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The pathology of alpaca fever

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RUNNING TITLE: Pathology of alpaca fever.

ABSTRACT

Alpaca fever is a condition of alpacas and llamas produced by *Streptococcus equi* subspecies *zooepidemicus*, characterized clinically by fever, depression, recumbence and death, and pathologically by polyserositis. Although a few cases of the disease have been reported, very little information about the pathology of this disease has been published and information on the pathology of alpaca fever is scant. In this study, a detailed gross and microscopic description of three cases of alpaca fever is presented. The three animals had disseminated fibrino-suppurative polyserositis with vascular thrombosis and intralesional gram positive cocci. In addition, two of the animals had severe fibrino-suppurative pneumonia, endocarditis and myocardial necrosis, while the third animal had transmural pleocellular enteritis with prominent lymphangitis. The lymphangitis observed in the latter suggests that dissemination of *S. equi* subsp. *zooepidemicus* occurred through lymphatic circulation and that at least in this animal, the portal of entry of infection was the alimentary system.

KEYWORDS: alpaca fever; lymphangitis; polyserositis; septicemia; *Streptococcus equi* subspecies *zooepidemicus*.

Alpaca fever is a disease of llamas and alpacas produced by *Streptococcus equi* subspecies *zooepidemicus*.[8] The disease is characterized clinically by elevated body temperature, depression, recumbency and death. Pathologically, alpaca fever is mainly characterized by polyserositis. In Perú, where this disease was first described, the morbidity in some alpaca herds has been estimated to be as high as 10%. It is hypothesized that stressors, including transport, may result in subclinical carriers developing clinical systemic infection.[7]

Although alpaca fever has been reported in South American camelids before, no detailed descriptions of the pathology of the disease have been published. We present here a detailed gross and microscopic description of three cases of alpaca fever.

Three alpacas from the same number of farms were submitted to the California Animal Health & Food Safety (CAHFS) Laboratory (San Bernardino and Davis branches) for autopsy and diagnostic work up, between May 2015 and May 2016. Animal 1 was a 7-day-old, 8.6 kg female; animal 2 was a 10-year-old, 73.0 kg male; and animal 3 was a 4-year-old, 51.5 kg male. Animals 1 and 2 had a history of sudden death, while animal 3 had a 5-day history of anorexia followed by death. In addition, animal 1 belonged to a herd of alpacas that was kept on a Thoroughbred horse farm, although direct contact with the horses was not reported.

A complete autopsy was performed on the three animals. The carcass of animal 1 was in good nutritional condition, and the carcasses of animals 2 and 3 were in fair nutritional condition, with a small amount of fat deposits and mild, generalized muscle atrophy. The most striking gross lesion in the three animals consisted of multiple strands of fibrin attached to the parietal and visceral pleura. The lungs were diffusely red, wet, soft and collapsed, and there was a moderate amount of froth in the trachea and lower airways. In addition, animal 1 had moderate hydrothorax, characterized by approximately 1 L of clear

pleural fluid with accompanying strands of fibrin. In animal 2, there were multifocal endocardial and epicardial ecchymoses, petechial and ecchymotic hemorrhages of the congested abdominal serosas and few intra-abdominal fibrin strands (Fig. 1). The liver of this animal had a prominent acinar pattern and the whole carcass was mildly yellow. No other significant gross lesions were observed in the 3 examined carcasses.

Ancillary tests were performed on samples from the three animals according to SOPs of CAHFS, unless otherwise specified. Samples of trachea, lungs, heart, liver, spleen, kidneys, adrenal glands, tongue, esophagus, gastric compartments, small intestine, cecum, colon and/or brain from the 3 animals were collected and fixed in 10%, buffered, pH 7.2 formalin for 48 h and processed routinely for the production of 4 µm-thick H&E sections. Selected sections were also stained with Gram, PAS, Giemsa, phosphotungstic acid hematoxilin (PTAH), or processed by immunohistochemistry for factor VIII. The microscopic findings in the most severely affected organs of the three animals were graded according to severity, using a scale between 1 (mild) and 4 (severe lesions) with intermediate scores showing progressive severity.

Samples of lung, liver, peritoneal exudate, small intestine and/or colon from the 3 animals were aseptically collected and subjected to aerobic or microaerophilic bacterial culture. Briefly, these specimens were inoculated onto 5% sheep blood Columbia agar plates (Hardy Diagnostics, Santa María, CA) and incubated aerobically or in 5-10% CO_2 at 37°C for 48 hours. A real-time PCR to detect a fragment of the *Salmonella*-specific invA gene was performed on intestinal content as previously described.[5] *Salmonella* culture was performed on bile, colon pool and/or intestinal content, using tetrathionate or selenite enrichment broth and selective plate media. Frozen sections of spleen were processed by RT PCR for bovine viral diarrhea, bluetongue and epizootic hemorrhagic disease viruses. Feces were examined for parasite eggs by a flotation method. A heavy-metal screen including lead,

manganese, iron, mercury, arsenic, molybdenum, zinc, copper, and cadmium was performed on liver samples by inductively-coupled argon plasma emission spectrometry. Selenium concentration in the liver was determined by inductively-coupled plasma spectrometry using hydride generation. Stomach content from animal 3 was grossly examined for toxic plant identification.

Microscopically, the most significant lesion was seen in the lungs of the 3 animals. Fibrino-cellular exudate lined the pleural surface and expanded the pleural stroma. This exudate was composed of fibrin admixed with cell debris, macrophages, lymphocytes, plasma cells, neutrophils and myriad gram positive cocci (Fig. 2). The pleural blood vessels were diffusely congested and had multifocal perivascular hemorrhages. Pleural lymphatic vessels were focally cuffed by macrophages and lymphocytes. The pulmonary parenchyma of the three animals was diffusely congested with multifocal areas of atelectasis and hemorrhage (Fig. 3). The interlobular septa were diffusely and mildly edematous, and infiltrated by small numbers of lymphocytes, macrophages and neutrophils. Arteries and veins were severely congested, and the lumina of many of them were partially to completely occluded by fibrinocellular thrombi with embedded neutrophils and colonies of gram positive cocci. Alveolar spaces were distended with an exudate composed of fibrin, neutrophils, macrophages, lymphocytes, plasma cells and colonies of gram positive cocci (Fig. 4). Additionally, the lungs of animals 2 and 3 had severe alveolar edema and multifocal hemorrhage. The grading of microscopic findings is summarized in Table 1.

Fibrino-cellular peritonitis, characterized by the presence of multifocal deposits of fibrin admixed with macrophages, lymphocytes, plasma cells, neutrophils and myriad gram positive cocci infiltrating the visceral and parietal peritoneum, was observed in all 3 animals. In animal 1, there was marked transmural congestion of the small intestine, and the intestinal submucosa was diffusely expanded by edema and hemorrhage, with multifocal thrombosis

of submucosal and mesenteric blood and lymphatic vessels which also contained myriad intraluminal Gram positive cocci (Figs. 5 and 6).

The heart of animals 2 and 3 had fibrinous epicarditis and endocarditis, characterized by stromal edema, multifocal hemorrhage and diffuse infiltration by numerous viable and degenerate neutrophils, fibrin and large numbers of gram positive cocci. In the myocardium of these 2 animals, discrete areas of myocardial degeneration and necrosis, neutrophilic infiltration and myriad intralesional gram positive cocci were observed. The myocardium of animal 1 had mild diffuse congestion and multifocal hemorrhage.

In the spleen, liver, kidney and adrenal cortex of animals 2 and 3, numerous large colonies of gram positive cocci were seen in the lumen of blood vessels. The surrounding parenchyma of these organs, contained few foci of lytic necrosis.

In all cases, *S. equi* subsp. *zooepidemicus* was isolated from liver, lung and peritoneal exudate. No other aerobic bacterial pathogens were isolated from any of the samples of the 3 animals cultured. PCR for Salmonella was negative in all 3 animals.

Animal 3 had marginally low levels of copper in the liver (19 ppm; normal range 25-100 ppm). The remaining heavy metals in this animal and all heavy metals in the other 2 animals were within normal range.

S. equi subsp. *zooepidemicus* is a gram positive, beta hemolytic, Lancefield group C organism, and is the microorganism most frequently isolated from the respiratory tract of clinically healthy horses and horses with pneumonia.[11] This microorganism has been associated with multiple syndromes in several animal species. *S. equi* subsp. *zooepidemicus* causes suppurative respiratory infections in young horses and uterine infections in elderly mares.[11] In dogs, this microorganism causes a disease characterized by sudden onset of pyrexia, dyspnea, hemorrhagic nasal discharge, pulmonary hemorrhage, pleural effusion and

death.[9] In pigs and non-human primates, *S. equi* subsp. *zooepidemicus* causes polyarthritis, bronchopneumonia, pleuritis, epicarditis, endocarditis and meningitis.[13] A case of polyserositis associated with *S. equi* subsp. *zooepidemicus* has been described in a camel.[14] In ruminants, this microorganism has been associated with sporadic mastitis.[12] In humans, *S. equi* subsp. *zooepidemicus* has been rarely isolated in association with consumption of contaminated food,[3] or after contact with affected animals, which confirms its zoonotic character.[6]

S. equi subsp. *zooepidemicus* is recognized as the cause of the so-called alpaca fever, one of the most significant diseases of alpacas and llamas.[4] In some countries, the morbidity of alpaca fever varies between 5% and 10%, and the lethality varies between 50% and 100%.[7] In alpacas, this disease may occur in acute, subacute or chronic forms. Acute and subacute forms are usually characterized by anorexia, depression and high fever. The chronic forms are characterized by focal infections, including abscesses in multiple locations and orchitis. The infection becomes systemic following ingestion of the organism, and death may occur from four to eight days following the onset of clinical signs.[2,7]

The pathogenesis of alpaca fever has not been completely elucidated. *S. equi* subsp. *zooepidemicus* is considered a mucous membrane commensal in alpacas,[7] and it has been suggested that transmission occurs orally via contaminated objects or direct contact with infected animals.[7]

In healthy carriers, onset of clinical systemic disease can be predisposed by viral infections, trauma, high temperatures or stressors such as transportation, inclement weather or malnutrition.[10,7] Systemic disease is characterized by polyserositis involving the serosas of the thoracic and abdominal cavities, and occasionally meningitis.[8] The clinical signs in

164 animals with polyserositis include dyspnea, colic, tense and tender abdomen, and
165 constipation.[7]

166 It has been proposed that young animals may be naturally predisposed to the
167 systemic manifestation of alpaca fever, while this form seems to be rarely seen in adult
168 animals.[8] However, two of the animals described in this study were adults and they suffered
169 from the systemic form of the disease, suggesting that this form of alpaca fever may be more
170 common in adult animals than previously thought. Animal 3 was mildly copper deficient;[1]
171 however, although we cannot rule out that the deficiency predisposed this alpaca to the
172 infection, it would seem unlikely as the hepatic copper level was only marginally low.

173 Lungs and abdominal and thoracic serosae are usually affected in acute and chronic
174 cases, with abscesses in multiple organs being observed in chronic cases.[7] No abscesses
175 were seen in any of the animals of our study, despite the fact that one of the cases was
176 characterized as sub-acute to chronic.

177 In the case of the neonatal cria in this study, the same personnel attended both the
178 equine and the alpaca facilities of the ranch, and it is possible that they acted as mechanical
179 vectors conveying *S. equi* subsp. *zooepidemicus* from the horses to the alpaca herd, since
180 this bacterium is ubiquitous in equine populations.[7] However, there is no record of previous
181 cases of *S. equi* subsp. *zooepidemicus* infections in horses on this farm, and as such this
182 possible interspecies transmission remains speculative. No other cases of alpaca fever had
183 occurred on this ranch for at least 12 months prior to the case reported here.

184 Although all three animals in this study had pulmonary lesions, the neonate had also
185 developed severe lesions in the intestinal tract, including enteric lymphatic vessels. This
186 difference may be associated with a different route of bacterial infection and dissemination,
187 although the number of animals in this study is not high enough to draw definitive
188 conclusions.

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DECLARATION OF CONFLICTING INTERESTS

The authors(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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REFERENCES

1. Andrews AH, Cox A. Suspected nutritional deficiency causing anemia in llamas (*Lama glama*). Vet Rec 1997;140:153-154.
2. Aubry P, et al. Septic orchitis in an alpaca. Can Vet J 2000; 41:704-706.
3. Bordes-Benítez A, et al. Outbreak of *Streptococcus equi* subsp. *zooepidemicus* infections on the island of Gran Canaria associated with the consumption of inadequately pasteurized cheese. Eur J Clin Microbiol Infect Dis 2006; 25:242-246.
4. Cebra CK, et al. Pathogenesis of *Streptococcus zooepidemicus* infection after intratracheal inoculation in llamas. Am J Vet Res 2000; 61:1525-1529.
5. Cheng CM, et al. Rapid detection of Salmonella in foods using real-time PCR. J Food Prot 2008; 71:2436-2441.

6. Eyre DW, et al. *Streptococcus equi* subspecies *zooepidemicus* meningitis - a case report and review of the literature. Eur J Clin Microbiol Infect Dis 2010; 29:1459-1463.
7. Fowler ME, Bravo PW. Infectious diseases. In: Fowler ME, Bravo PW, ed. Medicine and Surgery of Camelids. 3rd ed. Ames, Iowa: Blackwell Publishing, 2010:173-230.
8. Jones M, et al. Outbreak of *Streptococcus equi* ssp. *zooepidemicus* polyserositis in an alpaca herd. J Vet Intern Med 2009; 23:220-223.
9. Priestnall S, Erles K. *Streptococcus zooepidemicus*: an emerging canine pathogen. Vet J 2011; 188:142-148.
10. Timoney JF. The pathogenic equine streptococci. Vet Res 2004; 35:397-409.
11. Timoney JF, et al. Cloning and sequence analysis of a protective M-like protein gene from *Streptococcus equi* subsp. *zooepidemicus*. Infect Immun 1995; 63:1440–1445.
12. Sharp MW, et al. *Streptococcus zooepidemicus* infection and bovine mastitis. Vet Rec 1995; 137:128.
13. Soedarmanto I, et al. Identification and molecular characterization of serological group C streptococci isolated from diseased pigs and monkeys in Indonesia. J Clin Microbiol 1996; 34:2201-2204.
14. Stoughton WB, Gold J. *Streptococcus equi* subsp *zooepidemicus* pleuropneumonia and peritonitis in a dromedary camel (*Camelus dromedarius*) calf in North America. J Am Vet Med Assoc 2015; 247:300-303.

236 **Table 1.** Grading of microscopic lesions in tissues of 3 alpacas with alpaca fever*

	Severity of lesions*		
Organ	Animal 1	Animal 2	Animal 3
Pleura	3	2	2
Lung	2	4	4
Heart	2	3	3
Gastric compartments	0	2	2
Intestine	4	1	1
Peritoneum	4	2	2
Liver	1	2	1
Kidney	1	2	2
Adrenal gland	0	2	1
Spleen	0	2	2
Brain	0	1	1

237 *Severity of lesions was graded between 0 (no lesions observed) to 4 (severe), with 1, 2 and
238 3 indicating progressively severe lesions.

FIGURE LEGENDS

Figure 1. Alpaca with alpaca fever showing fibrin strands attached to abdominal serosal surfaces (arrows). The carcass is mildly icteric.

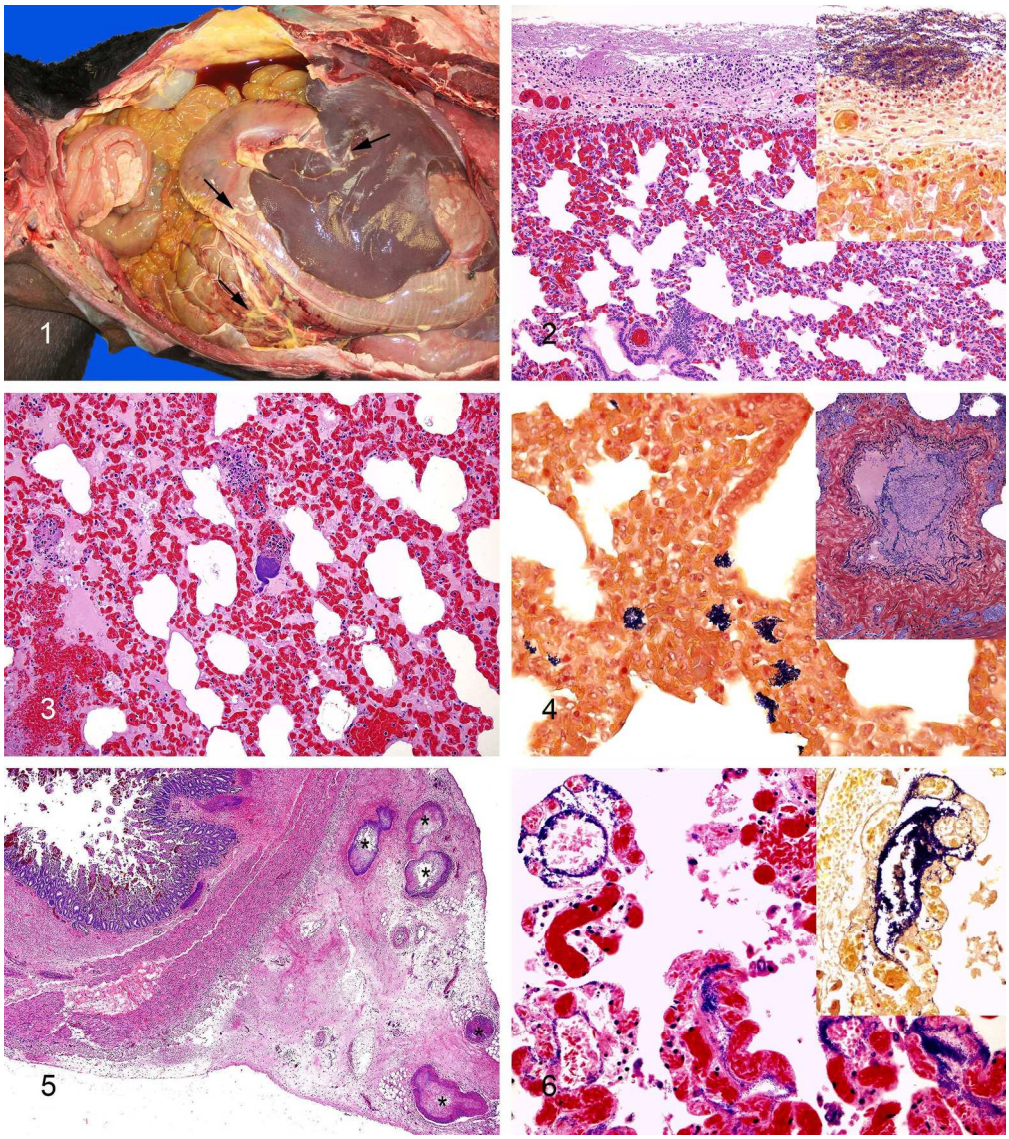
Figure 2. Fibrinosuppurative pleuritis in an alpaca with alpaca fever. Insert: Gram stain of the same lung showing myriad gram positive cocci.

Figure 3. Lung of an alpaca with alpaca fever showing diffuse congestion, alveolar edema, hemorrhage and colonies of cocci. H&E.

Figure 4. Lung of an alpaca with alpaca fever showing several colonies of gram positive cocci in the alveolar space and interstitium. Gram. Insert: Fibrin thrombus with embedded colonies of cocci occluding partially the blood vessel lumen. PTAH.

Figure 5. Small intestine of an alpaca with alpaca fever showing transmural pleocellular enteritis, including submucosal and serosal edema, hemorrhages and mesenteric thrombi with cocci in blood and lymphatic vessels (asterisks).

Figure 6. Small intestine of an alpaca with alpaca fever showing basophilic gram positive cocci (insert) covering the surface of the villi and the inner part of the dilated central lymphatic vessel. H&E.



Figures 1-6

191x216mm (300 x 300 DPI)