CHAPTER II

REVIEW OF THE RELEVANT LITERATURE

History

In 1923, Baudet reported an outbreak of an acute hemorrhagic disease in domestic ducks in the Netherlands. Bacterial cultures were negative. However, he reproduced the disease experimentally in domestic ducks by the injection of liver suspensions filtered through a Chambertand L_3 candle. He concluded that the disease was due to a specific duck-adapted strain of fowl plague (8). Later, in 1942, Bos observed similar occurences but was unable to reproduce the disease experimentally in chickens, pigeons, rabbits, guinea pigs and rats. Bos stated that the disease was unrelated to duckling hepatitis, fowl plague or newcastle disease and that it was a new and completely distinct viral disease of ducks, which he termed duck plague (9).

In 1967, the disease was detected for the first time in commercial duck flocks on the east coast of the United States and was called "duck virus enteritis". This latter designation was motivated by the fact that the clinical signs were to be found predominantly in the gastrointestinal tissue (10). At present "duck virus enteritis" is still acceptable as a designation.

In addition to the Netherlands and the United States, duck plague has been suspected in China (11) and confirmed in France (15), Belgium (14), India (12), England (17) and Canada (16).

The first outbreak of duck plague in Thailand occurred in September 1976 in Ampur Bangpakong in Chacherngsao. The disease broke out in a flock of 1-year-old-pekin ducks. This was followed by the rapid spread of the infection through 28 flocks within the same area, killing approximately 115,000 ducks (between 1 day old and 2 years of age) within 2 months (18, 19).

Disease Features

Duck plague is a disease of anseriformes i.e. birds belonging to the family Anatidae such as wild and domesticated ducks, geese and swans. The disease is spread by contact with infected birds and by both direct and indirect exposure to contaminated materials and equipment (20). Free access to ponds, moats and pools undoubtedly promotes the spread of contamination (21). The incubation period is 3 to 7 days and birds of all ages are susceptible. Egg production may drop by 20-100% (20). In some flocks, an inapparent form of the disease may prevail. Also, mortality may be the result of dual infection consisting both of duck plague and latent bacterial infection (22).

Macroscopic lesions of duck plague consist of tiny hemorrhagic spots in the esophageal mucosa. These spots are arranged in longitudinal rows and the esophageal mucosa is partly, or totally, covered with a yellow or grey pseudomembrane (diphtheritic plague). Similar lesions may appear in the cloaca mucosa. Annular bands in the small intestine are hemorrhagic and visible on the serosal surface. Extravasation of blood in the abdominal and thoracic cavities and petechiation on the heart are

also associated with the disease. Lesions in the esophagus are considered pathognomonic (11, 23-27). Key microscopic lesions of duck plague consist of hepatocytes and epithelial cells of the gastrointestinal mucosa, with the formation of intranuclear type A inclusion bodies. (28-30). These lesions may be considered suggestive of duck plague infection.

Psysical and Chemical Properties of Duck Plaque Virus

The virus will pass through a Chamberland L and a Berkfield N. Candle but not through a Seitz E.K. filter. The duck plague virus, however, will pass through a Seitz E.K. filter when treated with ethylenediaminetetraacetic acid (EDTA) (11). Virus in suspension may be passed through a Millipore membrane of 0.22 um porosity, but infectious virus is retained by a membrane of 0.10 um porosity (31).

Viral inactivation studies reveal that the virus is destroyed after heating 10 minutes at 56°C. The virus is rendered inactive after heating at 50°C for 120 minutes. The infectivity is lost after heating at 22°C for 30 days. The virus is also rendered inactive when it is dried over calcium chloride at 22°C for 9 days. Exposure of the virus for 6 hours at pH level of 7, 8 and 9 results in no loss of titer; however, exposure at pH 5, 6 and 10 results in a measurable reduction of titier. At pH 3 and 11, the virus is rapidly inactivated. The virus is sensitive to both ether and chloroform. Viral infectivity is reduced 4 logs by exposure of the virus in suspension to chymotrypsin, trypsin and pancreatic lipase (31).

An observation of thin sections of virus-infected cell cultures 48 hours after inoculation under an electron microscope revealed viral particles in both the nucleus and cytoplasm of the cell. In the nucleus, the particles were approximately 91 um in diameter, with a core approximately 48 um in diameter. Smaller viral particles of approximately 32 um in diameter were also observed in some nuclei. Larger particles, approximately 181 um in diameter, with a densely stained core of 75 um, were observed in the cytoplasm. These had a less densely stained envelope. The capsid structure of the virus had not been resolved (32). The virion of duck plague contained deoxyribonucleic acid as determined by means of enzymatic digestion and fluorescence technique (31,32). The duck plague virus has been classified as belonging to the herpesvirus group. Initially, the virus developed as an intranuclear particle that matured and moved into the cytoplasm and extracellular areas (32).

While differences in virulence between strains of duck plague virus have been noted, all strains appear immunologically identical (11,33,34). Thus there is only one known serotype of the virus.

Biological Properties of Virus

Virulent duck plague virus is cultivated on the chorioallantoic membrane (CAM) of 9-to-12-day-old duck embryos when blood or tissue suspensions from waterfowl carcasses are used (11). The embryo shows extensive hemorrhage and dies after 5 to 15 days (2).

Repeated attempts cultivate virulent duck plague virus on chicken embryo by using different routes failed. (35,36) After its 12th passage through embryonated duck eggs, the virus became lethal to chicken embryos. During the process, the pathogenicity of the virus with regard to ducks decreased rapidly. After the 10th chicken embryo passage, ducks could still be infected; after the 20th passage, however, all signs of virulence disappeared and thus the ducks were no longer endangered. The virus, however, retained its antigenicity and protected susceptible ducks against challenge inoculation (36).

Duck embryos inoculated on the CAM with virulent virus died 96 hours or more after inoculation. The mortality rate depended upon the virulence and viral concentration of the inoculum. The viral concentration was higher in the CAM suspension than in an embryo suspension or in yolk. Viral titers were as high as $10^{6.5}$ 144 hours and 168 hours into the postinoculation period in a CAM suspension of embryonating eggs inoculated via the chorioallantoic sac (CAS) and 144 hours into the postinoculation period in a similar suspension from embryonating eggs inoculated by the yolk sac route (37).

The mortality rate among the chicken embryo after inoculation with chicken embryo-adapted duck plague virus peaked between 72 and 192 hours after inoculation. There was, however, a lag phase of 48 hours before any of the embryos died. Viral concentration was highest in the CAM after 144 hours and in the amnioallantoic fluid (AAF) after 96 hours. The viral concentration

was highest in the CAM and AAF, whereas the viral titer was low in embryo and yolk suspensions (37).

Virulent duck plague virus was also propagated in duck embryonic fibroblast. Cytopathogenicity and eosinophilic, granular, intranuclear, inclusion bodies were observed in the infected cell. The inclusion bodies appeared as early as 12 hours after inoculation with the virus (38). The virus does not agglutinate chicken, duck, horse or sheep erythrocytes when saline or buffer solutions are used as the diluent (11).

Minute plaque observed in duck embryo cell cultures were inoculated with a suspension of CAM from duck embryos that had died subsequent to inoculation with virulent virus, on the 5th day after inoculation. The plaques were 1 to 2 mm in diameter, circular, and had irregular boundaries. The plaques did not increase in size, even after 5 days of incubation. When the virus was passaged 5 times in duck embryo cell cultures, it produce minute plaques, 1 to 2 mm in diameter, and small plaques, 3 to 6 mm in diameter, in a ratio of 1:2. The maximum number of plaques was found on the 6th day of the postinoculation period (DPI). The plaques formed with the chicken embryo attenuated virus formed a clear area; they resembled the relatively larger sized plaques produced by virulent virus. Avirulent viral plaques measured 1 to 3 mm at 4 DPI, 7 mm at 6 DPI and about 10 mm at 14 DPI. When the attenuated virus was plated on the chicken embryo cell cultures, the plaques that were produced were identical in size and morphologic properties to those produced in duck embryo cell cultures (39).

Recently, virulent duck plague virus was propagated by using American Type Culture Collection cell line 141 (CCL-141) - line of embryonic duck fibroblast cells. (40, 41, 42) As observed under a microscope, the sequence of changes produced by duck plague virus began 42 hours into the postinoculation period with cell fusion. By the 66th hour, holes had appeared in some foci, and after 76 hours nearly all foci exhibited a prominent hole and obvious cellular debris (41).

Laboratory Diagnosis

Animal Inoculation

Suspected tissue submitted for primary virus isolation should be homogenized and filtered or treated with antibiotic to prevent bacterial contamination. Susceptible, day-old ducklings, from a pathogen-free flock, can be inoculated intramuscularly in the leg muscle with 0.5 ml of the treated suspected material. Morbidity, as well as mortality, may be observed 3 to 12 days after inoculation. Typical duck plague lesions may be found on necropsy (2,3).

The inoculation of ducklings has been proved to be the most reliable method of confirming the disease (43) and as well as being a good indicator of duck plague virus (3).

Embryonating Duck Egg

Primarily, the virus can be isolated by propagation on the chorioallantoic membrane of 9-to-14-day-old embryonating duck eggs.

The infected embryo may die with typical lesions, 4 to 10 days into the postinoculation period. If initial attempts at isolation are negative, the chorioallantoic membranes can be harvested for further blind passages (26).

Cell Culture

The virus can be propagated in duck embryo cell cultures (44, 45) and duck embryo cell lines (CCL-141) (41,42). Both the cytopathic effect of the virus as well as its plaque forming ability have already been demonstrated (39,44,45). Muscovy duck cell cultures produced the best results in terms of virus yield and plaque forming ability (46), thus it is the most sensitive system for isolating the virus (43). Primary cultures produced a greater number of virus per cell than cell lines (41,46) but the latter do have a number of advantages in terms of availability, uniformity and the fact that their history is well-known. (41) In 1980, it was found that virulent duck plague virus plaqued at temperatures of between 37 - 41.5 °C, whereas the less-virulent isolated plaqued only at temperatures higher than 37 °C. The ability of duck plague isolate to grow within a whole range of temperatures may be a good indicator of its virulence and may help to differentiate isolates (48).

Serologic Identification

The neutralization test and microtitration in cell cultures are carried out (33,42). In addition, the use of embryonating egg has also been reported (36). Serologic identification of duck

plague virus is usually made by means of the virus neutralization test. Suspected samples are harvested and tested with specific duck plague virus antiserum by means of the alpha neutralization test using the "constant serum-diluted virus" method (49).

An increase in the serum neutralization titer following convalescence from the disease would demonstrate the progress of the disease within a particular flock. A virus neutralization index (NI) of 1.75 and more indicates an incidence of infection with duck plague virus (2-4,33). For neutralizing antibody detected by the beta neutralization method (constant virus-diluted serum) (50,51), titers of 1:8 or higher are considered significant (50).

The immunofluorescence technique (IF) has been used successfully in detecting duck plague antigen in infected tissue, particularly in some strains that do not form plaques (52). However, there are problems with IF, such as false positives and negatives, in addition to which fluorescence may be difficult to interpret (48).

The reverse passive hemagglutination (RPHA) test could be developed to detect duck plague virus. This procedure is simple, specific and sufficiently sensitive to be a useful diagnostic test, especially when used on the tissue of ducks dying of the disease (53).

Immunity

Birds which have already recovered from an attack of duck plague virus are immune to reinfection by the disease (3,4). Active

immunity has been demonstrated following use of a modified live virus vaccine (37). Parental (egg-transmitted) immunity has been shown in ducklings. It was then arranged that the progeny of breeder ducks, whose serum has a virus neutralizing antibody level of $10^{3.5}$, develop passive maternal immunity. This protection seems to diminish rapidly because most of the 13-day-old ducklings died when exposed to a challenge with virulent virus (54). Moreover, this immunity may interfere with the response to live virus vaccine (3). Passive immunization by the transfer of antibodies to susceptible ducks also protected them to a significant degree from lethal challenge (5). It is possible that the serum antibodies possessed the ability to kill the duck plague infected cells in the presence of complement (50).

Prevention and Control

There is no reported treatment for duck plague infection. Prevention is achieved by keeping susceptible birds in environments free from exposure to the duck plague virus. The disease which is introduced by wild, free-flying Anseriformes or caused by contaminated aquatic environments, must be prevented. Immunization has been employed as a preventive measure and also in controlling outbreaks of the disease (3).

Attenuated Live-Virus Vaccine

An embryo-adapted strain of duck plague virus that is avirulent in terms of domestic ducks has been developed by Jansen (2,3,4,57). This modified virus has been used extensively and

successfully in the Netherlands. This virus was propagated in chicken embryos and in chicken embryo cell cultures and was cloned by plaque selection. After an assay test for potency and safety, it was released for use by the duck industry for the immunization of their birds (36,58). It is possible to vaccinate ducks by means of intramuscular injection (36), by the oral method (6,61) or via the drinking water (62).

A study by Toth in 1970 revealed that $10^{3.58}$ per 0.5 ml of attenuated vaccine constituted a 90% protective dose (PD_{90}). Ducklings given this dose subcutaneously were subjected to challenge with $10^{5.58}$ duck lethal dose 50% (DLD_{50}) of virulent virus and withstood the challenge (7). A single vaccination of the virus administered to breeder ducks did not induce detectable antibody, whereas, two vaccinations induced a low level of neutralizing antibody (59). Susceptible ducks, placed with others that had been vaccinated with attenuated vaccine, did not develop neutralizing antibody (59). Susceptible ducks, placed with others that had been vaccinated with attenuated vaccine, did not develop neutralizing antibody. This meant that the vaccinated birds did not transmit the vaccine virus to the susceptible ducks (59).

In 1984, the newly isolated Sheridan 83 strain of duck plague virus was found to be apathogenic to ducks (50). Inoculation of ducks with this virus resulted in the production of antibodies that enabled the ducks to resist challenge with a virulent strain of duck plague virus. Passive immunization was also reported (55).

Inactivated Vaccine

A study was undertaken to compare the serologic and immunologic response of white Pekin ducks to a tissue culture of live attenuated and inactivated duck plague virus. The virus, inactivated by 0.05 acetylaziridine (AEI) at 37°C for 6 hours, induced as great a serologic response as the live attenuated virus. This inactivated preparation afforded the ducks protection against challenge. Preparations inactivated with 0.4% β-propiolactone (BPL) did not afford protection against a challenge inoculation with virulent virus (6). In another study, vaccine preparations not completely inactivated by AEI were found to be less protective than the live virus, and BPL-treated virus provided the ducks with no protection whatsoever (60).

The level of immunity provided by formalin-inactivated duck plague vaccine was 40%, increasing to 60% when the same vaccine was combined with incomplete Freund's adjuvant. It would seems that the adjuvant enhanced the immunity produced by the vaccine (5).

There is a lack of positive correlation between virus-neutralizing antidoty and the ability of vaccinated ducks to withstand challenge when inoculated with virulent duck plague virus (4,5,6,56,61). This then raises the question whether such protection is due to a cellular-mediated immunity (4). Vaccinated birds were resistant to infection as early as day 1 after vaccination, a fact which was considered attributable to the interference phenomenon (7,57,59,63).