AVIAN VIRAL TUMORS

DEFINITION

The several viral neoplastic diseases of chickens and turkeys, although previously considered a "complex," are actually distinct disease entities. In some cases a single tumor virus strain can induce multiple disease syndromes, thus causing uncertainty whether these neoplasms should be classified by etiology or by lesion type. Furthermore, some of the lesion types are so rare as to be of little concern.

In an attempt to simplify this situation, we will consider here only the four neoplastic disease syndromes that have economic importance: Marek's disease, a common lymphoproliferative disease of chickens caused by an alpha herpesvirus; avian leukosis/sarcoma, common retroviral diseases characterized by lymphoid or other neoplasias and lowered egg production in adult chickens; reticuloendotheliosis, which includes a runting disease and a chronic lymphoma in turkeys, chickens, and a variety of other avian species caused by a nondefective retrovirus; and lymphoproliferative disease, a retrovirus-induced disease of turkeys characterized by chronic lymphomas that although not yet reported in the United States, is found elsewhere and must be considered in a differential diagnosis.

I. MAREK'S DISEASE

DEFINITION

Marek's disease (MD) is a herpesvirus-induced neoplastic disease of chickens characterized by infiltration of various nerve trunks and/or organs with pleomorphic lymphoid cells.

OCCURRENCE

Marek's disease is important primarily in chickens, to a much lesser degree in quail, and has been rarely observed in turkeys, pheasants and jungle fowl. Turkeys and other species have limited susceptibility. The disease most commonly occurs in young, sexually immature chickens 2-7 months old, but can occur at virtually any age beyond 3 weeks. The disease occurs throughout the world and virtually all flocks are exposed to the causative virus.

HISTORICAL INFORMATION

A report in 1907 by a Hungarian veterinarian, Jozsef Marek, of paresis in roosters is the first description of the disease now called MD. The disease was first reported in the United States in 1914. Although forms of MD were an important cause of mortality in chickens prior to 1950, a sudden increase in mortality in the late 1950s and 1960s accelerated research. Reliable experimental transmission was achieved in 1962 and the causative herpesvirus was isolated and identified in 1967. Vaccines became available for use in the United States by 1970 and have been very effective in preventing the disease. However, sporadic losses and the fear of increased virulence of the virus have kept MD among the most important poultry diseases.

ETIOLOGY

1. Marek’s disease virus is a cell-associated alpha herpesvirus of subgroup a3. The herpesviruses associated with MD are classified into three serotypes. Serotype 1 isolates are ubiquitous in chickens and pathotypes vary from very virulent plus (vv+) (oncogenic) to nearly avirulent (mild). Serotype 2 isolates are common in chickens and are nononcogenic. Serotype 3 isolates, also known as turkey herpesvirus, are ubiquitous in turkeys and are nononcogenic. The three serotypes have considerable antigenic cross-reactivity.

2. The serotype 1 virus can be grown in cultured chick kidney cells prepared from 1-3 week old chicks and in duck embryo fibroblasts. It produces a distinct cytopathic effect with intranuclear inclusions in those cells.
Embryonal chick kidney cells and chick embryo fibroblasts are less effective for low-passage virus. Serotype 2 and 3 viruses can be isolated and propagated in chick embryo fibroblasts. The virus is usually tightly bound to living cells and in this form is very labile, but cell-free virus is released from the feather follicle epithelium and is relatively resistant to environmental factors. Both cell-associated and cell-free viruses are susceptible to a number of common disinfectants.

EPIDEMIOLOGY

Infected chickens shed virus-containing feather follicle dander, which is a source of infection for other chickens by the respiratory route. Infected carriers may or may not be clinically ill, and carrier birds can sporadically shed virus throughout their lifetimes. The disease is very contagious and infectious dander can be disseminated over long distances. Although excretions and secretions of infected chickens may contain virus, dander containing infectious enveloped virus particles is the most important means of transmission. Transmission of the virus through the egg does not occur. Hatchery transmission through shell contamination is also unlikely due to adverse environmental conditions for the virus.

CLINICAL SIGNS

Clinical signs occur in chickens affected with MD but are of little help in establishing a diagnosis. Birds with visceral tumors are depressed and often cachectic prior to death. Birds with lymphoid infiltration of peripheral nerves may demonstrate asymmetric partial paralysis [Fig. 1; Marek's Disease; NCSU] and/or dilation of the crop due to vagus nerve paralysis. Blindness is associated with lymphoid infiltration of the iris [Fig. 2; Marek's Disease; UC Davis]. Clinical signs usually do not appear prior to 3 weeks of age and peak between 2 and 7 months.

LESIONS

1. At least four different lesion patterns are recognized: gross enlargement [Fig. 3; Marek's Disease; UC Davis] and/or yellowing and loss of cross-striations of peripheral nerves [Fig. 4; Marek's Disease; NCSU]; discoloration of the iris [Fig. 5; Marek's Disease; NCSU]; enlargement of feather follicles [Fig. 6; Marek's Disease; Cornell U] with reddening (skin leukosis); and visceral tumors [Fig. 7; Marek's Disease; UC Davis] involving the liver [Fig. 8; Marek's Disease; UC Davis], heart [Fig. 9; Marek's Disease; UC Davis], spleen, gonad, kidney [Fig. 10; Marek's Disease; UC Davis], proventriculus [Fig. 11; Marek's Disease; NCSU], and other organs and tissues. Visceral tumors are the most frequent lesions, but combinations of lesion patterns are common.

2. Microscopically, the lymphomas are characterized by a mixture of pleomorphic lymphocytes [Fig. 12; Marek's Disease; UC Davis]. Some of these probably are true tumor cells that carry T-cell surface antigens and a tumor-associated antigen (MATSA). Others are probably host cells reacting against viral or tumor antigens and represent both T- and B-cells.

DIAGNOSIS

1. A diagnosis can usually be made after careful consideration of the history, the ages of the birds affected, and the location of the neoplastic lesions in a generous sample of typically affected chickens. Few epornitic diseases resemble MD with the exception of lymphoid leukosis and reticuloendotheliosis.

2. Marek's disease often occurs in 2-5-month-old (sexually immature) chickens but can also occur after the onset of egg production. Outbreaks after the onset of egg production in vaccinated stock have been called "late Marek's" and are often associated with newer, more highly virulent vv+ pathotypes.

3. Characteristics of MD lesions of importance in differential diagnosis include nerve involvement (when present), the absence of bursal lesions or, rarely, diffusely thickened bursas, and pleomorphic lymphocytes comprising lesions, some of which exhibit MATSA and only few of which are positive for immunoglobulin M (IgM). The ubiquitous nature of MD virus renders virology and serology of little value in diagnosis.
CONTROL

1. Commercial flocks are usually immunized via injection at 18 days of embryonation or at hatching. Care must be taken to insure that an effective dose is administered to every embryo or chicken. Because immunity from vaccination is not fully developed for 7-10 days, it is crucial to minimize early exposure. This requires careful sanitation and disinfection, particularly because MD virus survives well for months in poultry houses. Revaccination is not necessary and immunity is usually life-long. Appearance of the disease at older ages has been attributed to immunodepression due to environmental stress or infection with vv+ pathotype.

2. The most common vaccines consist of turkey herpesvirus (HVT), a serotype 3 virus, as a cell-associated preparation or bivalent vaccine consisting of turkey herpesvirus and a serotype 2 virus (SB-1 or 301 B). Attenuated serotype 1 vaccines are also used. Care must be taken in handling cell-associated vaccines as they are highly susceptible to adverse environmental conditions.

3. Genetic differences associated with the major histocompatibility (B) complex can aid both in resistance to MD as well as the response to vaccination.

TREATMENT

There is no effective treatment for MD. Birds with tumors or multiple skin lesions are condemned at slaughter.

II. AVIAN LEUKOSIS/SARCOMA VIRUSES

DEFINITION

The avian leukosis (ALV)/sarcoma group are retrovirus-caused, neoplastic diseases of semimature or mature chickens. The most common, lymphoid leukosis (LL) is characterized by a gradual onset in a flock, persistent low mortality, and neoplasia of the bursa of Fabricius with metastasis to many other internal organs, especially the liver, spleen, and kidney. A relatively new strain of ALV, “J”, probably resulting from the recombination of endogenous and exogenous viruses, primarily causes myeloid leukosis (myelocytomatosis).

OCURRENCE

Lymphoid leukosis associated mortality is most common in chickens 16 weeks of age or older. The disease is worldwide in distribution and widespread in the United States. Virtually all flocks are considered to be exposed to the virus but infection rates within some flocks have decreased due to efforts at eradication by primary breeder companies. Overall, the incidence of LL is low (1 or 2%), although occasional heavy losses can occur. A higher incidence of bursal disease virus may be associated with a reduced incidence of LL. With ALV-J, meat-type chickens appear to be more susceptible than layers.

HISTORICAL INFORMATION

The first report of LL is attributed to Roloff in 1868. However, the disease was not well characterized until a basis for its separation from MD was established in 1962.
ETIOLOGY

Avian leukemia is caused by a family of retroviruses known as avian leukemia viruses (alpha retroviruses), which have been classified into 10 subgroups—A, B, C, D, E, F, G, H, I and J. In the United States, subgroup A viruses are most common and are most frequently associated with LL with ALV-J myelocytomatosis next in frequency. Subgroup B viruses are occasionally isolated, whereas subgroups C and D are rare. Subgroup E viruses are common and are considered "endogenous" because they are derived from proviral genes permanently integrated into the host cell DNA; they rarely are associated with neoplasms. Subgroup F, G, H and I viruses primarily cause leukemia in species other than chickens. The viruses produce a group-specific antigen that can be detected in albumen of eggs and body tissues or fluids. ALV-J viruses have extensive antigenic variation within the strain. The avian leukemia viruses can be cultured in chicken embryo fibroblasts but most produce no cytopathology and are detected by antigen tests. Simple tests for antigen detection are available and are used in eradication programs in breeders. Antibody tests are also available and are used to monitor the status of flocks from which the virus has been eradicated.

EPIDEMIOLOGY

Egg transmission is an important mechanism of spread of avian leukemia viruses. The frequency of infected eggs is usually low but chicks hatched from infected eggs are permanently viremic (immune tolerant), do not develop antibody, have an increased risk of death from LL, may lay fewer eggs, and will probably shed virus into their own eggs thus perpetuating the infection. Chickens also can become infected by contact exposure, particularly with ALV-J, which is efficient at horizontal transmission. In meat type chickens, ALV-J viremia negative/antibody positive birds can shed virus and post hatch infected birds become tolerant shedders. Some chickens, particularly those of greater susceptibility due to endogenous virus infection or absence of maternal antibody, may transmit virus to progeny as a result of contact infection soon after hatch.

CLINICAL SIGNS

Chickens with LL may present with nonspecific or no clinical signs of disease. Many birds with tumors are unthrift or emaciated and have pale combs and wattles. Enlargement of the abdomen may result from massive enlargement of the liver. Some birds with tumors can be detected prior to death by palpation of an enlarged and lumpy bursa of Fabricius by insertion of a finger into the cloaca. Birds with skeletal myelocytomatosis may have observable masses on the shanks, head and thorax. Osteopetrosis of the long bones [Fig. 1; Leukosis; Cornell U] or “boot” shanks may occur. Flocks with high infection rates experience depressed egg production.

LESIONS

1. There are no unique external lesions. Lymphomas [Fig. 2; Leukosis; Cornell U] are seen in many organs in chickens 16 weeks of age or older, but are especially common in the liver, kidney, ovary, and bursa of Fabricius [Fig. 3; Leukosis; Cornell U]. The white-to-gray neoplastic lesions can be diffuse or are sometimes focal. If the bursa of Fabricius is incised, small nodular lesions can often be detected that would not otherwise be obvious. Myelocytomatosis [Fig. 4; Leukosis; UC Davis] is most common with ALV-J; however, other tumor types such as hemangiomas can also be seen.

2. Microscopically, the neoplastic cells in lymphoid tumors are uniformly lymphoblastic and the cells are pyroninophilic. Also, they are nearly all positive for surface immunoglobulin M (IgM). The tumors originate from bursal lymphocytes (B-cells) in which the proviral DNA of the virus integrates during the process of replication at a site in the host cell genome close to the c-myc gene, a normal host cell gene with homology to an oncogene present in avian retroviral strain MC29. Activation of this oncogene is believed to be the primary event in starting the neoplastic process.

DIAGNOSIS

1. Lymphoid Leukosis can usually be diagnosed after careful consideration of the age of the affected chickens, the course of the disease and the pattern of mortality in the flock, and the location of gross lesions
AVIAN VIRAL TUMORS

in a moderate number of typically affected chickens. Involvement of the bursa of Fabricius is nearly always present, although the lesions may not be detected without incision of the organ and examination of the epithelial surface. In contrast to MD, bursal tumors are intrafollicular, generally causing a more nodular enlargement. The characteristic tumor cell has B-cell and IgM surface markers. Molecular methods are available in research laboratories to detect in the DNA of tumor cells the proviral DNA of ALV located in close proximity to the c-myc gene.

2. Diagnosis is made more difficult because the lesions of LL often appear similar to those of MD, and can appear identical to those induced experimentally by reticuloendotheliosis virus. Because ALV is very widespread in chickens, virological and serological methods offer little help in confirming a diagnosis.

3. Diagnosis of ALV-J is achieved by gross and histopathologic examination of tumors and by virus isolation from cloacal or vaginal swabs or tumors. Although PCR tests have been developed, the virus mutates frequently requiring the production of new primers.

CONTROL

1. With LL, because egg transmission is so important and the disease is not very contagious, eradication is the preferred method of control. Most of the eradication efforts have been conducted by the primary breeding companies. Many breeders of egg-type chickens have reduced markedly the rate of congenital transmission in their primary breeders and grandparent stocks through a program of testing dams prior to egg production and removal of those considered likely to transmit virus to progeny. Some breeders have flocks from which the virus apparently has been eradicated. Commercial progeny from such breeders should have lower infection rates and thus should experience less tumor mortality and greater egg production.

2. Although LL is not a disease of commercial broilers, ALV-J is a problem and breeders have made significant progress in their eradication programs. However, due to the efficient horizontal transmission of ALV-J, control by eradication is more difficult.

3. Genetic resistance to infection with subgroup A viruses is common in meat-type stocks, but quite rare in egg-type stocks. When present, this resistance offers an alternative approach to control.

4. There is no vaccine that can protect against tumor mortality. Congenitally infected chicks are immunologically tolerant and cannot be immunized. Vaccines to immunize parent stock where ALV has been eradicated is being considered as a means to provide maternal immunity to progeny chicks.

TREATMENT

There is no effective treatment for LL.

III. RETICULOENDOTHELIOSES

(Re)

DEFINITION

Reticuloendotheliosis (RE) is a term used for a variety of syndromes caused by retroviruses that may be either defective or nondefective for replication in cell culture. Only a running syndrome and a chronic lymphoma, both caused by nondefective RE virus, are of economic importance.

OCCURRENCE

Nondefective RE virus is not ubiquitous, but infection is fairly widespread in chickens and turkeys, particularly in the southern states. The disease is uncommon. Running disease has been associated with the use
of RE virus-contaminated vaccines in chickens. Chronic lymphomas occur naturally in turkeys, including wild turkeys, ducks, quail, pheasants, geese, peafowl, prairie chickens and chickens but are rare. Exportation of seropositive birds to some countries is not permitted.

HISTORICAL INFORMATION

A virus was isolated from a field case of turkey lymphomas in 1958 that, after rapid serial passage in chickens and turkeys caused high neoplastic mortality within 3 weeks. Although this isolate, strain T, has been considered a prototype, it is not typical of field strains. Other isolates from ducks and chickens were recognized in 1974 to comprise a family of RE viruses.

ETIOLOGY

RE virus is a retrovirus with an unusually wide host range. It can be grown in cells from chickens, ducks, turkeys, quail, and other species, even some mammalian cells. It infects a variety of avian species. Non avian species are resistant to infection. All isolates are of a single serotype, but minor subtype differences have been noted.

EPIDEMIOLOGY

The virus is transmitted horizontally. Mosquitoes have been incriminated as passive carriers. Fowl pox viruses have also been found to harbor infectious REV. Transmission through the egg has also been identified, but usually occurs at a very low rate.

CLINICAL SIGNS

The runting syndrome, usually induced by inoculation of chicks at 1 week of age or less with RE virus-contaminated biologics, produces severe stunting and a feather abnormality characterized by compression of barbules to the shaft in its proximal portion. Signs associated with chronic lymphomas are few but birds may become depressed prior to death.

 LESIONS

1. The runting syndrome is characterized by severe atrophy of the thymus and bursa of Fabricius. The birds are immunodepressed and may show lesions of concurrent infections. Generally no tumors are noted but some birds may have enlarged nerves, proventriculitis, enteritis, and anemia.

2. The chronic lymphoma syndrome as produced experimentally in chickens with nondefective RE virus is identical in all respects with lymphoid leukosis. A different lymphoma has been induced experimentally in certain chicken strains that closely resembles MD because of nerve lesions, tumors in the liver [Fig. 1; Reticuloendotheliosis] thymus, and heart, and its occurrence as early as 6 weeks of age. Chronic lymphomas in species other than chickens are characterized by tumors of the liver and spleen, but bursal tumors are not particularly common.

DIAGNOSIS

1. A diagnosis of RE is probably best made on the basis of typical lesions and demonstration of infection with the causative agent by virus or antibody tests. Currently, the PCR test, an immunoperoxidase plaque assay, and an enzyme immunoassay are available.

2. In chickens, the disease must be distinguished from both LL and MD. Thus far, however, naturally occurring chronic lymphomas in chickens have not been documented. The runting syndrome must be distinguished from other immunodepressive conditions, especially infectious bursal disease and chicken infectious anemia.
3. In turkeys, the disease must be distinguished from lymphoproliferative disease in countries of occurrence. This can usually be accomplished by noting the age of occurrence, the absence of greatly enlarged spleens, and the uniform lymphoblastic morphology of the tumor cells on histology and using PCR assays.

CONTROL

No methods for control or treatment have been reported. Strict sanitation and insect control may help prevent infection from environmental sources. Eradication programs patterned after those developed for LL may be useful in breaking the egg transmission cycle.
## DIFFERENTIAL DIAGNOSIS OF AVIAN TUMORS

The differential diagnosis of tumors in chickens and turkeys is difficult and requires an adequate history and a careful postmortem examination of a representative sample of birds with typical lesions. In some cases, additional tests such as histology, immunofluorescent tests for surface antigens, and molecular hybridization will be helpful. The characteristics in the following table may be helpful in arriving at the correct diagnosis.

<table>
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<tr>
<th>Characteristic</th>
<th>Chickens</th>
<th>Turkeys</th>
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<tr>
<td><strong>Gross lesions</strong></td>
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<td>Bursa</td>
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^Abbreviations:  MD = Marek's disease, LL = lymphoid leukemia, RE = reticuloendotheliosis, LPD = lymphoproliferative disease.

^Two experimental syndromes are recognized: a bursal lymphoma with characteristics similar to LL, and a nonbursal lymphoma with characteristics similar to MD.