THE DIFFERENTIAL DIAGNOSIS OF

LYMPHOID LEUKOSIS AND MAREK'S DISEASE

SLIDE STUDY SET #3

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INTRODUCTION

Avian tumor viruses may be classified into three different groups. First, there is Marek's disease virus, a DNA containing enveloped herpesvirus which, until recently, was the most common cause of economic loss in the poultry industry. Secondly, there are the viruses of the leukosis/sarcoma group which are small RNA containing enveloped viruses often placed in the "leukovirus" group. Lastly, there is the reticuloendotheliosis virus group composed of RNA containing enveloped viruses different from the leukovirus group and occurring only very rarely in chickens. This narrative guide and slide set is designed to assist professional avian diagnosticians in distinguishing between lymphoid leukosis (LL) caused by viruses of the leukosis/sarcoma group and Marek's disease (MD) caused by a herpesvirus, the two most commonly occurring neoplastic diseases of poultry. Since the reticuloendotheliosis virus infection is not known to be a problem in the field, lesions induced by this group of viruses will not be discussed.

It is important to make a differential diagnosis between LL and MD because their epizootiology and the methods used for their prevention are entirely different.

Epizootiology

Both the leukosis/sarcoma viruses and MD virus are ubiquitous among chickens. They have been reported in other birds but they are not a problem. Sometimes morbidity and mortality from LL or MD may be as high as 50% in laying flock and this level of condemnation has been reported from MD in broilers.

The main means of spread of leukosis/sarcoma viruses is from the parent to the offspring through the egg. Horizontal transmission is important only in the very first few days of life. Thus, a high rate of congenital infection of genetically susceptible chickens with a virulent virus combined with an early rapid spread of infection in the incubator and brooder could result in a high mortality from LL.

In MD, transmission through the egg is exceedingly rare or does not occur at all. Most chicks become infected in the first few weeks of life by airborne virus or by virus from a contaminated environment. Early infection of genetically susceptible chickens under conditions which allow rapid horizontal airborne transmission may result in heavy losses from MD.

Prevention

Since LL is spread vertically, the only way to eliminate the infection is to break the egg transmission cycle. This may be accomplished by (a) testing eggs from breeding hens and eliminating those breeders found to be egg-transmitting the virus; (b) hatching small groups of birds from basic breeding lines and later

discarding all groups in which tests show infected birds and; (c) testing breeders for resistance to infection and employing only those which can not become infected with the viruses. Obtaining chickens from stocks that have had little or no loss from LL probably because they are genetically resistant or have a low level of viremia and rearing chicks in a sanitary environment are the best methods to keep the incidence of the disease down.

Large groups of chicks may be hatched free of MD virus, however, the virus is so highly infectious that almost all flocks become infected within the first few weeks of life. The usual method for prevention of disease, i.e. obtaining chickens from healthy resistant genetic stock and rearing them in a clean environment, have not always prevented losses from this disease. However, vaccines are now available and it is possible to prevent severe losses from MD by vaccination of chickens at the time of hatching and rearing them in a sanitary environment.

Differential Diagnosis

Since both viruses are ubiquitous and often persist in the host for long periods of time, virus isolation and serologic studies are of no assistance in distinguishing between the two diseases. The diagnostician must rely almost entirely on history, clinical examination and gross and microscopic lesions. The differences between LL and MD are outlined in Table 1. The reader will find it useful to have this table available while going through the slides. For each characteristic below, LL will be

discussed first and it will be followed by MD.

Anamnesis.

Although LL occurs almost exclusively in birds older than 16 weeks of age, MD may occur in chickens at any time after they are about 4 weeks of age. Between 4 and 16 weeks of age, a differential diagnosis between LL and MD is no problem. However, it is after that time that the two diseases may occur simultaneously in a flock.

Clinical signs.

In LL a greatly distended abdomen and penguin-like stance may occur. However, paresis or spastic or flaccid paralysis are usually absent. In MD, although paralysis is almost always present in a few birds in a flock, every bird is not paralysed. In more acute outbreaks where the viscera are predominately affected, there may be no paralysis. Birds are often dehydrated and depressed as in LL.

Gross lesions of bursa.

Unless complicated by simultaneous MD the nerves are not involved in LL. The liver and spleen often have large focal or diffuse lymphoid tumors and various other visceral organs may be involved simultaneously. The most important feature of LL is that focal or nodular bursal tumors are present in over 95% of the cases. Bursal tumors are occasionally 10 cm in diameter, however, they are often very small (1 to 2 mm in diameter) and the bursa may require careful dissection in order to detect them.

Slide 1. In LL, tumors of the bursa are always nodular and

focal in nature but there may be multiple tumors. There is a distinct nodular tumor at the point of the forceps in this picture and multiple tumors on the right hand side. On the left hand side are the normal plica of the bursa.

Slide 2. In MD there is often a pronounced atrophy of the bursa of Fabricius. The bursa on the left is normal whereas the one on the right is from a bird with MD. Bursal atrophy is particularly noticeable in young birds but is not pathognomonic for MD since it may occur with other infections, e.g. infectious bursal agent. Also, atrophy occurs in most normal birds at approximately sexual maturity.

Slide 3. In MD very occasionally tumors may be present in the bursa. In contrast to the nodular focal tumors of LL, those of MD are characteristically diffuse involving the entire plica and particularly the wall of the bursa. The bursa on the right has diffuse tumorous involvement of several plicae. There are several focal areas of necrosis which should not be confused with focal tumors. The bursa on the left is from an unaffected bird of about the same age.

Microscopic lesions of bursa.

Slide 4. (Methyl green pyronin stain. Magnification X 25. The first sign of LL is in the bursa of Fabricius where histological transformation of the cells of a single follicle may occur as early as 12 weeks. A transformed follicle with larger, more pryoninophilic (or eosinophilic) cells, particularly in the cortex, is shown at the top of this slide. The remaining follicles

are normal.

Slide 5. (Hematoxylin and Eosin (H & E) stain X 10). The transformed follicle of LL enlarges until it may extend over large areas of the bursa. In this slide a small portion of the transformed follicle is shown at the bottom and there are many normal appearing follicles in the remainder of the bursa.

Slide 6. (H & E X 100). This is a high power view of a normal follicle on the right and a transformed follicle of LL on the left. In the transformed follicle the cells are larger, more uniform and immature in appearance.

Slide 7. (H & E X 40). In MD, the most common lesion in the bursa is a degeneration, necrosis and cyst formation of the follicles (not shown here). Occasionally there is an infiltration and proliferation of lymphoid cells between apparently normal appearing bursa follicles. This inter-follicular infiltration in MD should be contrasted with the intra-follicular transformation in LL. In MD this results in the diffuse thickening of the plica and the wall of the bursa whereas in LL there is the characteristic nodular tumor.

Gross Lesions of nerves.

The most characteristic lesion in MD is the enlargement of peripheral nerves. It is occasionally infrequent in outbreaks where there are predominantly visceral lesions. Nerve lesions are never associated with LL and in the field where reticuloendotheliosis must be disregarded, are pathognomonic for MD. Any one or more of the peripheral or autonomic nerves may be

involved.

Slide 8. Here, the sciatic plexus and nerve from a bird with unilateral MD involvement are shown. The involved nerve is enlarged, yellowish, and translucent in contrast to the glistening white unaffected nerve on the right. It is common to find selective involvement of only a few nerves.

Microscopic lesions of nerves.

In LL, since there is no involvement of the peripheral nerves or brain, most nervous tissues, except for dorsal root ganglia, are completely devoid of lymphocytes.

Slide 9. (H & E X 160). This is a section of a nerve from a chicken with MD. Based mainly on the morphology of infiltrating cells. MD lesions may be classified as types A, B, or C. The type A lesion observed in this slide occurs earliest in the disease and consists of an infiltration of rapidly proliferating pleomorphic lymphoid cells between the neurons. Large lymphocytes which at times may be very darkly stained (MD cells) are characteristic of this type of lesion.

Slide 10. (H & E X 160). The type B lesion seen in this slide occurs in older birds with MD and consists of an edema and infiltration with plasma cells and small lymphocytes. Sometimes, as in the type A lesion there is a demyelination and Schwann cell proliferation.

Slide 11. (H & E X 160). Type C lesions, the mildest type, occur in mature birds with MD that are often clinically normal. There is only a light infiltration with small lymphocytes and

occasional plasma cells. In the central nervous system, i.e. brain and spinal cord, similar infiltrations and proliferations may occur, however, they are often difficult to distinguish from those of Newcastle disease and avian encephalomyelitis.

Gross lesions of viscera.

The liver is frequently involved in both LL and MD and it is not possible to distinguish between the 2 diseases on gross examination of the liver. Also, in both LL and MD many other visceral organs may be involved.

Microscopic lesions of viscera.

Slide 12. (H & E X 400). Beginning at about 16 weeks of age the transformed cell of LL in the bursa metastasize to other organs of the body, e.g. the liver. The cells retain their characteristic uniformity and an aplastic nature. They have a large cytoplasm and a vesicular nucleus with one or more highly prominent magenta staining nucleoli. Mitotic figures are common and medium and small lymphocytes are rare.

Slide 13. (H & E X 160). When post-mortem decomposition has set in LL tumors, there is often a pronounced karyorrhexis of the lymphocytes.

Slide 14. (H & E X 160). The cells involved in all lesions of MD, including the liver shown here, are highly pleomorphic lymphoid cells. They consist of large, medium and small lymphocytes. The large lymphocytes with the abundant cytoplasm, vesicular nuclei and prominent nucleoli are in the minority. Medium lymphocytes with chromatin in clumps throughout the nucleus

and a less abundant cytoplasm and small lymphocytes with the darkly stained nuclei and minimal cytoplasm are in abundance. Mitotic figures are frequent and should not be confused with small lymphocytes and/or MD cells.

Slide 15. (H & E X 400). In MD large lymphoblastic cells with very dark staining cytoplasm and often a clear area surrounding as if they had retracted during fixation occur sporadically in nerves and tumors, e.g. of the lung shown here. They are referred to as MD cells and are usually common in this slide. There is still a predominance of small and medium lymphocytes.

Slide 16. (H & E X 160). After post-mortal decomposition of tissues with MD lesions has set in, nuclei of lymphoid cells tend to undergo pyknosis in contrast to LL where Karyorrhexis occurs. In MD the pleomorphic nature of the lymphoid cells can still be seen by the variation in size of nuclei and cell outlines.

Lesions of the skin, muscle and eye.

In LL skin infiltration with lymphoid cells may occur on a small scale and may be due to concurrent MD virus infection. Tumors in LL are very rarely found in the subcutaneous tissue.

Slide 17. One of the most common causes of condemnations in poultry is MD tumors of the skin. In an advanced case, as is shown here, most of the feather follicles over the entire body may be enlarged due to lymphoid cell infiltrations. However, only one or a few feather tracts may be affected. Sometimes there are large subcutaneous lymphoid tumors such as the one at the base of the

comb in this chicken.

Slide 18. (H & E X 40). In MD lesions of the skin lymphoid infiltrations in the subcutis are often prominent and characteristically follicular. They are increased in amount and the cells contain more lymphoblasts than the lymphoid aggregations in the skin of normal birds. In LL, tumors of the muscle are very rare. However, they are not uncommon in chickens with MD.

Slide 19. The deep pectoral muscle of this chicken has a large lymphoid tumor of MD.

Slide 20. Ocular lesions, as shown here, are generally considered to be a sign of MD. Usually there is a lymphoid infiltration of the iris which causes a white discoloration, the pupil is often irregular and does not respond to changes in light intensity.

Using the above criteria it is possible to make a satisfactory differential diagnosis on almost all cases. There will always be occasional chickens in which one will not be able to distinguish between the two diseases based on the above criteria. The number of these should decrease with increasing experience in the field. Also, there are the rare instances where lymphoid leukosis and Marek's disease occur simultaneously in the same bird. Statistically and practically these instances are so rare that they do not create a diagnostic problem.

Table 1. Differential Diagnosis between Lymphoid Leukosis and Marek's Disease.

	Lymphoid Leukosis	Marek's Disease
Age of onset	16 weeks of age	4 weeks of age
Clinical signs		
Paralysis or paresis	Absent	Usually present
Gross lesions		
Peripheral nerve and ganglion involvement	Absent	Usually present
Bursa of Fabricius	Nodular tumors	Diffuse enlargement or atrophy
Skin and muscle tumors	Usually absent	May be present
Microscopic lesions		
Peripheral nerve infiltration	Absent	Present
Cuffing in white matter of cerebellum	Absent	Present
Skin infiltration with follicular patterns of lymphoid cells	Absent	Present
Cell proliferation in bursa of Fabricius	Intra-follicular	Inter-follicular
Cytology of lymphoid cells	Uniform "blast" cells	Pleomorphic mature and immature cells

References for further reading.

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Acknowledgements.

The authors thank Dr. M. Peckham for slide 8, Dr. F. J. Siccardi for slid 12 and Dr. R. L. Witter for slide 14.