ducks.

Histopathological examination revealed the presence of a limited number of necrotic foci of the acinar cells of pancreas in the geese and, to an even lesser extent, in the Muscovy ducks. Mild haemorrhagic duodenitis was observed in both geese and in the Muscovy ducks, while necrosis of the caecal tonsils was observed in the geese only. The latter also presented with congestion, mild hydropic degeneration and focal granuloma in the liver. Mild to moderate lymphocytic encephalitis with perivascular cuffing was observed in the brain of geese and Muscovy ducks (Figure 2).

Mild positive immunohistochemical reaction against the viral nucleoprotein antigen was detected in the acinar cells of pancreas of the geese (Figure 3). Similarly, nuclei and cytoplasm of the neurons and astrocytes in the grey matter of the central nervous system (CNS) of geese showed an intense, positive immunohistochemical reaction (Figure 4), while in the Muscovy ducks a positive reaction was restricted to a few individual neurons and glial cells. Lymphocytic perivascular cuffing never showed a positive reaction on immunohistochemistry. The remaining organs for both species were negative by immunohistochemistry.

Discussion

The data presented indicate that viral replication of HPAI virus has occurred in some vital organs of naturally infected domestic geese and Muscovy ducks. Although infectious virus was not recovered from the ducks, evidence of viral repli-

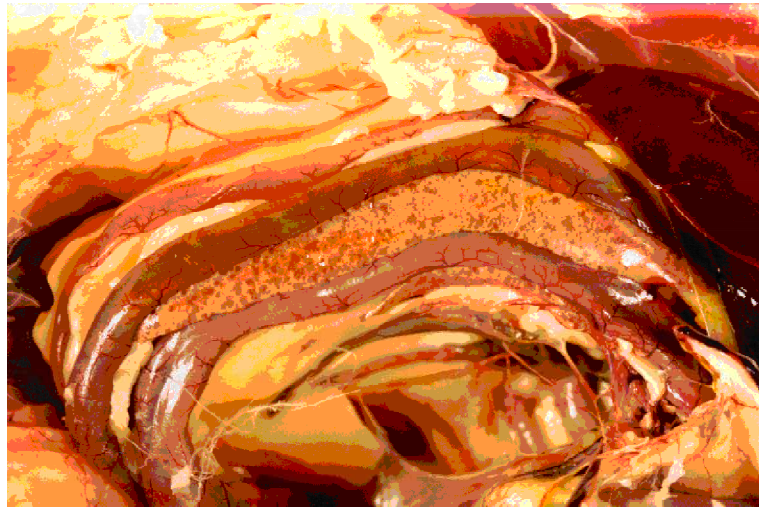


Figure 1. Enlarged, hardened and yellowish pancreas of one of the dead geese. The surface exhibits a foamy appearance with small, rounded greyish vesicles.

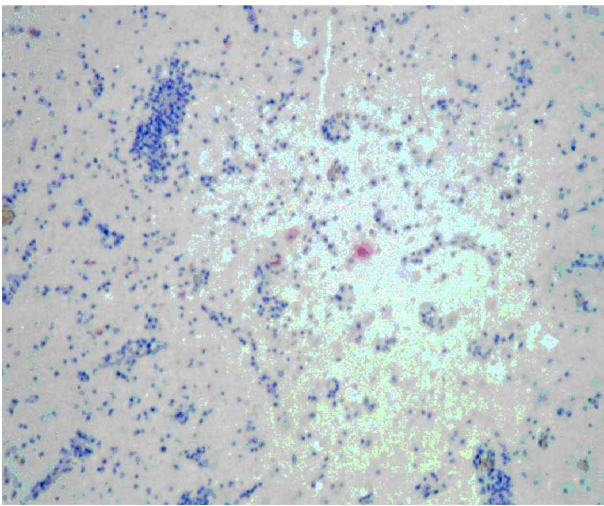


Figure 2. Lymphocytic perivascular cuffing in the brain of a Muscovy duck naturally infected with highly pathogenic avian influenza virus of H7N1 subtype. Individual positive neurons exhibit mild immunohistochemical staining. EnVision AP/NFR. Bar = 140 μ m.

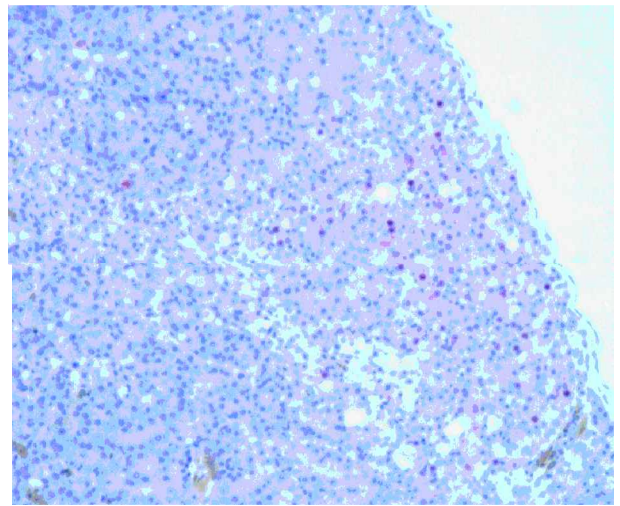


Figure 3. Acinar cells of the pancreas of a goose showing mild immunohistochemical positivity of the nucleus. EnVision AP/NFR. Bar = 70 μ m.

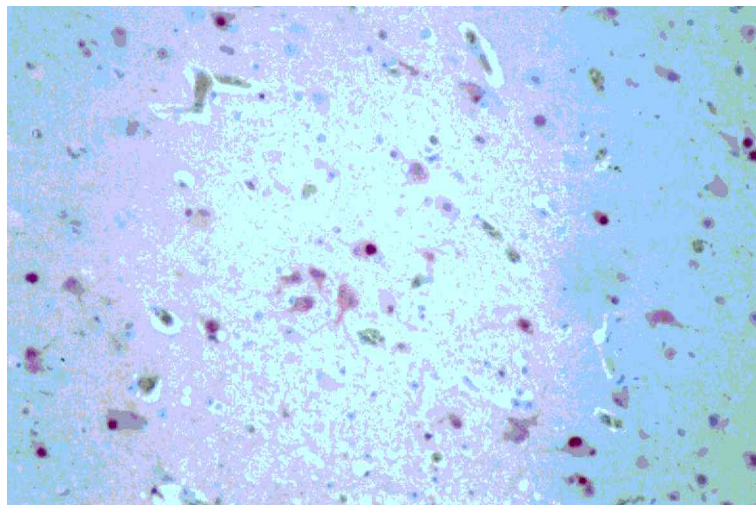


Figure 4. Intense immunohistochemical positive reactions in the neurons in the brain of a goose infected with highly pathogenic avian influenza virus of H7N1 subtype. EnVision AP/NFR. Bar = 70 μ m.

cation was obtained by immunohistochemistry. A similar experimental finding was previously recorded by Wood *et al.* (1995), who infected Pekin ducks with HPAI viruses and reported virus isolation from the brain, kidney, liver and spleen of birds infected with a virus of H5N2 subtype, and spleen, liver and kidney of ducks infected with an H7N7 virus. However, in these experiments, the infected Pekin ducks remained clinically healthy, despite the presence of the virus in vital organs.

The gross and histopathological findings recorded in the present investigation indicate that the pancreas is a target organ for viral replication in domestic waterfowl. Similar findings have been reported for other species in the Italian and other epidemics (Hooper & Selleck, 1987; Capua *et al.*, 2000b; Swayne & Suarez, 2000). However, in contrast to necrosis of the pancreas, spleen and brain of chickens and turkeys, and of the spleen, kidney, liver and brain of ostriches submitted during the epidemic (Capua *et al.*, 2000a,b), similar lesions were not detected in domestic waterfowl. The histopathological lesions detected in the brain of the Muscovy ducks suggest that the viral damage caused the clinical signs observed in the field. With reference to the mortality observed in the domestic waterfowl, viral replication in vital organs, as detected by immunohistochemistry in the pancreas and in the CNS, did not determine necrosis. Therefore, whether or not viral replication was the cause of the death of the birds remains unclear. In fact, the lack of pancreatic lesions and the presence of only mild perivascular inflammatory reaction in the CNS are in contrast to the severe histopathological findings observed in chickens, turkeys and ostriches. Moreover, similar findings, such as mild inflammatory lesions associated with immunohistochemically positive neurons, were the only finding in the CNS of convalescent broiler breeders affected by avian influenza (Mutinelli *et al.*, 2000), which had survived natural infection. The natural resistance of waterfowl to infection with HPAI could possibly be explained by the lack of severe, necrotic lesions in the pancreas and in other vital organs as opposed to the lesions present in other susceptible bird species.

In our opinion, further investigations are necessary to elucidate the pathogenesis of HPAI in waterfowl, with particular reference to the general absence of clinical signs and of mortality despite viral replication in target organs.

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RÉSUMÉ

Mortalité associée à une infection naturelle à virus influenza hautement pathogène de sous-type H7N1 chez le canard de Barbarie (*Cairina moschata*) et chez l'oie domestique (*Anser anser var. domestica*)

Parmi les 413 cas d'influenza aviare hautement pathogène (HPAI) dus à un virus de sous-type H7N1 qui ont été observés en Italie en 1999-2000, un cas diagnostiqué dans un élevage fermier a mis en évidence de la mortalité et des symptômes nerveux chez les canards et les oies. Les animaux morts : oies (*Anser anser var. domestica*) et les canards de Barbarie (*Cairina moschata*) ont été adressés au laboratoire pour réaliser des examens bactériologiques, virologiques, histologiques et immunohistochimiques. Les examens bactériologiques de routine n'ont rien révélé, tandis qu'un virus de l'HPAI de sous-type H7N1 a été isolé à partir des oies. Des lésions au niveau du pancréas ont été observées chez les oies et les canards, et l'examen immunohistochimique réalisé à partir du pancréas des oies s'est révélé positif. Les lésions histopathologiques ont été observées au niveau du système nerveux central chez les deux espèces ; ces résultats ont été confirmés par des réactions positives en immunohistochimie révélant la présence du virus.

ZUSAMMENFASSUNG

Mortalität bei Moschusenten (*Cairina moschata*) und Hausgänsen (*Anser anser var. domestica*) im Zusammenhang mit natürlicher Infektion mit einem hoch pathogenen aviären Influenzavirus vom Subtyp H7N1

Unter den 413 Ausbrüchen von hoch pathogener aviärer Influenza (HPAI), die 1999 bis 2000 in Italien auftraten und durch ein Virus des Subtyps H7N1 verursacht wurden, war ein in einem Kleinstbestand diagnostizierter Ausbruch durch Mortalität und nervöse Symptome bei Enten und Gänsen charakterisiert. Tote Gänse (*Anser anser var. domestica*) und Moschusenten (*Cairina moschata*) wurden dem Labor für bakteriologische, virologische, histologische und immunhistochemische Untersuchungen eingeschickt. Die routinemäßigen bakteriologischen Untersuchungen verliefen negativ, wohingegen HPAI-Virus vom Subtyp H7N1 aus den Gänsen isoliert wurde. Eine Pankreasschädigung wurde sowohl bei den Gänsen als auch den Enten festgestellt, und der Pankreas war bei den Gänsen auch immunhistochemisch AI-positiv. Histopathologische Veränderungen wurden im Zentralnervensystem beider Spezies festgestellt, und dieses Ergebnis wurde durch positive immunhistochemische Befunde hinsichtlich des Vorhandenseins des Virus untermauert.

RESUMEN

Mortalidad en patos de Muscovy (*Cairina moschata*) y en gansos domésticos (*Anser anser var. domestica*) asociada a una infección natural con virus de influenza aviar de alta patogenicidad del subtipo H7N1

De entre los 413 brotes de influenza aviar de alta patogenicidad (HPAI) causados por un virus del subtipo H7N1, que ocurrieron en Italia durante el periodo 1999–2000, un brote diagnosticado en un grupo de aves de corral se caracterizó por mortalidad y sintomatología nerviosa en patos y gansos. Se remitieron gansos (*Anser anser var. domestica*) y patos de Muscovy (*Cairina moschata*) ya muertos al laboratorio para realizar los estudios bacteriológicos, virológicos, histológicos e inmunocitoquímicos. Los estudios bacteriológicos rutinarios resultaron negativos, pero se aisló un virus HPAI subtipo H7N1 de los gansos. Tanto en gansos como en patos se observó daño pancreático, y en el páncreas de los gansos se detectó positividad a AI mediante inmunocitoquímica. Se observaron lesiones histopatológicas en el sistema nervioso central de ambas especies y este resultado fue corroborado por la detección, mediante técnicas inmunocitoquímicas, de la presencia del virus.

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