INFECTIOUS BURSAL DISEASE

SLIDE STUDY SET #14

A CONTINUING EDUCATION PROGRAM

DEVELOPED BY P. D. LUKERT
DEPARTMENT OF MEDICAL MICROBIOLOGY
UNIVERSITY OF GEORGIA
ATHENS, GA 30602

REVISED BY AHMED MUTALIB
ANIMAL HEALTH RESEARCH
COLLEGE OF VETERINARY MEDICINE
MISSISSIPPI STATE UNIVERSITY
MISSISSIPPI STATE, MS 39762

IN COOPERATION WITH THE CONTINUING EDUCATION COMMITTEE

OF

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AAAP BUSINESS OFFICE
UNIVERSITY OF PENNSYLVANIA
NEW BOLTON CENTER
KENNETT SQUARE, PA 19348-1692

INTRODUCTION

Infectious bursal disease (IBD) is an acute viral infection of young chickens that affects the lymphoid tissue particularly the bursa of Fabricius. The disease was first reported by Cosgrove in 1962 (5) and was referred to as "avian nephrosis" because of severe kidney damage in affected birds. In the same year, Winterfield and Hitchner (9) and Winterfield et al. (10) recognized that avian nephrosis and IBD are two different syndromes and identified the causative agent of avian nephrosis as an isolate of infectious bronchitis virus with nephrotoxic tendencies and referred to the causative agent of IBD as "infectious bursal agent". The disease was also referred to as "Gumboro disease" because it was first observed on farms near Gumboro, Delaware. Hitchner (7) proposed the name for the disease that we now recognize as infectious bursal disease (IBD). The disease has a world-wide distribution.

ETIOLOGY

Infectious bursal disease virus (IBDV) is a member of the Birnaviridae family (3). Two serotypes of IBDV, designated 1 and 2, and many strains have been recognized. The virus is highly contagious and spreads rapidly through a flock. Spread of infection is through contamination of feed and water by droppings from actively infected birds (1). There is no evidence that IBDV is transmitted through the egg or that a true carrier state

exists in recovered birds. The virus is very stable and this explains its long, persistent survival in poultry houses (2).

CLINICAL FEATURES

Chickens are the only species known to develop clinical disease. Chickens up to 18 wk of age are susceptible, but the disease is commonly diagnosed in chickens between 3 and 6 wk of age. Chickens younger than 3 wk do not exhibit clinical signs but develop a severe immunosuppression that is economically more important than the clinical disease. Sequelae to this immunosuppression include gangrenous dermatitis, inclusion body hepatitis, *E. coli* infections, and vaccination failures.

The onset of the disease is rapid and chickens exhibit ruffled feathers, anorexia, depression, whitish or watery diarrhea, soiled vent feathers, vent-picking, severe prostration, and finally death (5). In severe cases, the entire flock will exhibit signs. Mortality may be as high as 20-30% but is quite variable. The course of the disease is usually 5 to 7 days.

Slide 1. A typically depressed chicken with ruffled feathers and evidence of watery diarrhea.

PATHOLOGIC FEATURES

Gross Lesions. Gross lesions are primarily seen in the bursa of Fabricius and musculatures of thigh and breast. The renal tubules may be distended with urates; however, these lesions are secondary and are most probably due to dehydration. Occasionally,

hemorrhages are detected in the kidneys, and mucosa at the juncture of the proventriculus and gizzard.

Slide 2. Swollen and edematous bursa. By the 2nd or 3rd day postinfection, the bursa is covered with a gelatinous yellowish transudate.

Slide 3. A caseous exudate may sometimes be found in the lumen of the bursa as a result of the extensive necrosis and inflammation of the bursal follicles during the acute phases of the disease.

Slide 4. Some bursas exhibit extensive hemorrhage; in these cases birds may void blood in droppings.

Slide 5. Hemorrhages of the leg, thigh, and breast muscles are common, but they may be absent in some birds.

Histopathology. Histopathologic lesions are most evident in the bursa, but changes may also be seen in the spleen, cecal tonsil, thymus, and gland of Harder (4).

The bursa undergoes extensive necrosis of the lymphocytes making up the follicles. Changes can be seen as early as 1 day postinfection, and all lymphoid follicles are affected by 3 or 4 days. Degeneration and necrosis of lymphocytes are followed by edema, hyperemia, and accumulation of heterophils. As the inflammation declines, cystic cavities develop in the medullary

areas of follicles, and there is fibroplasia of the interfollicular connective tissue. Ultimately there is chronic atrophy of the follicles, and some follicles remain cystic. The bursal epithelial layer becomes more prominent and glandular in appearance, with columnar epithelial cells containing globules and mucin.

The spleen will exhibit lymphoid necrosis of germinal centers and the periarteriolar lymphoid sheath on the third day postinfection. The spleen recovers rapidly and there is no sustained damage to germinal centers. The damage to the thymus and cecal tonsil is similar and rapid repair and recovery occur by 5 to 6 days postinfection.

The gland of Harder is also affected. After an infection of 1-day-old chicks, the populations of plasma cells are 5-to-10-fold less than in uninfected birds (6).

Slide 6. Normal bursal follicles showing the cortical (darkly stained), medullary (pale) and corticomedullary regions.

Slide 7. Necrosis and inflammation of a bursal follicle. Note pyknotic nuclei of necrotic lymphocytes.

Slide 8. Cystic cavities in the necrotic bursal follicles as the inflammatory reaction subsides in the follicular areas of the bursa.

Slide 9. Bursa from a bird recovered from IBD showing the

chronic atrophy with fibroplasia of the interfollicular tissue and absence of follicles.

DIAGNOSIS

Presumptive diagnosis can be based on the rapid onset of infection, high morbidity, spiking mortality curve, and rapid recovery (5-7 days) from clinical signs. The gross and histologic changes in the cloacal bursa are characteristics.

Isolation of the virus. The cloacal bursa and spleen are the tissues of choice for the isolation of IBDV. Several methods may be used to isolate IBDV. The embryonating egg from susceptible flocks or lymphoid cell culture derived from the bursa are the most sensitive methods for cultivating the virus (8). The chorioallantoic membrane of 9-to-11-day-old embryos is the preferred route and age of inoculation, and deaths of some embryos occurs in 3 to 5 days. The IBDV produces cytopathic effects in cell culture, and plaques are formed under agar after 3 or 4 days incubation. The disease may be diagnosed rapidly by staining impression smears of the cloacal bursa with fluorescein-labelled anti-IBDV antibody. Samples for staining should be collected during the acute phase of the disease. It should be noted that this test does not differentiate between the two serotypes of the virus.

There is a difference between the sites of replication of virulent and avirulent strains of IBD in the cloacal bursa. Virulent strains replicate in the lymphocytes of the medullary and cortical areas of the follicles, whereas avirulent strains

replicate only in the interfollicular tissue of the bursa. This explains why the virulent viruses are immunosuppressive and avirulent vaccine strains are not. However, some vaccine strains retain some pathogenicity and are capable of causing immunosuppression in susceptible birds that are not protected by maternal antibody.

Slide 10. Fluorescent antibody (FA)-stained lymphocytes in an acetone-fixed impression smear of the cloacal bursa 3 days postinfection with IBDV. The staining of such smears with fluorescein-conjugated antibody may be used to diagnose IBD rapidly and definitively. If smears are made of the bursa during the recovery stages of the disease, there may be no intact infected cells present, and therefore, no FA staining. Virus, however, can be isolated for at least 10 to 14 days postinfection.

Slide 11. Immunofluorescent staining of a section of the bursa infected with a virulent strain of IBDV 2 days postinfection. The virus is replicating in cells throughout the follicles.

Slide 12. Immunofluorescent staining of a section of the bursa infected with avirulent vaccine strain of IBDV 2 days postinfection. The virus is replicating in cells of the interfollicular tissue.

Slide 13. Electron micrograph exhibiting IBD viral particles

with an approximate diameter of 68 nm. They are non-enveloped and have icosahedral symmetry. Many particles are empty, incomplete, and non-infective.

Slide 14. Plaques are produced in cell culture by three different strains (C,T, and V) of IBDV. The C & T strains have a mixture of large and small plaques. The V strain is a cloned strain of IBDV and has only the large plaque.

Slide 15. Cell-culture cells stained by FA and are showing that the virus replicates in the cytoplasm and the nucleus is unstained.

Serology. Several serologic procedures are available to detect antibodies to IBDV. The agar gel precipitation test is rapid, but is only qualitative. Virus neutralization test and the enzyme-linked immunosorbent assay (ELISA) are quantitative and preferable to the agar gel precipitation test. The ELISA is sensitive and available commercially but unlike the virus neutralization test it will not differentiate between antibodies to the two serotypes of the virus.

Slide 16. An agar gel precipitation test with antigen prepared from infected bursas placed in the center well. Serum is added to surrounding wells. Of the six sera represented in this slide, five are positive.

Slide 17. A microtiter cell-culture virus-neutralization test. Infected cell control wells are represented in the 12th well of each row at the far right. Virus controls are in well 11. Antibody is diluted two-fold starting at 1/2 on the left. The eight sera in this slide, rows A through H, have titers of 64, 32, 128, 128, 16, 32, 16 and 16 because at those dilutions they protect the cell monolayer from destruction by 100 TC ID50 of virus. The plates are fixed in ethanol after 72 hours of incubation and stained with 1% aqueous crystal violet solution to visualize the cell monolayers.

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