Chapter 3

Macroscopic Pathology of the Lymphoreticular System of Ruminants

Philip W. Ladds

In common with the circulatory and nervous systems, the lymphoreticular system (LRS) consists of central, and less conspicuous but equally important peripheral components which are present to a greater or lesser degree, in almost all organs throughout the body of normal animals. Also, tissues of the LRS can arise *de novo,* as and when required, at sites of chronic inflammation. It is important to always be aware of this ubiquitous presence of the LRS because diagnosis of diseases primarily affecting it, such as lymphosarcoma, are often made, or at least suspected when changes are seen first in other, non-lymphoid, organs.

Essentially, the LRS consists of an intimate association of lymphocytes with reticular cells and fibres - the so-called macrophage phagocytic system whose primary role is to recognise, trap and process antigens and "present" them to lymphoid elements so that immune reactions may proceed.

The major, central, components of the LRS are the thymus, spleen and haemolymph nodes, the lymph nodes, and the tonsils.

Thymus

Age-associated changes

In normal animals thymus weight is maximal in the very young or up to puberty, after which there is progressive involution. Studies in cattle have confirmed that in contrast to other LRS tissues, thymus weight-bodyweight ratio decreased progressively from birth (Figure 1). Irrespective of the degree of involution, however, some remnant of the thymus always remains – although this author's experience is that it may not always be possible to find! In age-associated atrophy of the thymus, functional tissue is replaced largely by fatty tissue although cysts filled with serous fluid may also develop.

Haemorrhages

The thymus, perhaps because of its pale colour, is frequently recognised as a conspicuous site of haemorrhages – especially petechiae and ecchymoses. The causes of such haemorrhages are varied and include septicaemia (eg leptospirosis, listeriosis) or toxaemia (enterotoxaemia, cyanobacterial poisoning).

Developmental disturbances

There are early reports of accessory thymus tissue, associated with the thyroid gland in both cattle and sheep. Also, a more recent report describes large (4 cm diameter) bilaterally symmetrical masses of accessory thymus located at the angles of the mandible in a Nubian kid goat; herd history suggested the condition was heritable

Small thymus

Although there appears to be no report of thymic aplasia or hypoplasia associated with clinically apparent immune deficiency in cattle or small ruminants, profound thymic atrophy has been described in one breed of cattle in association with disturbed zinc metabolism.

Much more important is the striking atrophy of the thymus that may occur in response to "stress". Acute infection, severe injury, X-irradiation or a large dose of cortisone can all cause the thymus to shrink to half its size or less within a day or so – hence the erroneous interpretation, early in this century, of status *thymo lymphaticus* in children who died suddenly - without prior illness. The normal, large, thymus in these children was mistaken as a lesion; there had simply not been sufficient time for thymus involution to occur. Probably in disease of varied cause, rapid thymic involution reflects both a stress response (presumably cortisone mediated) as well as direct destruction of LRS components by eg toxins of infectious agents, particularly viruses.

Large thymus

Obvious enlargement of the thymus is probably most often due to neoplasia (see below). Quite significant thymic enlargement may occur in cattle after castration. Endocrine influences would seem to be involved; it is recognised that the thymus and therefore cell-mediated immunity, are influenced during development and in adult life by endocrine hormones.

Thymic hyperplasia is a diagnosis that mostly requires histopathological examination as there may be proliferation of particular elements such as follicles without any increase in overall size of the thymus. Repeated vaccination of calves, however may induce hyperplasia such that the thymus may fill the anterior mediastinum and extend up the neck.

Thymitis

Whereas swelling is an expected change in most organs in acute inflammation, for the reasons given above, thymic inflammation is probably more often associated with atrophy. Such is the case in epizootic abortion in calves, in which there is involution in spite of concurrent cellular infiltration.

Neoplasia

In contrast to the spleen or lymph nodes, the thymus contains epithelial elements; so-called thymoma is a tumour derived from such epithelium. Thymic tumours composed only of epithelium are however quite rare so in most such tumours lymphoid proliferation predominates. In general, these lymphoid tumours of the anterior mediastinum and thoracic inlet of bovine yearlings are aggressive whereas those in sheep and goats are encapsulated masses that because of their large size may cause clinical signs but do not metastasise.

Thymic lymphoma in cattle is not associated with bovine leukaemia virus infection. Swelling at the base of the neck may be massive, and the tumours, which may weigh in excess of 20 kg, can extend from the base of the heart to the rami of the mandible. There is invasion of tumour tissue into adjacent structures.

Lymphoid and lympho-epithelial lymphomas in sheep and goats are usually incidental "space-occupying" lesions found at necropsy or slaughter. They form well-encapsulated irregular masses up to ~15 cm in diameter that on section may resemble normal thymus but with prominent fibrous septae.

Spleen

Involution

As with other lymphoid organs, splenic involution occurs with advanced age such that the spleen of an old animal is firm, and is seen histologically to be composed largely of connective tissue rather than lymphoid elements. In one study in cattle for example, the spleen-bodyweight ratio (obtained by weights measured after animals had been "bled out" in an abattoir), decreased from a maximum of 38 ($x10^{-4}$) at approximately 5 month-of-age to 18 ($x10^{-4}$) in animals older than 7 years (*Figure 1*).

Atrophy of the spleen may also occur with starvation or as a consequence of protracted wasting disease. Rapid involution of the spleen may occur in fulminating disease such as bovine virus diarrhoea. Changes in overall size of the spleen in disease will reflect concurrent circulatory events and an increase or decrease in LRS parenchyma. In rinderpest, for example, there is early splenomegaly but microscopically there is marked breakdown of lymphocytes – so-called lympholysis.

Developmental disturbances

A variety of such disturbances occur rarely in ruminants. These include complete absence (aplasia), hypoplasia, duplication (in association with other defects), and accessory spleens. There is a recent report of accessory (supernumerary) spleen in an otherwise normal mature goat;

both spleens were of similar weight and were supplied independently by separate splenic vessels at the hilus.

Pigmentation

Haemosiderosis of the spleen, which results from the excessive breakdown of erythrocytes, is likely to be observed in areas where piroplasmoses are endemic. For haemosiderosis to be apparent grossly, however, very large amounts need to be present. Affected spleens are slightly enlarged, firm in consistency, and dark brown on the cut surface.

Enlarged spleen (splenomegaly), and splenitis

Essentially the spleen may be enlarged because of one or more changes, perhaps occurring simultaneously – increased blood, hyperplasia of the macrophage phagocytic system, inflammatory oedema and cell infiltration, and lymphoid hyperplasia. Although histology is needed to clarify the type of change, careful gross examination may provide some indication.

The most common cause of severe congestion of the spleen at necropsy, is when barbiturate drugs are used for euthanasia. Distension of the spleen with blood also occurs as acute hyperaemia in acute systemic infections and some acute bacterial intoxications such as in clostridial enterotoxaemia.

Of particular interest in ruminants, however, is the acute hyperaemic swelling of the spleen that occurs in anthrax, in which the reaction is almost solely vascular. Grossly the capsule is tense and encloses excessive soft pulp of tarry consistency and dark colour. Note however that this change can to some extent be caused by post mortem autolysis, so case history and caution in interpreting findings, are needed. The appearance in acute babesiosis is similar to anthrax, and in endemic areas, is largely diagnostic. In less acute conditions there is hyperplastic splenomegaly, manifested grossly as the cut surface of the enlarged spleen exuding less blood but having a more "meaty" consistency.

Such change is a feature of salmonellosis in calves, in which histology confirms that there is diffuse hyperplasia and hypertrophy of reticular elements. An enlarged spleen, "meaty" as a result of hyperplastic changes, may also be seen in bovine malignant catarrh (malignant catarrhal fever), and is particularly striking (almost to the point of resembling neoplasia) in Jembrana Disease – a lentivirus infection of Bali cattle.

Nodular hyperplasia

This condition is seen occasionally in old bulls, and was also the subject of a specific report in New Zealand where it was observed in 0.8% of aged ewes from one source. Grossly, shape of the spleen was irregular and nodular, shown microscopically to be due a mix of localised

hyperplasia of reticular elements, congestion, haemorrhage and thrombosis.

Neoplasia

Although uncommon in ruminants, no doubt because they are slaughtered before reaching "cancer age", haemangioma-haemangiosarcoma may occur and may metastasise to the liver. Lymphosarcoma may cause diffuse splenic enlargement with total weight in cattle being 50 kg or more. In one review of bovine lymphomas, the spleen was involved in 30% of cases. Rupture and haemorrhage are likely complications.

As in other species, and presumably because of the peculiar vascularity of the spleen, it is, in ruminants, an uncommon site for secondary neoplasms. Superficial (capsular), implants of squamous cell carcinoma and other neoplasms, are however, observed.

Haemolymph (haemal) nodes

These nodes which are often intimately associated with lymph nodes, are typically found only in ruminants and rats. Although there is debate about communications between lymph and blood vessels in these nodes, there are probably no such connections.

Haemolymph nodes have germinal centres and because they filter blood, one expects their function and pathology to parallel that of the spleen. Marked enlargement of these nodes occurred in experimantal *Trypanosoma vivax* infection in sheep and goats. Haemangiosarcoma in haemal lymph nodes of sheep has also been observed.

Lymph nodes

The normal node:

There is considerable variation in the size, colour and shape of lymph nodes in apparently normal animals and such variation is, to a large extent, unexplained. Because of this, one has to recognise a "range of normality", particularly if nodal changes are generalised throughout the body. In interpreting more localised changes, however, comparison of the node in question with its opposite, contra-lateral node, is especially useful.

Some variation in nodal morphology can be related to "non-disease" factors such as age, anatomical location and physiological state. The nodes of foetuses and neonates are pale and lack the irregular capsular contours of nodes in older calves, indicative of follicular development.

Dark pigmentation of the deeper, medullary regions is a feature of bovine nodes. With increasing age the amount of pigment (probably the "wear

and tear" pigment, lipofuscin) increases in all nodes but is most pronounced in the mesenterics, probably because of material draining directly from the gut.

Deposition of a striking orange carotenoid pigment is sometimes observed in the lumbar nodes of cattle and is presumed to originate from the corpora lutea of the ovary (cows) or gut. Dark brown to black melanin pigment in nodes is mostly secondary to melanomas located in their drainage area, but may be congenital. In the case of melanoma, histology of the node is necessary to distinguish simple pigment drainage from metastasis.

Pale, lipid accumulations are not uncommon in the mammary nodes of lactating animals, and their firm nature may cause confusion with infectious granulomas. In very fat animals there may be considerable encroachment of fat into the hilar region of nodes.

General reactions of lymph nodes:
Regional lymph nodes may respond to a wide variety of stimuli so that ultimately they are quite different from their contra-lateral node or other nodes in the same animal. Clinically, and on macroscopic examination the most obvious change is increased size, but changes in shape, consistency and colour may also be observed. Perhaps unfortunately, microscopic examination is needed to ascertain precisely which nodal component has proliferated. In this regard it needs to be stressed that lymph nodes are physically rather "fragile" so that if histology is to be done, the node must be handled gently (not squeezed or tugged!) and slices for fixation in formalin should be obtain using a very sharp knife, or scalpel.

In very general terms, enlargement associated with a uniformly pale colour and soft consistency is likely indicate lymphoid hyperplasia or neoplasia, whereas an enlarged node that on cutting is seen to be oedematous and have a discoloured, perhaps mottled surface, is more likely to be acutely inflamed. Also, quite extensive sclerosis with hardening of the node is a common feature of chronic change both in inflammation and neoplasia, sometimes even leading to the formation of bone, so-called osseous metaplasia.

Non-infectious, non-neoplastic changes:
Whereas the spleen has the role of filtering the blood, the lymph nodes act as filters for extravascular fluid. Non-infectious, non-neoplastic morphological changes in nodes primarily involve this filtering function.

Lipid drained from a site of injury to a regional node may appear as distinct firm foci that are pale, or yellow if discoloured by bilirubin from haemorrhage in the same area.

Emphysema, with the presence of distinct bubbles, is occasionally seen in the mediastinal nodes of cattle with interstitial pulmonary emphysema.

Although the normally pale colour of nodes facilitates the recognition of haemorrhage, additional information and histology are needed to decide whether erythrocytes (mostly in nodal sinuses) represent actual haemorrhage within the node or simply drainage from an injured area. Studies on bruising in cattle indicate that many, perhaps most erythrocytes drained to a regional node continue through the afferent lymphatics to re-enter the blood. Therefore erythrocytes that remain in a lymph node would appear to have been defective as a consequence of eg toxaemia or inflammation. Erythrocytes retained in this way are converted by macrophages in the node to the pigment haemosiderin, and this process (haemosiderosis) is detected grossly by the colour of the node, and especially its medulla, changing from red to rusty brown.

Another pigment which may result in a striking green colour of eg the parotid lymph node of calves is an exogenous one used for ear marking. Similar changes occur in nodes draining tattooed areas in humans.

Local or generalised non-inflammatory oedema of nodes that are enlarged and on cutting have a moist appearance and perhaps increased fibrosis, is occasionally observed. Impaired drainage from the node, possibly due to damaged afferent lymphatics, would seem to be the cause.

Lymphadenitis:

As explained above under 'general reactions', microscopy is needed to determine the cause of nodal enlargement but such enlargement seen grossly to be associated with suppuration, oedema and increased "redness" due to active hyperaemia will suggest *acute lymphadenitis*. This is especially so if similar changes are observed in any primary lesion within the drainage area. It is important to note that acute inflammation of the parenchyma of the larger nodes in cattle (and presumably small ruminants) may not be uniform; this is because afferent lymphatics to different parts of the node originate from different body regions. The superficial cervical (prescapular) node for example, is considered to be a composite of at least four nodes, each draining separate, large areas.

As discussed in relation to the spleen, changes in acute inflammation will be a combination of fluid and cellular accumulation (to dilute and remove the irritant) and purely immunological events – both of which can occur with great rapidity such that inflamed nodes can treble in size in several days. Rarely, however, the gross appearance my be suggestive of a particular pathogen. Acute lymphadenitis in anthrax is diffuse, exudative and haemorrhagic. Similar changes are observed in both lymph node and peri-nodal tissue in infection with *Clostridium septicum* but frequent gas bubbles are an additional finding.

In *sub-acute lymphadenitis* continued nodal enlargement is most likely to represent a balance between inflammation per se and immunological responses; the gross appearance of these lesions is unlikely to be suggestive of any particular pathogen.

With subsequent development of lesions however, and progression to *chronic lymphadenitis*, observed changes are more likely to be of value in diagnosis. Essentially the lesion may localise to form an abscess – with varying degrees of capsular fibrosis and inspissation of necrotic debris (such as in caseous lymphadenitis in sheep and goats), or a granulomatous response will result. Fortunately, the microscopic, and sometimes the gross appearance of the so-called "infectious granulomas" are sufficiently characteristic to permit precise diagnosis – except in old, largely resolved lesions.

Included among the causes of infectious granulomas are microorganisms such as *Rhodococcus equi, Propionibacterium* sp., *Nocardia-Streptomyces,* to so-called "club-forming" granulomatous organisms (*notably Actinobacillus lignieresi), Mycobacteria* spp., *Brucella* spp., fungi and algae, and parasites ranging from protozoa through helminths to pentastomes. These specific infections will be illustrated.

More difficult to diagnose and categorise than those diseases mentioned above is the response of lymph nodes to several viral pathogens in cattle. In Malignant Catarrhal Fever, and even more so in Jembrana Disease, the nodal response to infection is essentially enlargement with a massive proliferation of lymphoid cells, which in many respects resembles neoplasia.

Neoplasia:

Once again, lymph node changes in neoplasia are usually detected as enlargement. If gross examination of the enlarged node reveals that the shape, colour and consistency of the node are more or less normal, the immediate problem is to decide whether the change is neoplasia, and in particular lymphoma, or reactive hyperplasia. Knowledge of any associated lesion in the drainage area of the node, or elsewhere in the body, will usually help resolve this question..

Typically, however, neoplastic proliferations in a lymph node will appear as nodules which distort its shape from normal, which bulge on cutting and tend to compress adjacent lymphoid tissue, and have a consistency and colour which is different from normal node. This appearance of course may vary considerably depending on the age and degree of malignancy of the lesion, and concurrent inflammation, necrosis and haemorrhage. Particularly in the absence of any primary lesion, it may be impossible on gross examination to distinguish between a neoplasm and a granuloma.

Neoplasia in lymph nodes may be primary but is more often secondary, reflecting the importance of nodes as filters of lymph from their drainage areas.

Lymphoma is a general term applied to any *primary neoplastic disorder* of lymphoid tissue; the terms lymphosarcoma and malignant lymphoma are used to describe those tumours which are clearly malignant. It may

be impossible on gross examination, and often very difficult histologically to differentiate these tumours from reactive hyperplasia.

The presence and type of *secondary tumours* in lymph nodes are somewhat easier to diagnose. This is because a less diffuse and more nodular appearance of lesions with a consistency and perhaps colour contrasting with normal node can be expected. Even though most farm livestock are slaughtered before reaching "cancer age", an impressive variety of tumours forming metastases in lymph nodes is described. These include melanomas, squamous cell carcinoma (SCC) and adenocarcinoma (especially of uterine origin), neurofibroma, and heart-base tumours. Especially because the keratinised masses (epithelial "pearls") in metastatic SCC may induce a foreign body type granulomatous response, they may, even histologically, be mistaken for granulomas of infectious origin, so history, and knowledge of the primary lesion are important.

Age-associated changes in bovine lymphoid system

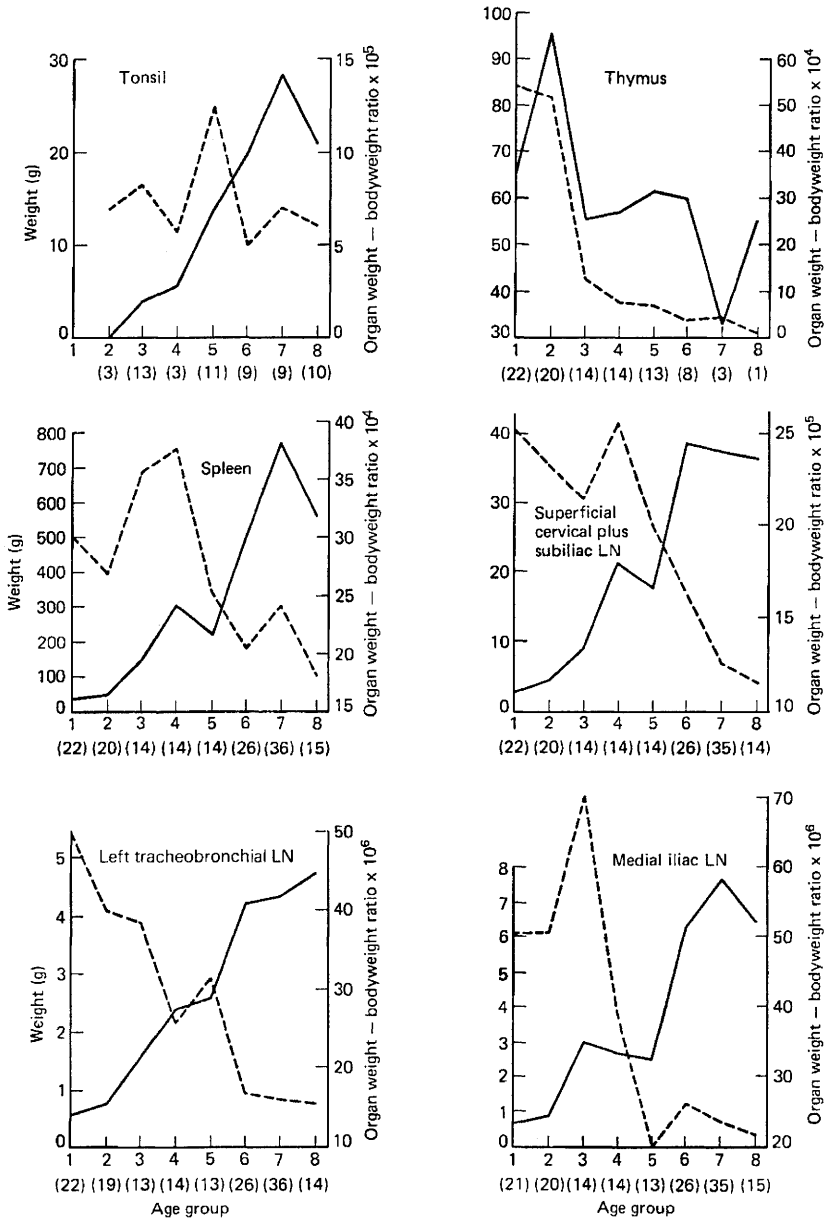

FIG 1: Changes in organ weights (————), and organ weight-bodyweight ratios (— — — —) in relation to age. Approximate ages were groups 1 and 2 — 'young' and 'mature' fetuses, respectively; groups 3, 4 and 5 — calves newborn to one month, four to six months, and seven to nine months, respectively, and groups 6, 7 and 8 — heifers or cows nine months to three years, four to six years and seven to nine years, respectively. Figures in brackets indicate number of animals within each age group