INCLUSION BODY HEPATITIS OF CHICKENS

SLIDE STUDY SET #2

A CONTINUING EDUCATION PROGRAM PREPARED BY

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INTRODUCTION

Inclusion body hepatitis of chickens (IBHC) was initially described by Helmboldt and Frazier (1) in 1963 as an apparently rare condition of uncertain etiology. In the late 1960's the disease was recognized with increasing frequency in commercial flocks. Recent reports by Howell et al. (2), Bickford (3) and Pettit and Carlson (4) indicate that IBHC is currently a disease of economic significance occurring quite frequently in several regions of the United States and Canada. In view of the fact that IBHC appears to be an emerging disease, this program was prepared as a summary of current knowledge which hopefully will provide useful guidelines for the diagnostician.

CLINICAL FEATURES

FLOCK HISTORY

Breed Incidence. IBHC has occurred in both layer replacement and broiler chickens although most reported cases have involved the latter (1,2,4).

Influence of Commercial Strain. Numerous commercial strains of both light and heavy breeds have been affected.

Influence of Breeder Flock. While there is little evidence implicating specific breeds or strains, there is evidence in several integrated operations suggesting that chickens breaking with IBHC originate from certain breeder flocks. There is some documentation that the IBHC agent is transmitted from dam to progeny (5).

Age Incidence. IBHC is a disease of young chickens with most outbreaks occurring from 5 to 10 weeks of age. The great majority of cases have occurred at 6 to 7 weeks of age.

Seasonal Incidence. There appears not to be a strict seasonal incidence but, in temperate climates, the incidence tends to be greatest in spring and summer.

Presenting Signs. The initial evidence of disease in affected flocks is usually a sudden and drastic increase in mortality. In the early stages there may be a 3 to 10 fold increase in mortality in flocks which otherwise appear quite healthy.

Mortality Pattern. Generally, the mortality rate increases for 3 to 5 days, plateaus at a high level (0.5 to 1.0% per day) for

the next 3 to 5 days then falls to normal levels in an additional 3 to 5 days.

Cumulative Mortality Rate. The disease persists in affected flocks for approximately 1 to 2 weeks and the cumulative mortality ranges from 2 to 10%.

Influence of Complicating Diseases. In several cases IBHC has been complicated with other diseases (particularly respiratory and enteric infections) and, in such cases, the mortality rate and the duration of the disease may be extended considerably.

Influence of Housing. Nearly all of our cases of IBHC have occurred in floor-reared chickens. However, a few affected flocks were reared on wire floors.

CLINICAL SIGNS

Appearance of Affected Chickens. As noted above, the initial upturn in mortality may occur without previous observation of overtly sick birds in the flock. As the disease progresses, however, clinically affected chickens may be found. The clinical signs in affected live chickens include pallor and/or jaundice of the unfeathered skin, marked depression, weakness, eventual prostration and death.

Clinical Course. The course of clinical disease in individual chickens is quite rapid - usually 24 hours or less. It is noteworthy that affected live birds selected from a flock for diagnosis will usually die in transit to the diagnostic laboratory

if more than a few hours travel time is involved. Another observation suggesting a rapid course is the fact that throughout the duration of IBHC in a flock, the great majority of the chickens appear bright and alert (i.e. there is not a high morbidity rate).

Slide 1. A typically affected chicken is shown. The photograph, while depicting little more than a typical "sick chicken" does serve to make the point that clinical features alone are by no means diagnostic. With anamnestic data considered, however, the pale, jaundiced appearance of the facial skin should establish an index of suspicion which should cause IBHC to be included in the differential diagnosis.

PATHOLOGIC FEATURES

Gross Lesions. A variety of gross tissue alterations have been associated with field outbreaks of IBHC. While the name of the disease implies primary liver involvement, lesions are by no means restricted to liver. Often gross changes in other organs and tissues are as important as hepatic lesions in causing the diagnostician to include IBHC in his differential diagnosis. Gross lesions included in this program are presented in the order in which they would likely be observed during the necropsy of affected chickens.

Slide 2. Pallor in any animal should suggest anemia and, in the chicken as in other species, this is readily confirmed by determination of the packed erythrocyte volume (hematocrit) in heparinized whole blood. Generally chickens with IBHC are anemic

with hematocrit values ranging from 1/2 to 1/10 of normal. The anemia in IBHC is attributable to 2 major lesions which are detectable at necropsy. The first is hemorrhage which is sometimes evident even in the subcutis of the intact chickens. This slide shows several areas of suffusive type hemorrhage in the skin over the thigh and wing.

Slide 3. When the overlying skin is reflected subcuticular and intramuscular hemorrhages are often extensive. Hemorrhage tends to be most severe in the musculature of the tibial area, the thigh and the breast. Marked pallor of the musculature is also evident in this slide in that the thigh musculature is approximately the same color as the breast muscle.

Slide 4. In addition to a "paling out" of the musculature and scattered hemorrhages in the breast and thighs, there is a definite icteric cast to the musculature and fat deposits of the chickens in this slide. This is a common finding in chickens in advanced stages of IBHC. It is noteworthy that these are 9-week-old white leghorn pullets and they have not been fed supplemental carotenoid or xanthophyll pigments as might be the case in some broiler birds. Jaundice, of course, might be expected in diseases where there is extensive liver damage and hemorrhage.

Slide 5. The second major lesion responsible for the clinically detectable anemia is aplasia of the bone marrow. This photograph illustrates the appearance of bone marrow in the split proximal femur of one of the chickens in slide 4. The bone marrow in field cases of IBHC varies from a red gelatinous consistency to

the white-grey appearance evident in this photograph. Regardless of the gross appearance, a significant degree of aplasia can be detected histologically and the discoloration is due to replacement of hematopoietic elements by adipose tissue.

Slide 6. Hemorrhage may also be observed beneath the serosa of the abdominal organs. In this slide numerous petechial subserosal hemorrhages are evident in the intestine. A note of caution might appropriately be expressed at this point regarding the multiplicity of causes of aplastic anemia and widespread hemorrhaging in chickens, perhaps the most prevalent of which are the mycotoxins. All lesions must be interpreted in conjunction with anamnestic and clinical observations and the pattern of changes in other organs and tissues.

Slide 7. The next organ likely to be examined in the post-mortem examination is the liver. As might be expected, the liver is the most consistently involved of all visceral organs. This slide shows the appearance of a severely affected liver as seen in some chickens in the terminal stages of IBHC. The liver is moderately enlarged with blotchy areas of hemorrhage scattered throughout the yellow-tan degenerative parenchyma. This appearance comes very close to being pathognomic of IBHC but, unfortunately, this degree of involvement is by no means constant in chickens with this disease. Such severe hepatic lesions seem to occur most frequently at the midpoint of the outbreak (about 1 week after the initial upsurge of mortality) and are almost always restricted to moribund or dead chickens.

Slide 8. The liver in this slide illustrates the gross changes of moderate hepatic involvement. The liver is somewhat lighter in color than normal and there is a reticulate pattern of degeneration in the parenchyma. Even milder changes may be found in livers of affected chickens and, in these instances, the only alterations may be mottling, slight yellow-tan discoloration or scattered petechial hemorrhages. Such minimal hepatic changes are shown in slides 6 and 7. Despite the variation in gross appearance, histopathologic changes and intranuclear inclusion bodies typical of IBHC were observed in all of these livers. It should be emphasized that wide variation in liver involvement may be noted in groups of chickens submitted for diagnosis.

Slide 9. Lesions in the heart have been observed in about 25% of all chickens examined from affected flocks. Gross cardiac changes include hydropericardium, subepicardial hemorrhages and milky discolored areas in the epicardium. In this slide a portion of the liver has been excised to expose the heart in which milky areas of epicardial discoloration are evident. These white areas on the surface of the heart have been examined histologically and represent areas of subepicardial edema and inflammation.

Slide 10. Swelling and yellow-tan discoloration of the kidneys and atrophy of the spleen and bursa of Fabricius are also frequent gross findings in chickens with IBHC. As in this slide, affected kidneys tend to bulge from their bony recessed in the synsacrum and have a prominent lobular pattern. The bursa of Fabricius, also evident in this slide, is reduced to about 1/3 of

the expected size in a chicken of this age (9 weeks).

Slide 11. The kidneys, spleen and bursa of Fabricius of a 5-week-old chicken are shown in this photograph. Swelling and discoloration of the kidneys and atrophy of the bursa of Fabricius can be seen. The spleen in this chicken appears normal in size but splenic size is related as much to the degree of sinusoidal engorgement as to the status of its lymphoid elements. Lymphoid depletion was demonstrated histologically.

Microscopic lesions. Histopathologic alterations have been observed in variable degrees in all the organs and tissues with gross lesions as described above. Complete histologic surveys of chickens with IBHC indicate that certain tissues are not altered to any significant degree; these tissues include: brain, eye, tissues of the respiratory system and upper digestive tract, thymus, pancreas, endocrine glands and gonads. In general terms the histopathologic changes in skin, muscle and intestine tend to reflect loss of vascular integrity (hemorrhage). No specific vascular lesions have been found in these locations, however, nor has there been any inflammatory, degenerative or proliferative changes in these sites. Microscopic lesions are occasionally encountered in heart and spleen and the observed changes have been presented in the discussions of slides 9 and 11, respectively. The more definitive and consistent histopathologic changes of IBHC have occurred in bone marrow, liver, kidney and bursa of Fabricius and these are presented in the following slides.

Slide 12. Affected bone marrow is always aplastic to some

degree. In this specimen hematopoietic elements have been completely lost and only adipose tissues and a few islands of undifferentiated mesenchymal cells remain.

Slide 13. Microscopic lesions in livers include: 1) various degrees of diffuse degenerative change in hepatic cells, 2) biliary hyperplasia (hyperplasia of the lining epithelium of ducts and proliferation of bile ductules or capillaries) and 3) infiltration of inflammatory cells (primarily lymphocytes and plasma cells) around portal triads. With severe hepatic involvement the hepatocellular degeneration progresses to overt necrosis with areas of parenchymal collapse resulting in pooling of blood in sinusoids or hemorrhage. Such advanced parenchymal damage is shown in this slide. There is marked hyperplasia of the epithelium lining the large bile duct on the left. Marked vacuolar degeneration and disorientation of hepatocytes can be seen and an area of parenchymal collapse and hemorrhage is present on the right.

Slide 14. Bile ductular proliferation and periportal inflammatory cell infiltration can be seen around a portal triad.

Slide 15. The diffuse swelling and dissociation of hepatic cells shown in this slide represent the type of parenchymal change often seen in livers with relatively mild gross alterations.

Slide 16. The unique histologic change in the liver is the presence of Cowdry type A intranuclear inclusion bodies in hepatic cells. The large red to light violet staining inclusion bodies in a severely affected liver are clearly shown in this slide. There is a distinct clear space between the inclusion body and the

marginated chromatin distributed along the nuclear membrane. Intranuclear inclusion bodies may be much fewer and much more difficult to find in livers with milder changes.

Slide 17. Microscopic lesions in the kidney include swelling and degenerative changes in epithelial cells of the convoluted tubules, interstitial edema and occasionally interstitial hemorrhages. Severe nephrosis and interstitial edema are evident in this kidney section from a chicken with severe IBHC.

Slide 18. Variable degrees of lymphocytic depletion are consistently seen in the bursa of Fabricius. In this bursal plica the follicles are much reduced in size and the epithelial folding indicates the degree of atrophy that has taken place due to loss of lymphoid tissue. The bursal involvement in IBHC is always bland - i.e. there is no inflammatory reaction such as is seen in infectious bursal disease.

ETIOLOGY

Inoculation of Embryonated Chicken Eggs. The cause of IBHC has been demonstrated by Fadly and Winterfield (5) to be a virus. Four- to seven-day-old chicken embryos inoculated by the yolk sac route with infective liver suspensions will usually die in 5 to 10 days showing cutaneous congestion and hemorrhages. It is important, however, that the embryos originate from IBHC susceptible parent stock or failure will result in propagating the virus. In this regard, the majority of breeder flocks studied by the authors have proved immune. The agent also causes opaque

plaques on the chorioallantoic membrane when embryos are inoculated at 10 days of age and examined 5 to 7 days later. Infected embryos often have a focal necrotizing hepatitis and basophilic intranuclear inclusion bodies are evident in hepatic cells.

Inoculation of Susceptible Chicks. Yolk from infected embryos when inoculated into susceptible young chickens results in gross hepatic lesions (degeneration, disseminated necrotic foci, and hemorrhages) within a few days. Death may be seen in some of them if the chickens are inoculated subcutaneously; others will be stunted. Together with the hepatitis, bone marrow aplasia and intramuscular hemorrhages may be noted in some of the chickens that are necropsied Histologically, focal areas of hepatic necrosis and intranuclear inclusion bodies are seen. Electron microscopy of ultrathin sections has revealed the presence of virus particles in affected liver cells of experimentally infected chickens during the early stages of infection.

Other Approaches to Isolation and Identification. The virus may also be propagated in chicken embryo kidney cell cultures in which a cytopathic effect and intranuclear inclusions are observed. The best results in isolating the virus from diseased chickens are obtained in the very early stages of infection. Virus neutralizing antibodies are formed in the blood after infection (5). Hence, the serum-virus neutralization test can be an aid in detecting infection by the causative agent as convalescent serums will neutralize over 3 logs of virus.

Slide 19. This slide shows numerous raised plaques induced by

the IBHC virus within 5 to 7 days after inoculating the agent on the dropped chorioallantoic membrane. The plaques tend to have a smooth surface and several areas of coalescence are obvious.

Slide 20. Focal necrotizing hepatic lesions, as seen in this slide, are typical of those occurring in up to 50% of SPF chicken embryos that die after inoculation via the yolk sac at 4 days of incubation.

Slide 21. Blotchy hemorrhages and disseminated foci of necrosis have been consistently observed in livers of susceptible chicks that die or are killed within 3 to 7 days after subcutaneous inoculation with the IBHC agent. The hepatic lesion pattern shown in this slide shares many common features with that of naturally infected chickens (see slide 7).

Slide 22. This slide illustrates some of the histopathologic changes induced in livers of susceptible chickens by the IBHC agent. There is a small central focus of necrosis. Several of the hepatic cells surrounding the necrotic area contain intranuclear inclusion bodies in various stages of development. A mild degree of bile ductular proliferation can be seen in the lower left corner of the slide.

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