

The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2005-2006

CONFERENCE 15
8 February 2006

Conference Moderator: LTC Terrell Blanchard, DVM, Diplomate ACVP and ACVPM
Armed Forces Institute of Pathology
Washington D.C.

CASE I – 457966A (AFIP 2992393)

History: Five ewes were observed to have loss of appetite, poor body condition and progressive worsening respiratory distress. One of the ewes was euthanized, the clinician performed the necropsy and submitted lung samples to the pathology department at Kimron Veterinary Institute in Bet-Dagan.

Gross Pathology: The lungs were enlarged, heavy and failed to collapse when the thorax was opened. According to the clinician there were multifocal areas of consolidated lung tissue of various sizes. The lesions were grayish-white with a firm consistency.

Laboratory Results: Marked leukocytosis (neutrophilia and lymphocytosis).

Histopathologic Description: There are multifocal areas of epithelial cell proliferation on a fine connective tissue stroma showing papillary aspects or forming acinar structures. Adjacent to this proliferation, randomly compressed alveoli are present as well as multifocal areas of lung tissue infiltrates with histiocytes and lymphocytes. There are also areas of peribronchiolar lymphoid infiltrates and bronchioles filled with neutrophils and macrophages as well as multifocal areas of type-2 pneumocyte and smooth muscle cell proliferation can be seen.

Contributor's Morphologic Diagnoses: 1. Lung: Pulmonary carcinoma, adenomatosis, ovine.
2. Lung: Bronchointerstitial pneumonia, suppurative with alveolar atelectasis, subacute, moderate.

Contributor's Comment:

JAAGSIEKTE

(Synonyms: Sheep pulmonary adenomatosis, Ovine pulmonary carcinoma)
Jaagsiekte (JS) is a contagious bronchiolo-alveolar carcinoma of the lungs caused by a Retrovirus.

Diseases of livestock caused by members of the family *Retroviridae*:

SUBFAMILY	HOST ANIMAL (uncommon host) *	DISEASE
Oncovirinae	Sheep, goat *	Jaagsiekte
	Cattle, sheep *	Enzootic bovine leukosis
Lentivirinae	Sheep, goat *	Maedi- visna
	Goat, sheep *	Caprine arthritis-encephalitis
	Horse, donkey	Equine infectious anemia
Spumavirinae	Various animals	Non- pathogenic, "foamy viruses" in cell culture

Brief history and Etiology: First recognized in South Africa more than a century ago. A detailed macroscopic pathology was published by Hutcheon in 1891, with evidence of infectivity and attempts to control it, followed by reports from England, Germany, and France.

The disease is now known to have an almost worldwide distribution and to have considerable economic impact in countries with substantial sheep populations. In South Africa, a survey over a 40-year period indicated that JS is by far the most common neoplasm reported in sheep (64 percent). (7)

Even though JS was known to be contagious long before its neoplastic nature had been elucidated, many years of research in various countries failed to reveal its causal agent, although filtration experiments suggested a viral aetiology.

The first successful experimental transmission of the disease by co-habitation was reported by De Kock. An ovine herpes virus was the first candidate virus isolated, but transmission and molecular hybridization experiments proved that this virus is a passenger and not directly involved in the aetiology of the disease.

The possibility of a retroviral aetiology was first suggested by the observation in Israel of typical retrovirus particles in adenomatous lesions. (1) Retroviruses were also found in cell cultures established from JS-affected lungs, and reverse transcriptase (RT) activity in extracts of tumor tissue. No transmission attempts were made in these studies and the results were difficult to interpret in view of the possible presence of maedi-visna lentivirus in the material studied.

Several lines of evidence (transmissions studies, serological studies, electron microscopy, genomic clone and sequence of a JSRV and genomic organization typical of type D and B retroviruses shown and the evidence that JRSV was found to be consistently, specifically and absolutely associated with JS) cumulatively implicated a retrovirus called Jaagsiekte Sheep Retrovirus (JSRV) in the aetiology of JS.

The above evidence did not prove unequivocally that infection with JSRV is sufficient to induce JS, and the possibility existed that JSRV was acting as a helper virus, complementing a hitherto undiscovered acutely transforming retrovirus.

In 1996, Palmarini and co-workers constructed an infectious molecular clone derived from an integrated proviral exogenous JSRV sequence (called JSRV-21) isolated from a spontaneous case of JS. Concentrated stocks of JSRV-21 obtained from transfected 293T cells were used for intratracheal inoculation of new born-lambs. Two of the lambs developed JS (confirmed at the histological, immunological and molecular level), thus proving conclusively that JRSV is both necessary and sufficient to induce the disease.

Jaagsiekte sheep retrovirus exists in two closely related but molecularly distinct forms. The first is an infectious exogenous retrovirus which is transmitted horizontally from one animal to another and is the aetiological agent of JS. The second is a group of endogenous retroviral loci that resemble exJSRV at the nucleotide level, but are transmitted/ inherited vertically through the germ line. These endogenous sequences will be referred to as enJSRV. enJSRV are widely distributed in ungulates, with 15 to 20 copies of closely related sequences being found in the genomes of all domestic sheep and goats. Three different loci have been cloned and completely sequenced. No sheep population that lacks enJSRV has been identified, and the possible role of these sequences in disease, as well as their importance to the host organism, has yet to be elucidated. Jaagsiekte is associated absolutely with its aetiological agent exJSRV, whereas enJSRV by themselves are not associated with disease. (1,2,3,7,9)

Epidemiology: Jaagsiekte occurs in sheep as a sporadic or endemic disease in all continents of the world where sheep occur, except in Australia. It displays an extremely variable prevalence between countries. Natural disease with a low prevalence does occur in goats in some countries, but it has not been diagnosed in this species in southern Africa (it has been experimentally transmitted to kids in South Africa). It would appear that goats are less susceptible to infection by JSRV than are sheep. Transmission studies in Europe have provided similar results and in Ireland, during the epidemic of JS in the 1930s and 1940s, JS was not seen in goats despite close contact with sheep suffering from the disease. Jaagsiekte is endemic throughout South Africa and Namibia, and observations suggest that more animals die as a result of the disease in winter or after sudden drop in temperature, possibly as a result of secondary bacterial pneumonia.

Outbreaks of JS occur when affected sheep are introduced into clean flocks. In Ireland it was observed that approximately 5.5 to 8 months elapsed between the introduction of an infected animal into a "clean" flock. It seems that there are breed differences in susceptibility: Iceland- Gottorp breed were far more susceptible than Adalbol breed. In the U.K, there is higher prevalence in Scotland than in England. In South Africa; Merino and Karakul sheep and their cross-breeds are apparently more susceptible than are English breeds. When these genetic differences were recognized, farmers were urged to breed from resistant families and breeds, and this led to a decrease in the prevalence of the disease. A survey in South Africa indicated that the annual mortality due to JS in infected flocks varied from less than 1 to 24 per cent (average 3.6 percent).

The sex of the animal does not seem to play a role in its susceptibility. Although sheep of all ages are susceptible to experimental infection, neonatal lambs are more susceptible than are lambs more than a few days old. It is likely, therefore, that affected sheep contract the disease at a very early age from infected dams, some of which are asymptomatic. Experimental results suggest that intra-uterine transmission does not occur. Therefore, embryo transfer has a potentially important role in creating "clean" flocks, while enabling the introduction of new genes. (1,3,4,7,9)

Pathogenesis: Experimental transmission of JS suggests that the respiratory system is the natural route of infection. The primary lesion in JS is a well-differentiated multifocal tumor that originates from the transformation of type- 2 pneumocytes and the non-ciliated bronchiolar (Clara) cells. Transformed cells, in which replication of the virus has been observed, proliferate and form clusters that invade and eventually obliterate the alveolar lumen, resulting in death due to hypoxia. The transformed cells retain their secretory function, producing large amounts of surfactant-containing, clear, viscous fluid which accumulates in the air passages and aggravates respiratory distress. It also leads to coughing and the

formation of aerosol droplets containing the virus or tumor cells, which can infect other animals. As mentioned, natural cases of JS are often complicated by secondary infections, and in such cases the immediate cause of death is usually an acute or chronic pneumonia.

A characteristic histological feature of JS is the presence of large numbers of macrophages, usually in the areas adjacent to the adenomatous lesions. Various studies of their possible role in the pathogenesis of the disease have failed to provide a clear-cut answer. On the one hand, it has been clearly demonstrated that JS tumor cells produce a chemotactic factor which stimulates proliferation of macrophages; while on the other hand, activated alveolar macrophages produce substances which stimulate the proliferation of type-2 cells. After chemotactic stimulation, macrophages have even been shown to produce both growth-stimulatory and inhibitory factors, depending on their interaction with other lymphoid cells. No evidence for activity of the alveolar macrophages against tumor cells has been found.

Another factor in the pathogenesis of JS about which little is known is the apparently close association of the disease with lentivirus infection. Lentiviruses remain latent in alveolar macrophages and replicate only after these cells are activated. The presence of large numbers of activated macrophages in JS-affected lungs may therefore create ideal conditions for lentivirus replication, possibly aided by an immunosuppression in sheep, the degree of which correlates well with the latent period before the appearance of JS signs in co-infected animals. It is possible that the lentivirus, in addition to causing a mild interstitial pneumonia, may also predispose animals to secondary infections, including JS. (2,7)

Morphology and morphogenesis of the virion:

Mature virions have an average diameter of 110 nm and, when negatively stained, appear as pleomorphic, enveloped particles with spikes on the surface, typical of most retroviruses. The morphology of positively stained JSRV is distinct from that of any other retrovirus. It possesses a slightly eccentric nucleoid with an electron-dense perinucleoidal space. The virus has a density of 1.17 to 1.18 g/ml, similar to that of mouse mammary tumor virus (MMTV) and Mason-Pfizer monkey virus (MPMV), respectively type B and type D retroviruses. Replicating JSRV particles are occasionally seen in tumor cells. Immature particles, 75 nm in diameter, assemble in the cytoplasm and are then transported to the cell membrane, where budding of mature particles takes place. The morphogenesis is similar to that of types B and D retroviruses, but is quite distinct from that of type C oncoviruses (e.g. bovine leukemia virus) and lentivirus (e.g. maedi-visna virus), where the virions are assembled at cell membrane in a crescent shape before budding as an immature particle. (5,7)

Oncogenic potential of exJSRV: The mechanism of transformation in JS has not yet been elucidated. Owing to the recent discovery that the JSRV provirus had integrated into a part of the sheep genome that does not align with any known cellular sequences (including proto-oncogenes), the possibility of insertional mutagenesis being the transforming mechanism is strongly indicated. JSRV clone was able to induce JS by itself, in the same time frame as field isolates; exJSRV is not a helper virus for an acutely transforming retrovirus. It was concluded that the genome of exJSRV itself harbours its oncogenic potential.

Hecht and his co-workers suggested three possible alternatives:

1. One of the exJSRV protein products may still lead to cellular transformation.
2. One of the viral products may act as a transcription factor to stimulate a cellular oncogene; exJSRV may harbour a more potent activator than those found thus far.
3. Binding to the type-2 pulmonary epithelial cell receptors by their retroviral ligands could result in signal transduction, thereby stimulating the production of growth factors, followed by cell proliferation and subsequent neoplasia. (6,7)

Clinical signs, Pathology and Histopathology: In natural disease, the incubation period has been estimated approximately five to six months, with two- to four-year old sheep being most commonly affected. Clinical disease is therefore rarely seen in lambs under the age of six to nine months.

The onset of clinical signs is insidious in natural cases. Initially, while the habitus is normal and the condition of the animal is good, the respiratory rate is more rapid than normal after an animal has been driven. As the disease progresses, affected animals have reduced appetite, lose body weight, and lag behind the flock when it is driven. Marked respiratory distress is evident on exercise, the respiratory movements being short and jerky. Tachypnoea and dyspnoea eventually become evident even at rest. Spasmodic bouts of coughing occur and there is a great increase in the amount of secretion from the lungs. This is regarded as almost pathognomonic for JS. Moist rales are heard on auscultation of the thorax, sometimes even without the aid of a stethoscope. There is generally no fever, but pyrexia may occur as result of pneumonia following secondary bacterial infection. Average duration of clinical disease is two months, with a range of a few days to six months. There are cases on record where affected animals have survived for more than a year. Presence, nature and extent of secondary bacterial pneumonia and the environmental conditions under which the animal is kept are important factors in the variations in the duration of clinical signs. Once clinical signs manifest, the disease is irreversible. (1,3,7,8,9)

No consistent hematological abnormalities have been reported in animals suffering from JS. However, increased blood levels of IgG occur in cases with advanced

lesions, and these have been ascribed to the presence of numerous plasma cells in the lungs.

The presence of secondary lesions in the lungs often obscures those due to the primary condition and renders the diagnosis of the disease tenuous on gross pathological evidence alone. In advanced cases of JS, the lungs are three or more times their normal weight and almost fill the thoracic cavity. They do not collapse when the thorax is opened. Both lungs are usually involved, but not necessarily to the same extent. The cranial and middle lobes and the cranial part of the caudal lobes are commonly affected, but any part of the lung may be involved. Nodules and areas of diseased tissue of various sizes (less than one millimeter in diameter) are sometimes scattered throughout the normal lung tissue. The lesions consist of very dense tumorous tissue that is grayish-white with a relatively firm consistency due to fibroplasia, which in some cases can be extensive.

The distribution of lesions suggests that the primary lesion or lesions grow by expansion. Intrapulmonary spread of the infection with the development of new foci probably occurs both acrogenously and via the lymph and blood stream. Each new focus is seen grossly as a small grayish-white, semi-transparent nodule which at first is barely visible to the naked eye. As it expands it may coalesce with neighboring nodules and, in this way, large lesions eventually develop. Intra- and extrathoracic metastasis of the tumor may occur. In South Africa extrapulmonary metastasis is rare, whereas it frequently occurs in sheep of the Awassi breed in Israel, where metastases both within and outside the thorax may be found in 30 to 50 percent of cases. This variation may also be linked to different viral strains. The most frequent metastasis sites are the bronchial and mediastinal lymph nodes (approximately 10 percent), peritoneum, skeletal musculature, liver, spleen, kidneys, heart and mesenteric lymph nodes. Spread to visceral and parietal pleura may also occur. (1,3,7,8,9)

Ovine pulmonary carcinoma (OPC) lesions are those of well-differentiated, multicentric, bronchioloalveolar carcinoma. The tumor grows as a simple cuboidal to columnar epithelium, (early stages of tumor development where alveolar type-2 cell proliferation is the initial stage), on a thin connective tissue stroma forming acinar or papillary architecture. These masses tend to compress adjacent alveoli and may be associated with a moderate lymphohistiocytic alveolar infiltrate. Continued proliferation obscures this pattern, and fibroplasia often occurs in more disorganized and degenerative regions. The papillary proliferations involve both alveoli and bronchioles in many nodules.

Lesions of OPC must be differentiated from progressive pneumonia (lentivirus). While both can have type-2 cell hyperplasia (papillary proliferations of cuboidal and columnar epithelium) and interstitial fibrosis in the absence of significant interstitial

pneumonia , the ovine lentivirus-induced lesion has a prominent peribronchiolar and perivascular lymphoid inflammation and lacks the papillary alveolar ingrowths of epithelium present in early OPC lesions. (Dual infections with JSRV and ovine lentivirus are present in many flocks!). In some cases, the connective tissue stroma is very prominent and may proliferate to form thick layers between the tumor acini or beneath the pleura. This stroma generally consists of mature collagen but may appear myxomatous. There may be evidence of exudative pneumonia due to secondary invaders, represented by varying amounts of a fibrinous or purulent inflammatory reaction which may obscure the primary lesion. Abscesses and pleuritis may be present.

Ultrastructural characterization of the component epithelial cells shows a predominance of alveolar type-2 cell morphology with microvilli, desmosomes, and intracellular lamellar bodies, whereas the columnar cells have secretory granules and glycogen compatible with origin from secretory bronchiolar epithelial cells (Clara). Cytoplasmic dense bodies suggestive of Clara cell differentiation can also be present in some cases, and these cells may coexist with cells of type-2 cell morphology. Most neoplastic cells originate from granular type-2 pneumocytes, are well differentiated on the periphery of the lesion, contain many free polysomes and show nuclear margination, indicating that they are in a state of rapid protein synthesis and cell division. Intracytoplasmic JSRV particles, when seen, are often associated with centrioles, suggesting that the virus replicates in actively dividing cells. Nuclei and mitochondria are often abnormal in the malignant cells. In the cytoplasm, numerous secretory granules contain varying amounts of osmiophilic material and myelinoid bodies. These granules are associated with surfactant production and are responsible for the large amount of lung secretion characteristic of JS. (1,3,6,7,8,9)

Differential diagnosis, Diagnosis and Control: In the early literature, clinically and pathologically, JS was often confused with various forms of chronic pneumonia. Pasteurellosis, in South Africa is the most commonly encountered lung condition in sheep, and is also the most common secondary complication in cases of JS. Other organisms causing lung lesions include *Arcanobacterium (Actinomyces) pyogenes*, *Corynebacterium pseudotuberculosis*, *Mycoplasma* spp., and lungworms. In countries where maedi (synonyms: zwoegerzidkte, bouhite Montana progressive pneumonia, and Marsh's progressive pneumonia) occurs, which is caused by a lentivirus and characterized by chronic, nonneoplastic inflammatory lesions must also be differentiated from JS. Today it is known that the two diseases are closely associated in many countries and are often present as co-infections in the same animal, which adds to the existing confusion. (1,3,7,9)

The clinical diagnosis of JS cannot be made with certainty, especially in the first case to be encountered in a flock. A history of long-standing, progressively worsening respiratory distress syndrome in a flock, coupled with the results of

clinical examination of affected animals, should alert one to the possible presence of JS. Up-ending an affected animal by its hind legs (often called the wheel-barrow test) may, in cases with extensive lung pathology, induce the flow of a clear viscous fluid from the nostrils, supporting a diagnosis of JS.

The diagnosis of JS must, at least initially, be confirmed by histopathological examination of the lung lesions. Several specimens of diseased tissue from different locations should be collected because lung neoplasia may be obscured, both macroscopically and microscopically, by secondary pneumonia.

The absence of circulating antibodies means that classical serological methods used as an indirect means to confirm infection cannot be used in the case of JS. The absence of a humoral response to exJSRV probably precludes the possibility of effective vaccination. Tools such as the complete nucleic acid sequence and an infectious clone are now available. With the complete genome sequence of both exogenous and endogenous viruses known, it should be a formality to identify those genes/ antigens that can be used to screen specifically for the presence of the exJSRV.

Eradication is not economically feasible. The prevalence in a flock can be reduced to less than 1 per cent (under southern African conditions) by strict isolation and removal of animals showing early clinical signs. If the lambs of infected ewes are eliminated together with their dams, the prevalence of JS can be further reduced. The probability that the etiological agent does not cross the placental barrier enhances the possibility of building up a clean flock by either embryo transfer or by removing lambs from their dams by caesarian section or immediately after birth and subsequently raising them in a disease-free environment. As natural infection by droplet inhalation, it requires close contact and retroviruses are relatively unstable when the atmosphere is dry and temperatures high. Good management practices can contribute considerably to the control of the disease. (7)

AFIP Diagnoses: 1. Lung: Carcinoma, multicentric, breed not specified, ovine.
2. Lung: Pneumonia, bronchointerstitial, suppurative and histiocytic, chronic, diffuse, severe with peribronchiolar lymphoid infiltrates.

Conference Comment: The contributor provides a thorough overview of jaagsiekte. Attendees discussed the etiology of the bronchointerstitial pneumonia and considered a secondary bacterial infection as well as concurrent infection with ovine lentivirus (Maedi) as plausible causes. In particular, interstitial pneumonia with peribronchiolar lymphoid infiltrates is a characteristic finding in cases of ovine lentiviral pneumonia (Maedi). As noted by the contributor, jaagsiekte and maedi can occur in the same flock and often complicate the diagnosis.

The family *Retroviridae* includes many viruses of veterinary importance. All retroviruses contain reverse transcriptase (RNA-dependant DNA polymerase) which enables reverse transcription of virion RNA into double-stranded DNA. The retrovirus genome encodes three major genes: The group-specific antigen (gag) gene; the reverse transcriptase or polymerase (pol) gene; and the virion peplomer proteins or envelope (env) gene. Some retroviruses, such as the lentiviruses, also encode several other accessory genes (10).

A list of some of the more important retroviruses in man and animals and the genus they belong to follows (10):

Retroviridae

- *Alpharetroviruses*
 - Avian leukosis, carcinoma and sarcoma viruses
 - Avian myeloblastosis viruses
 - Rous sarcoma virus
 - Duck spleen necrosis virus
- *Betaretrovirus*
 - Mouse mammary tumor virus
 - Ovine pulmonary adenomatosis virus (Jaagsiekte)
- *Gammaretrovirus*
 - Feline leukemia virus
 - Feline sarcoma virus
- *Deltaretrovirus*
 - Bovine leukemia virus
 - Simian T lymphotropic viruses
 - Human T lymphotropic viruses 1 and 2
- *Epsilonretrovirus*
 - Walleye dermal sarcoma virus
- *Lentivirus*
 - Human immunodeficiency viruses 1 and 2 (HIV)
 - Simian immunodeficiency viruses (SIV)
 - Maedi/visna virus
 - Caprine arthritis-encephalitis virus (CAEV)
 - Feline immunodeficiency virus (FIV)
 - Equine infectious anemia virus(EIA)
 - Bovine immunodeficiency virus (BIV)
- Spumavirus
 - As mentioned by the contributor, these are called “foamy viruses” and are not known to cause disease but can contaminate cultured cells

For a more complete listing of retroviruses in animals, readers are encouraged to review reference 10.

Contributor: Ministry of Agriculture and Rural Development, Veterinary Services, Kimron Veterinary Institute, PO Box 12, Bet Dagan, Israel 50250.

References:

1. K. Perk. Slow Virus Infection of Ovine Lung. *Advances in Veterinary Science and Comparative Medicine*. 1982; 26: 267- 287.
2. M. Palmarini, J. Michael Sharp, Marcelo De Las Heras, and H. Fan. Jaagsiekte Sheep Retrovirus Is Necessary and Sufficient To Induce a Contagious Lung Cancer in Sheep. *Journal of Virology*. 1999; 73: 6964- 6972.
3. J. G. Wandera. Sheep Pulmonary Adenomatosis (Jaagsiekte). *Advances in Veterinary Science and Comparative Medicine*. 1971; 15: 251-283.
4. B. N. J. Parker, A. E. Wrathall, R. W. Saunders, M. Dawson, S.H. Done, R. Bradely. Outbreak of sheep pulmonary adenomatosis in the Irish republic. *The Veterinary Record*. 1989; 18: 177.
5. P. Anna- Lise, D. W. Verwoerd, Helen M. Garnett. The morphology and morphogenesis of Jaagsiekte Retrovirus (JSRV). *Onderstepoort Journal of Veterinary Research*. 1983; 50: 317-322.
6. T. A. Nobel, F. Neumann and U. Klopfer. Histological patterns of the metastases in Pulmonary Adenomatosis of sheep (Jaagsiekte). *Journal of comparative Pathology*. 1969; 79: 537- 540.
7. J. A. W. Coetzer & R. C. Tustin. *Infectious Diseases of Livestock*, second edition, Oxford, Great Clarendon Street: Oxford University Press; 2004.
8. K. V. F. Jubb. Peter C. Kennedy, Nigel Palmer. *Pathology of Domestic Animals*, 4th ed, Academic Press. INC; 1993.
9. Donald J. Meuten. *Tumors in Domestic Animals*, 4th ed, Iowa State Press; 2002.
10. Murphy FA, Gibbs EP, Horzinek MC, Studdert MJ: *Veterinary Virology*, 3rd ed., pp. 363-389. Academic Press, London, England, 1999

CASE II – WN05/945 (AFIP 2996888)

Signalment: Adult, male, breed unknown, *Bos taurus*, cattle.

History: A nodular enlargement of the mediastinal lymph node was detected during routine meat inspection at an abattoir.

Gross Pathology: Mediastinal lymph node: A circumscribed 15 mm diameter nodule enlarges one pole of the 10 mm diameter node. On section, this nodule contains multiple gritty off-white foci, 0.5 to 2 mm in diameter.

Laboratory Results: Microbiology: Mediastinal lymph node: A single colony of *Nocardia pseudobrasiliensis* was isolated.

Histopathologic Description: *On each glass slide there are two (2) tissue sections: the smaller section is undecalcified and the larger section is decalcified.*

Mediastinal lymph node (**undecalcified** smaller section): Within the circumscribed, sparsely encapsulated nodule at one pole of the node, normal node architecture is almost completely replaced by multiple mineralized foci bordered by a prominent zone of syncytial cells and epithelioid macrophages, and separated by irregular fibrous septa heavily infiltrated by plasma cells and lymphocytes. There are occasional small foci of neutrophilic infiltration associated with the mineralized foci. The margins of many of the mineralized foci comprise a mass of branching filaments of variable diameters, with the coarsest having an irregular staghorn-like appearance.

Mediastinal lymph node (**decalcified** larger section): Similar changes as in the undecalcified section, except that the branching filamentous structures bordering the mineralized foci are less coarse and therefore less distinct following decalcification.

Gram stain (**undecalcified** smaller section): No bacteria are detected. The mineralized foci and the surrounding coarse branching filaments have identical orange-brown staining. *This is consistent with the coarse mineralized filamentous structures representing mineralized 'gravestones' of an 'actinomycete' that is no longer demonstrable by the Gram stain.*

Contributor's Morphologic Diagnosis: Mediastinal lymph node: Granulomatous lymphadenitis, with mineralization and mineralized filamentous bacterial casts. Nocardiosis (*Nocardia pseudobrasiliensis*).

Contributor's Comment: The striking feature of this lesion is the staghorn-like mineralized filamentous structures at the periphery of the mineralized foci within the lymph node granuloma. Their filamentous mineralized morphology is different from the radiating club-like structures seen in the typical 'club colonies' of actinomycosis or actinobacillosis.

The significance of the single *Nocardia* sp colony isolated from this lymph node case is arguable. However the morphology of the mineralized filamentous structures surrounding the mineralized foci within the granuloma are consistent with them being 'gravestones' of an 'actinomycete' that is not club-forming.

Nocardia pseudobrasiliensis was designated as a new species in 1996, after a new taxon of *Nocardia brasiliensis*-like organisms was found in 1995 associated with cutaneous and disseminated human disease (1). It is an uncommonly recognized human pathogen, with approximately one case per year being detected in Queensland, Australia.

AFIP Diagnosis: Lymph node, mediastinal (per contributor): Lymphadenitis, granulomatous, multifocal to coalescing, marked, with mineral and filamentous bacilli, breed not specified, bovine.

Conference Comment: There are three main species of *Nocardia*: *N. asteroides*, *N. brasiliensis* and *N. otitidiscaviarum*; *N. asteroides* is the species most commonly isolated.

Nocardia are saprophytic actinomycetes that are present in most environments and produce a suppurative to granulomatous opportunistic infection. *Nocardia* sp. are Gram-positive, non-motile, aerobic, filamentous rods that are partially acid-fast. They are also facultative intracellular pathogens that survive within phagocytic vacuoles of macrophages and neutrophils by inhibiting phagosome-lysosome fusion, neutralizing phagosome acidification, resisting oxidative burst, and altering lysosomal enzymes. Most commonly, *Nocardia* sp. cause an opportunistic infection as a result of wound contamination and spread via direct extension or hematogenous dissemination. Typically, *Nocardia* sp. infections present as ulcerated nodules, abscesses and draining tracts on the distal limbs and head, especially at sites of previous wounds and injury. Occasionally, *Nocardia* sp. infections can present as a subacute to chronic respiratory infection with mucopurulent oculonasal discharge, dyspnea, diarrhea and hyperthermia. In dogs, coinfection with canine distemper virus is commonly reported.

Attendees discussed the presence of filamentous bacteria surrounding mineralized debris within the section of lymph node. Although present on H&E stained sections, the filamentous bacteria were best seen with a GMS stain performed at the AFIP. Although *Nocardia* sp. rarely form granules, as the contributor notes their morphology in this case is different from the radiating club-like structures seen in the typical 'club colonies' of actinomycosis or actinobacillosis.

Attendees developed a differential diagnosis based on those organisms which cause granulomatous to pyogranulomatous inflammation. Other etiologies considered include: actinomycosis, *Rhodococcus equi*, foreign body reactions, deep mycotic infections, mycobacteriosis, botryomycosis, or other chronic bacterial or fungal infections.

Contributor: NSW Department of Primary industries
Wollongbar Agricultural Institute, Regional Veterinary Laboratory
Bruxner Highway, Wollongbar
NSW Australia 2477

References:

1. Wallace JW, Brown BA, Blacklock Z, Ulrich R, Jost K, Brown JM, McNeil MM, Onyi G, Steingrube VA, Gibson J. New *Nocardia* taxon among isolates of *Nocardia brasiliensis* associated with invasive disease. J Clin Microbiol 1995;1528-1533.

CASE III – 1042/05 (AFIP 2988212)

Signalment: Adult, female, Nelore, cow.

History: This cow is from a Nelore herd of 85 cows, 24 calves, and 3 bulls kept on pasture with mineral supplement ad libitum. Between April 2004 and June 2005, four cows and one bull developed clinical disease characterized mostly by progressive weight loss. The cow examined in this case calved four months ago and began losing weight two months after parturition.

Gross Pathology: The cow had a marked cachexia, the apparent mucosal surfaces were pale, and there was an ulcer in the tongue approximately 2 cm in diameter. Large numbers of *Haemonchus contortus* were found in the abomasum. There was a fibrous adhesion between the liver and the diaphragm. The pancreas was whitish, firm, and had a rough surface (Figs. 1 and 2). On the cut surface of the pancreas a large number of parasites of the genus *Eurytrema* were observed (Fig 3).

Histopathologic Description: Pancreas. Several intra-ductal trematodes, and accumulation of operculated eggs into dilated ducts and interstitium. Multifocal accumulation of epithelioid macrophages and multinucleated giant cells associated surrounding eggs in the interstitium (Fig. 4). Multifocal interstitial lymphocytic infiltration. Mild accumulation of inflammatory cells into the pancreatic ducts. Moderate multifocal to coalescing periductal and interstitial fibrosis with ductal hyperplasia. The parasites have no body cavity, tegument with few spikes and 5 to 15 μm squared crystal-like structures adhered to the tegument (Fig. 5), distinct oral sucker apparently located at one of the extremities of the body (Fig. 6), and both male and female genital organs in one single parasite, including the vitellaria,

testes with mature spermatozoa (Fig 7), and an elongated and tortuous uterus filled operculated eggs with a yellow or brown shell.

Lymph node. Multifocal accumulation of a few eggs into the sub-capsular and paracortical sinuses with minimal inflammatory reaction (Fig. 8), and moderate lymphoid hyperplasia.

Contributor's Morphologic Diagnosis: Pancreas. Pancreatitis, granulomatous, chronic, multifocal to coalescing, severe, with intraductal trematodes (*Eurytrema* sp.), Nelore, bovine.

Contributor's Comment: The parasite described in this case has morphological features compatible with those described for trematodes in histological sections. (1) Considering its gross morphology and location, the parasites were recognized as belonging to the genus *Eurytrema*. *Eurytrema pancreaticum* and *E. coelomaticum* are trematode parasites of ruminants, pigs, and man. The adult form is usually located in the pancreatic ducts, or occasionally in the biliary ducts. *Eurytrema* sp. has a broad geographic distribution, including Europe, Asia, and South America. Two intermediate hosts are required for the life cycle of this parasite. In Brazil, the first intermediate host is the snail *Bradybaena similaris*, and the second intermediate host is a grasshopper of the genus *Conocephalus*. Cattle become infected by accidentally ingesting the second intermediate host. (2)

Eurytrema pancreaticum has been recognized as more pathogenic for cattle than *E. coelomaticum*. However, severe chronic pancreatitis, such as in the present case, has been described as a result of *E. coelomaticum* infestation. (2) Considering its morphology and geographical location (3,4), the parasite in this case was considered to be *E. coelomaticum*.

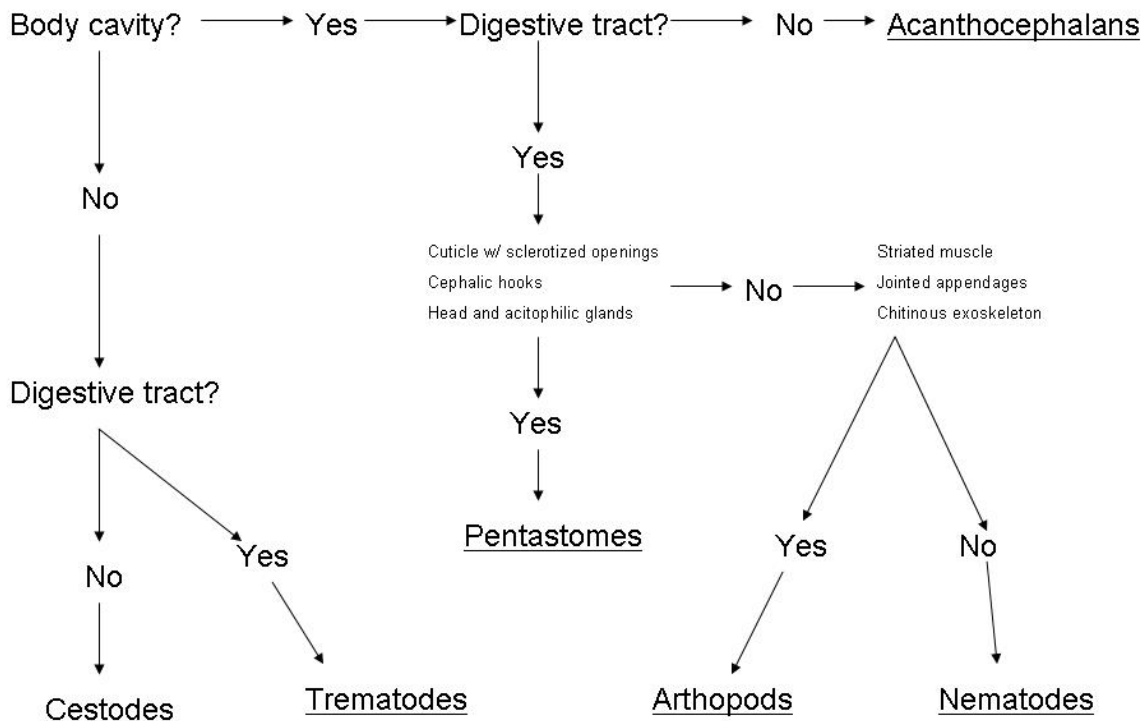
Eurytrema sp. is a frequent incidental finding at necropsy or in slaughterhouses in Brazil. (5) The frequency of *Eurytrema* sp. in slaughtered cattle in some areas of Brazil may reach 70%. The official Brazilian meat inspection service has reported more than 400,000 rejected pancreases out of more than 55 million cattle slaughtered over a 10-year period

In spite of being an incidental finding at necropsy, *Eurytrema* sp. often causes a chronic pancreatitis in ruminants that may be clinically associated with a wasting condition despite good nutritional supply (2,6), as reported in the present case.

AFIP Diagnoses: 1. Pancreas: Ductal ectasia, multifocal, marked, with intraluminal adult trematodes, periductal fibrosis, acinar atrophy and granulomatous pancreatitis centered on trematode eggs, Nelore, bovine.

2. Lymph node, sinuses: Trematode eggs, multifocal, few.

Conference Comment: The contributor provides an excellent overview of *Eurytrema* sp. infection in cattle. There is some variability among slides with respect to the sections of the parasites and the degree of granulomatous inflammation surrounding trematode eggs. Attendees discussed the parasite's morphology and how it was identified as a trematode. Below is a simple algorithm (adapted from reference 1) to aid in classification of metazoan parasites in tissue section.



This case was reviewed by Dr. C.H. Gardiner, parasitology consultant for the Department of Veterinary Pathology, AFIP. In North America, *Eurytrema procyonis* can be found within the pancreatic duct of small carnivores, including raccoons, cats, and foxes. Clinical signs and histologic lesions are similar to those seen in ruminants infected with *E. pancreaticum*.

Readers are encouraged to review case 4, conference 29 from the 1995-1996 Wednesday Slide Conference for another case of *Eurytrema* sp. in the pancreas of an 18-month-old Holstein ox.

<http://www.afip.org/vetpath/WSC/WSC95/95wsc29.htm>

Contributor: Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.
<http://www.vet.ufmg.br>

References:

1. Gardiner CH, Poynton SL. An atlas of metazoan parasites in animal tissues. Washington DC: Armed Forces Institute of Pathology; 1999.
2. Ilha MRS, Loretto AP, Reis ACF. Wasting and mortality in beef cattle parasitized by *Eurytrema coelomaticum* in the State of Paraná, southern Brazil. Vet Parasitol. 2005; in press.
3. Mattos Jr. DG, Vianna SSS. O *Eurytrema coelomaticum* (Trematoda: Dicrocoeliidae) no Brasil. Arq Flum Med Vet. 1987; 2:3-7.
4. Travassos L, Freitas JFT, Kohn A. 1969. Trematodeos do Brasil. Mem Inst Oswaldo Cruz. 1969; 67:1-886.
5. Azevedo, JR, Mannigel RC, Agulhon AZ, Borba TR, Barbieri AW, Oliveira DCL, Headley AS, Janeiro V. Prevalence and geographical distribution of bovine eurytrematosis in cattle slaughtered in northern Paraná, Brazil. Pesq Vet Bras. 2004; 24:23-26.
6. Graydon RJ, Carmichael IH, Sanchez MD, Weidosari E, Widjayanti S. Mortalities and wasting in Indonesian sheep associated with the trematode *Eurytrema pancreaticum*. Vet Rec. 1992; 131:443.

CASE IV – 04 H 6608 (AFIP 2984847)

Signalment: 7.8 year old, female, German Shepherd, *Canis familiaris*, dog

History: One year and 8 months previously this dog had presented with limping and a painful bony mass on the right distal tibia. The only hematologic abnormality was a very mild hypercalcemia. Thoracic radiographs were within normal limits. At the time of first presentation, a fine needle aspirate (FNA) and biopsy specimen of the right tibia was done and revealed macrophages and branching septate fungal hyphae with parallel sides suspected to be *Aspergillus sp.* Similar findings were obtained from a FNA of a prescapular lymph node consistent with systemic mycotic disease.

Culture of the bone yielded rapid growth of an unidentified fungus that was submitted to the University of Texas Health Science Center Fungus Testing Laboratory (San Antonio, TX). Growth consistent with a basidiomycete was reported along with a susceptibility to itraconazole. Antifungal therapy with

itraconazole (Sporanox®) resulted in some decrease in pain and limping after two months of treatment. Slow progression of the swelling of the right tibia over the next 18 months in spite of intermittent treatment occurred with progressively worsening ataxia. Computer tomography of the brain revealed lesions in the thalamic region and enlarged lateral and third ventricles suggesting disseminated disease. Because of worsening CNS signs, euthanasia was requested and necropsy performed.

Gross Pathology: Lungs were heavy, dark red, rubbery, and failed to collapse. In the heart the mitral valve had rounded, nodular, to roughened margins. The endocardium of the pulmonary outflow track was roughened, granular, and pale in a small patch (granulomatous endocarditis and myocarditis). The liver was slightly enlarged and pale (steroid hepatopathy). The spleen had siderocalcific plaques. Bone changes within left and right tibias consisted of irregular thickening of the periosteum and cortices with multiple irregular pale nodules within the marrow (granulomatous osteomyelitis). In the brain, the left to mid-thalamic area had a 2 cm dark tan circular lesion that pressed dorsally into the left ventricle (malacia and encephalitis).

Laboratory Results: Previous culture of the bone revealed a basidiomycete (University of Texas Health Science Center Fungus Testing Laboratory (San Antonio, TX)). Further characterization of the culture was done with genetic testing revealing a 94% correspondence with the basidiomycete fungus *Oxyporus populinus*, a decay shelf mushroom typically found on damaged maple trees.

Contributor's Morphologic Diagnoses: Bone: osteomyelitis, granulomatous and necrotizing, chronic, multifocal, severe, with intralesional fungal hyphae consistent with basidiomycosis
Artery: arteritis, granulomatous, chronic, multifocal, with intralesional fungal hyphae consistent with basidiomycosis

Contributor's Comment: This animal had been diagnosed with basidiomycosis over one year previously and treated with itraconazole but without resolution and with slow progression. Basidiomycetes are fungi that include puffballs, shelf fungi, rusts, smuts, and mushrooms. The hyphae detected at necropsy were assumed to be the same organism as that originally isolated. After euthanasia, histopathology revealed dissemination of the infection to many organ systems including heart, blood vessel walls, thyroids, adrenals, and kidneys, as well as bone. No organisms were detected in liver, lung, and spleen. Basidiomycete hyphae in this case appeared to have an affinity for growth within endocardium, myocardium, arterial walls, arterial adventitia, and endocrine organs (adrenals, thyroids) with relatively scant inflammation, possibly due to the recent steroid therapy. Inflammation was most abundant in bone and periosteal vessels. The brain lesion was an area of

malacia, likely due to ischemia from blood vessel damage, but no hyphae or macrophages were found within brain.

The hyphae in this case are generally uniform in size bearing more or less parallel walls and frequent septa and occasionally perpendicular or dichotomous branching. In some areas the hyphae are slightly bulbous with undulating walls between septa. No clamp connections were noted on the hyphae by histopathology.

Basidiomycosis due to *Schizophyllum commune* has recently been reported in a dog from Japan, apparently the first report of its kind in a dog.(1) Basidiomycetous fungi, especially *Schizophyllum commune*, is reported to be more and more frequently isolated from humans with pulmonary and sinus disease as well as occasional systemic disease with brain involvement.(2,3) Infections in humans have also been reported due to a basidiomycete *Coprinus cinereus*, notably as endocarditis.(3) The infections tend to be associated with immunosuppression; in human cases this is thought to be due in part to HIV infections.(2)

Disease due to basidiomycetes may be under-diagnosed due to misdiagnosis as *Aspergillus sp.*, which the hyphae in sections closely resemble. Identification of cultured specimens as basidiomycetes can be done based on tests such as the benomyl test and morphologic characteristics. *Schizophyllum*, for example, has characteristic clamp connections on hyphae in culture. These characteristic structures, however, may or may not be present or detectable on hyphae within tissue sections, adding to the difficulty with diagnosis. (2)

Molecular analysis of DNA can be done to more specifically identify isolates. The present case, for example, has 94% correspondence to *Oxyporus populinus*, a shelf mushroom found on damaged maple trees. Further testing of the isolate is being done, and if the tentative species is verified, this would be the first reported case of systemic disease due to this organism in any species.

AFIP Diagnosis: Bone, distal right tibia (per contributor): Osteomyelitis, granulomatous and necrotizing, multifocal to coalescing, severe, with myriad fungal hyphae, German Shepherd Dog, canine.


Conference Comment: The contributor provides an interesting case of fungal osteomyelitis. There is some variability among slides, with some sections of bone marrow having higher numbers of neutrophils and necrotic debris. Attendees discussed the presence of fractured and scalloped trabeculae with empty osteocyte lacunae forming sequestra of necrotic bone. Attendees agreed that *Aspergillus sp.* is the top differential diagnosis based on both fungal morphology and frequency of

occurrence. This case serves as a valuable example of why additional diagnostic tests, such as culture, are important in accurately identifying both fungal and bacterial microorganisms.

Contributor: Department of Veterinary Pathology, College of Veterinary Medicine
Iowa State University, Ames, IA 50011-1250
<http://www.vetmed.iastate.edu/departments/vetpath/>

References:

1. Kano R, et al: First report on *Schizophyllum commune* from a dog. J Clin Microbiol 40: 3535-3537, 2002.
2. Rihs JD, Padhye AA, Good CB: Brain abscess caused by *Schizophyllum commune*: an emerging basidiomycete pathogen. J Clin Microbiol 34:1628-1632, 1996.
3. Kern ME, Uecker FA: Maxillary sinus infection caused by the homobasidiomycetous fungus *Schizophyllum commune*. J Clin Microbiol 23:1001-1005, 1986.

Signature Authenticated by Approve 
Approved by: Carl I Shaia,
r: Sunday, 12 February, 2006 at 18:55:5

Carl I. Shaia, DVM
Major, Veterinary Corps, U.S. Army
Wednesday Slide Conference Coordinator
Department of Veterinary Pathology
Armed Forces Institute of Pathology
Registry of Veterinary Pathology*

*Sponsored by the American Veterinary Medical Association, the American College of Veterinary Pathologists and the C. L. Davis Foundation.