An adenovirus infection of poultry in Pakistan

A.H. CHEEMA, J. AHMAD and M. AFZAL *

Summary: A previously unknown disease which has spread among broiler poultry farms in Pakistan, attacks well-growing chicks at 3-5 weeks of age, with 30 to 60% mortality. At necropsy, the most prominent lesion is hydropericardium. Lungs are congested and oedematous, and liver and kidneys are pale and swollen. Histologically, there was necrosis and severe vascular changes in the heart as well as congestion, oedema and inflammatory cell infiltration in the lungs. Focal necrosis and mononuclear cell infiltration were present in the liver and many hepatocytes contained large, basophilic, intranuclear inclusions. The disease was reproduced in 15-day-old broiler chicks by subcutaneous inoculation of liver homogenate. The liver homogenate also caused stunted growth and haemorrhages when inoculated into embryonating eggs. Virus particles with icosahedral symmetry were seen by electron microscopy in purified liver extract. The disease is believed to be caused by a hitherto unreported adenovirus. However, the role of some other potentiating factor cannot be ruled out at this stage.

KEYWORDS: Aviadenovirus - Broilers - Hepatitis - Hydropericardium - Pakistan - Poultry diseases - Viral diseases.

INTRODUCTION

A previously unknown poultry disease has spread throughout Pakistan. Although sporadic cases of this disease were observed as early as 1985, the present outbreak started in August 1987 at Karachi and then gradually appeared all over Pakistan (M.S. Jaffery, 1988, personal communication). The disease has been seen almost exclusively in broiler chicks 3-6 weeks old, although rare cases have been reported in layer and breeder pullets (1, 2). The disease appears suddenly in well-growing broilers at around 20 days of age. The course of the disease is usually 10-14 days during which a mortality of 30-60% has been reported. At necropsy, the most prominent gross lesion is the accumulation of clear, watery or jelly-like fluid in the pericardial sac. The fluid is white, amber or occasionally green. This has given rise to the common name "hydropericardium syndrome" (1). This report describes the gross and histopathological lesions of the natural disease. Experiments on transmission, serology and electron microscopy to elucidate the aetiological agent are also reported.

PATHOLOGY OF THE DISEASE

Affected poultry farms in the Rawalpindi-Islamabad area were visited. Necropsy was performed on diseased birds soon after death. The most prominent gross lesion

* Animal Sciences Institute, National Agricultural Research Centre, Islamabad, Pakistan.
was the accumulation of clear, watery or jelly-like fluid in the pericardial sac. The heart was misshapen and flabby. Petechial and ecchymotic haemorrhages appeared in the heart, musculature and other organs. There was congestion and variable degree of oedema in the lungs. Liver was swollen, pale and friable. The kidneys were enlarged and pale and tubules prominent.

For histopathology, paraffin sections (5-7 µm thick) were cut and stained with haematoxylin and eosin (HE). Microscopic changes included multiple necrotic areas in the myocardium of ventricular walls, especially in the papillary muscles. The muscle fibres in the affected areas were shrunken, fragmented and more eosinophilic. Accumulation of mononuclear cells occurred in some areas. Calcification of affected myocardial fibres was observed. Deposition of proteinaceous material and extravasation of erythrocytes between the muscle bundles and fibres were consistently observed. In some birds there were large aggregates of mononuclear cells in the epicardium, its mesothelial cells being activated and enlarged. Prominent changes took place in the arteries. The intimal cells were swollen, rounded, vacuolated and projected in the lumen. Cellular vacuolation, mild proliferation, inflammatory cell infiltration and deposition of proteinaceous material were present in the media, but especially in the adventitia of these vessels (Fig. 1).

The most characteristic histological lesions occurred in the liver. There were small, multifocal areas of coagulative necrosis. Many hepatocytes had large, round, basophilic, intranuclear inclusion bodies (Fig. 2). The chromatin material was granular and margined. The nuclear membrane and the inclusions were surrounded by clear halos. Many other cells had large, dense, hyperchromatic nuclei (Fig. 2). The bile ducts were prominent and their lining epithelium showed hydropic degeneration and necrosis. Accumulation of histiocytic cells occurred in the portal areas. Fatty change and congestion were usually present.

In the lungs, there was congestion and oedema. Infiltration by mononuclear cells and heterophiles occurred in the alveolar walls. The vascular changes seen in the heart were also present in the lungs. The changes in the kidneys were variable. In some birds, there was massive necrosis of epithelium in all renal tubules. In other birds, the tubular epithelium was intact but lifted up from the basement membrane by oedema fluid. In some birds a bluish, granular material was deposited on the surface of renal epithelial cells.

**EXPERIMENTAL TRANSMISSION**

For experimental transmission, lungs, liver, bursa and a 3-7 cm long caudal portion of large intestine were collected from birds showing typical gross lesions. Each of these organs was homogenised separately in normal saline using glass tissue grinders. The suspensions were centrifuged at 5000 g for 30 min to remove the cell debris. Antibiotics (streptomycin 100 µg/ml and penicillin 1000 IU/ml) were added to the supernatant fluid. This fluid was used to inoculate embryonating eggs and broiler chicks.

Fifteen-day-old healthy broiler chicks were used in experimental transmission. These birds, maintained on a commercial broiler feed, were divided into five groups each of four birds. Extracts from lungs, liver, intestine and bursa were inoculated
Fig. 1

A large myocardial artery with pronounced vacuolation and activation of intimal cells and oedematous thickening of adventitia (HE × 250)

Fig. 2

Section of liver with necrosis of scattered hepatocytes, mild infiltration by mononuclear cells, intranuclear, basophilic, inclusion bodies (arrow a) and large, dense hyperchromatic nuclei (arrow b) (HE × 400)
in separate groups subcutaneously in the wings in a dose of 0.35, 0.7, 0.25 or 0.5 ml per bird. Chicks in the fifth (control) group were each inoculated with 0.5 ml normal saline.

Mortality was seen only in groups inoculated with liver and bursal extracts. In the liver extract group, two birds died four days after inoculation and one bird after nine days. In the bursa-inoculated group, one bird died on the fifth day and one on the ninth. Post-mortem and histopathological studies revealed typical lesions of the disease only in the birds inoculated with liver extract. Birds given the lung extract, intestinal extract and the control group remained healthy until ten days after inoculation when the experiment terminated.

The same tissue extracts were also inoculated into the chorioallantoic membrane (CAM) and yolk sac (YS) of eggs in the eighth day of incubation. Embryos inoculated with liver extract died after 4-7 days and the embryos were haemorrhagic and stunted. Some embryos inoculated with other extracts also died, but these seemed to have been contaminated with bacteria.

VIRUS CHARACTERISATION

Agar-gel precipitation was carried out in 1% Noble agar in petri plates with serum from naturally-infected birds. Liver and lung extracts gave strongly positive precipitation, while weak precipitation was seen with bursal and intestinal extracts.

Liver extract was used as a source of virus for electron microscopy. The homogenate was freeze-thawed twice to disrupt intact cells. It was centrifuged at 12,000 rpm for 30 min at 4°C in Beckman Centrifuge Model J2-21, using a J-20 rotor, to remove cell debris. The material was then centrifuged at 40,000 rpm for 1 hr at 4°C in Beckman Ultracentrifuge Model L8-80, using a 50 Ti rotor. The pellet was suspended in phosphate-buffered saline and washed once. This purified material was mounted on copper grids and stained with uranyl acetate and phosphotungstic acid for electron microscopy. Viral particles having the characteristic hexagonal morphology of adenovirus were seen (Fig. 3).

DISCUSSION

The present epidemic has caused considerable economic loss in the nascent but fast-developing poultry industry of Pakistan, which has thus suffered a great setback. As a result of this disease, about 25% of broiler farms have been closed. The cause of the disease is obscure, and many elements including the presence of mycotoxins, rancid fat, excess salt, organophosphorus compounds in feed and antagonistic drug action of coccidiostats, have been considered (1, 2). The causative agent was suspected to be a virus because intranuclear inclusion bodies were present in hepatocytes. Experimental transmission of the disease, haemagglutinating ability and precipitation reaction in gel diffusion further supported the idea. The virus resembled an adenovirus upon electron microscopy. This preliminary investigation indicates that the disease is inclusion-body hepatitis (IBH) caused by an adenovirus.

Many hepatocytes with large, dense hyperchromatic nuclei were consistently observed in natural cases as well as in experimentally-infected birds. This increase
The diagnosis of IBH-adenovirus infection raises a number of questions. The presence of disease almost exclusively in broilers 3-5 weeks old, high mortality and post-mortem lesions (particularly hydropericardium) have not been reported in the literature in typical inclusion-body hepatitis of poultry (4). Adenoviruses of poultry are usually considered as orphan viruses with insignificant disease potential. However, there are many serotypes of avian adenoviruses, and the pathogenicity of different isolates varies greatly (3, 4). Due to a lack of reference material, we are unable to determine whether this virus is a new avian adenovirus, or belongs to an existing serotype. Furthermore, the possibility of some other potentiating factor cannot be ruled out. Studies on characterisation and antigenicity of the isolate are in progress.

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Résumé : Une maladie précédemment inconnue s'est répandue parmi les élevages de poulets de chair au Pakistan. Elle attaque les poulets âgés de trois à cinq semaines qui se développent bien, et entraîne une mortalité de 30 à 60%. A l'examen nécropsique, la lésion la plus notable est l'hydropéricarde. Les poumons sont congestionnés et oedématexes, le foie et les reins sont pâles et gonflés. L'examen histologique a révélé une nécrose et des lésions vasculaires graves dans le myocarde, ainsi qu'une congestion, un œdème et une infiltration cellulaire inflammatoire dans les poumons. Une nécrose focale et une infiltration de cellules mononucléaires étaient présentes dans le foie, et de nombreux hépatocytes contenaient de volumineuses inclusions intranucléaires basophiles. La maladie a pu être reproduite chez des poulets de chair de 15 jours par inoculation sous-cutanée de broyat de foie. Le broyat de foie a également provoqué une inhibition de la croissance et des hémorragies après inoculation à des œufs embryonnés. Des particules virales à symétrie icosaédrique ont été observées au microscope électronique dans l'extrait purifié de foie. On pense que la maladie est causée par un adénovirus qui n'a pas été signalé jusqu'à présent, sans qu'il soit possible d'exclure, au stade actuel, l'intervention d'un autre facteur potentielisant.


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Resumen: En Pakistán, en las granjas avícolas, se ha propagado una enfermedad hasta ahora desconocida, que ataca a los pollos de carne de tres a cinco semanas de edad que presentan un desarrollo normal, causando una mortalidad situada entre el 30 y el 60 por ciento. En el examen necrópsico, la lesión más notable es la del hidropericardio. Los pulmones se congestionan y son edematosos, el hígado y los riñones son pálidos y se hinchan. El examen histológico reveló necrosis y lesiones vasculares graves en el miocardio, al igual que congestión, edema e infiltración celular inflamatoria en los pulmones. Se encontró necrosis focal e infiltración de células mononucleares en el hígado y numerosos hepatocitos contenían voluminosas inclusiones intranucleares basófilas. La enfermedad ha podido reproducirse en pollos de carne de 15 días por inoculación subcutánea de homogenado de hígado. El homogenado de hígado también provocó una inhibición del crecimiento y hemorragias después de su inoculación en huevos embrionados. En el microscopio electrónico, se observaron partículas virales de simetría icosaédrica en el extracto purificado de hígado. Se piensa que la enfermedad es causada por un adénovirus que no se había señalado hasta ahora, sin que haya sido posible excluir, en la situación actual, la intervención de otro factor potencializante.

PALABRAS CLAVE: Adenovirus aviar - Enfermedades aviares - Enfermedades virales - Hepatitis - Hidropericardio - Pakistán - Pollos de carne.

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