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Histophilus somni myocarditis and leptomeningitis in feedlot cattle: case report and occurrence in South America

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Abstract. We investigated deaths in a group of feedlot steers in Argentina. The main findings in 3 steers autopsied were pulmonary congestion and edema, necrotizing myocarditis, pericarditis, suppurative leptomeningitis, and bronchopneumonia. *Histophilus somni* was detected by bacterial culture and immunohistochemistry in the hearts of the 3 animals. Partial sequences of the 16S rRNA gene of a *H. somni* isolate had 99% similarity with other *H. somni* sequences in GenBank. Most reports of *H. somni* septicemia in cattle originate from North America and western Europe. There is scant information about cardiac histophilosis in South America. A survey of diagnostic laboratory personnel in 7 South American countries documented various forms of bovine histophilosis in Argentina, Brazil, Uruguay, and Venezuela.

Key words: bovine; *Histophilus somni*; meningitis; myocarditis; pneumonia; sudden death.

Histophilus somni is an opportunistic bacterial pathogen in the family *Pasteurellaceae*.[1] The organism may be found in the lower urogenital and upper respiratory tract of healthy cattle, but it is responsible for several clinical syndromes in cattle that are referred to collectively as histophilosis or *H. somni* disease complex (HSDC).[18] HSDC includes respiratory disease (pneumonia with or without laryngitis),[6,8] septicemia (thrombotic meningoencephalitis [TME], myocarditis, and/or arthritis),[4,5,16,17] reproductive disease (abortion and infertility),[2,9,11] and other conditions including otitis and mastitis.[18]

HSDC occurs worldwide and is well documented in regions of intensive cattle production.[4,6,18] Myocarditis and encephalitis caused by *H. somni* are also well recognized in cattle,[18] but there is limited published information about the complex in South America. We describe herein the epidemiologic and pathologic features of cardiac and meningeal histophilosis in a group of feedlot cattle in Argentina. Given the absence of published reports of cardiac histophilosis in Latin America, we surveyed pathologists in several South American countries to determine the components of the HSDC that they had diagnosed.

In June and July 2014, the owner of a cattle feedlot in the department of Calamuchita, Cordoba province, Argentina, reported sudden deaths in 7- to 9-mo-old beef cattle in 3 pens. Mortality in individual pens was 6 of 149 (4.0%), 5 of 143 (3.5%), and 2 of 98 (2.0%). Field autopsies performed by a veterinary practitioner revealed only heavy dark edematous lungs. He reported that similar sudden fatal episodes had occurred in the past, but the cause had not been determined.

The feedlot was visited twice by one author (CA Margineda) to collect clinical history and epidemiologic information and to perform postmortem examinations (Table 1) on 3 carcasses (cases 1–3; one from each affected pen). The feedlot contained 16,591 steers at the first visit and 19,649 at the second. The feedlot was well-managed with low annual mortality (0.68%). The proportion of dead cattle subjected to postmortem examination was ~85%. Weaned beef and dairy calves from northern and central Argentina entered the feedlot at 5–7 mo old. The typical feeding period was 3–4 mo. Calves were vaccinated within 48–72 h of arrival using a multivalent product containing inactivated *H. somni, Mannheimia haemolytica, Pasteurella multocida*, bovine herpesvirus 1, bovine parainfluenza virus 3, and bovine viral diarrhea virus (Providean Respiratoria; Tecnovax, Mercedes,

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Table 1. Clinical history, epidemiologic information, pathology, bacterial culture, and immunohistochemistry (IHC) in spontaneous cases of sudden death caused by *Histophilus somni* in cattle.

Case	Breed/body weight	Clinical sign	Days on feed	Lesion			Bacterial culture/IHC		
				Heart	Lungs	Brain	Heart	Lung	Brain
1	Aberdeen Angus/~320 kg	Sudden death	34	Acute multifocal myocarditis	Edema and congestion	Absence of lesions	*Hs*/+	*Hs*/–	ND
2	Crossbreed/~310 kg	Sudden death	102	Acute focal infarction	Bronchopneumonia	Absence of lesions	*Hs*/+	*Hs*/+, *Tp*/ND	ND
3	Holstein/~360 kg	Sudden death	33	Acute focally extensive myocarditis	Edema and congestion	Leptomeningitis	*Hs*/+	*Hs*/–	*Hs*/+

Hs = *Histophilus somni*; ND = not done; *Tp* = *Trueperella pyogenes*; – = negative; + = positive.

Argentina). Cattle were revaccinated with the same product 23–25 d after arrival.

Routine autopsies were performed on 3 animals that died spontaneously. Samples of heart and lungs, and the whole brain, were fixed in 10% neutral-buffered formalin for 72 h. Six samples were collected from each heart and lungs, including representative specimens from multiple areas of both organs. After fixation, brains were sliced at 1-cm intervals, and subsamples were collected from frontal and occipital cerebral cortex, thalamus, rostral colliculi, pons, medulla oblongata, and cerebellum. Tissues were processed routinely for the production of hematoxylin and eosin (H&E)- and Gram-stained sections.

The 3 animals had similar gross cardiac lesions, which consisted of focally extensive areas of red-purple (Figs. 1A–C) or white-yellow discoloration (Fig. 1B), surrounded by a 2-mm wide bright red zone. The lesions (2 × 2 × 3 cm) affected both left papillary muscles in case 1 and one left papillary muscle in case 2. Case 3 had subendocardial lesions involving much of the inner half of the left ventricular myocardium (4 × 3 × 5 cm), including both papillary muscles, and focal lesions in the interventricular septum. There was severe diffuse fibrinous pericarditis in case 1 (Fig. 1D). The lungs of the 3 steers were heavy, purple, and edematous. In addition, the lungs of case 2 had consolidation of ~20% of the cranioventral lobes of each lung. In this steer, 5–10-mm abscesses were observed in the consolidated areas of the lungs, and there was fibrinosuppurative exudate in bronchi and fibrinous pleuritis overlying areas of consolidation. In case 3, there was fibrinosuppurative exudate in the leptomeninges at the base of the brain and over both cerebral hemispheres. No other significant gross abnormalities were observed in any of the carcasses; in particular, the joints appeared grossly normal.

The histologic lesions in the heart were similar in the 3 animals. They consisted of large discrete areas of acute necrotizing myocarditis, characterized by coagulative necrosis, neutrophilic inflammation, hemorrhage, and fibrinous exudate. Intralesional colonies of coccobacilli were present (Figs. 2A–C). Necrotic myocardium was surrounded by a rim of hemorrhage and an inflammatory cell infiltrate comprised of predominantly neutrophils, lymphocytes, plasma cells, and macrophages. Thrombosis and vasculitis affected medium- and small-caliber arteries and veins (Fig. 2A). The heart of case 2 had a discrete area of acute infarction with mineralized cardiomyocytes. No histologic lesions were observed in the papillary muscles of the right ventricle. Histology confirmed the presence of fibrinous pericarditis in case 1, and leptomeningitis in case 3 (Fig. 2D). The pericardium had fibrin effusion with enmeshed neutrophils. The leptomeningitis was distributed throughout the meninges of cerebral cortex and medulla oblongata. Serofibrinous exudate mixed with macrophages and neutrophils filled the subarachnoid space. Perivascular cuffing by similar inflammatory cells was prominent. The lumen of blood vessels in the subarachnoid space, especially small arterioles, contained large numbers of macrophages and neutrophils with fewer lymphocytes, and had hypertrophy of endothelial cells and subendothelial inflammation. The classic lesion of TME (i.e., randomly distributed septic infarcts throughout the brain) were not seen in any of the 3 animals. No lesions were present in the brain parenchyma of any of the 3 steers or in the meninges of cases 1 and 2.

Both lungs of cases 1 and 3 had moderate, disseminated congestion and intra-alveolar exudation of fibrin and neutrophils. In case 2, chronic-active bronchopneumonia with multifocal necrosis was evident in both lungs. Alveoli were filled with neutrophils, fibrin, and fewer macrophages, lymphocytes, and plasma cells, in a seemingly random lobular pattern. The pleura had fibrin effusion with enmeshed neutrophils and macrophages. Large septic thrombi occluded pleural and interlobular lymphatics. H&E- and Gram-stained sections revealed bacterial colonies in aggregates suggestive of biofilms. The gram-negative coccobacilli were consistent with *Histophilus* sp.

Selected paraffin sections of heart, lung, and brain were processed for *H. somni* immunohistochemistry (IHC) as described previously.[8] The positive control block was from a heart from which *H. somni* had been isolated. Negative controls consisted of the heart blocks of the 3 affected steers

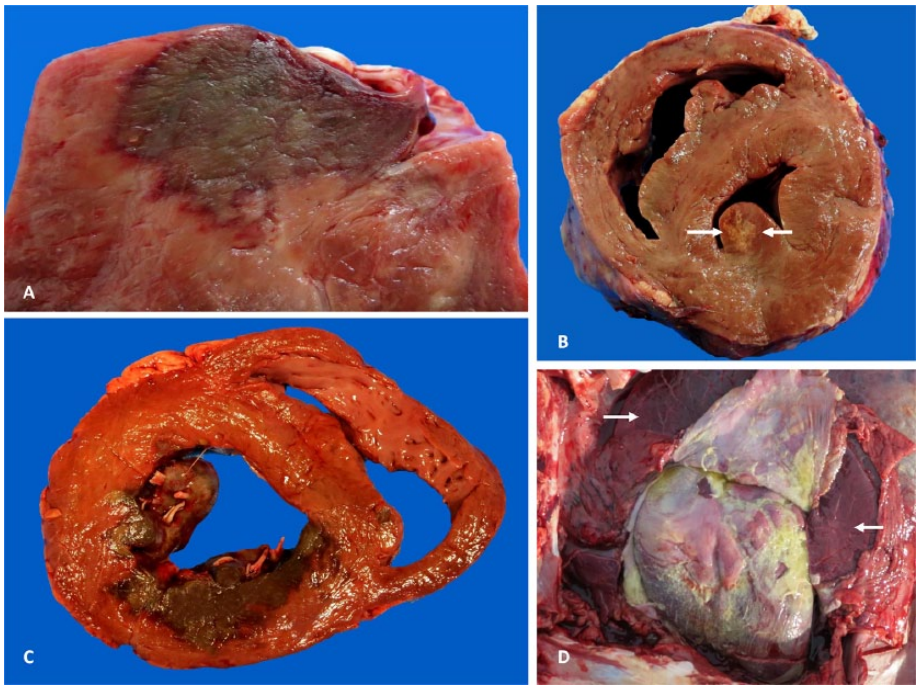

Figure 1. Gross lesions in feedlot steers with *Histophilus somni* infection. **A.** Cut surface of the heart in case 1 with a discrete red-purple lesion in the papillary muscle of the left ventricular myocardium. **B.** Transverse section of the heart in case 2 with an infarct in one papillary muscle (between arrows). **C.** Transverse section of the heart in case 3 with subendocardial infarction involving much of the inner half of the left ventricular myocardium, including both papillary muscles. **D.** Extensive fibrinous pericarditis in case 1; pericardial sac reflected. There is also pulmonary congestion and edema (arrows).

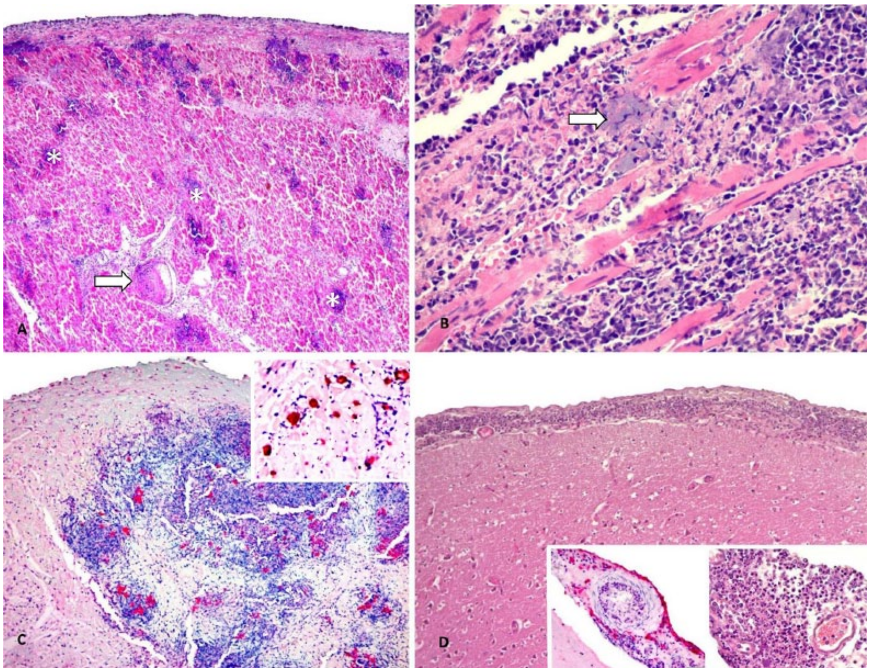

Figure 2. Histologic lesions in histophilosis in feedlot steers. **A.** Acute necrotizing myocarditis in case 3 with multiple small colonies (asterisks) of *Histophilus somni*, and thrombophlebitis (arrow). H&E. 50×. **B.** Focal myocarditis in case 1, with a large colony of *H. somni* (arrow). H&E. 400×. **C.** Disseminated bacterial colonies (stained red) in a discrete area of myocarditis in case 1. *H. somni* IHC. 50×. Inset: abundant *H. somni* antigen lines the capillaries and veins. *H. somni* IHC. 400×. **D.** Leptomeningitis over the occipital cerebral cortex in case 3. H&E. 50×. Left inset: meningitis with IHC staining of *H. somni*; the meninges are stained intensely. 400×. Right inset: inflammatory reaction predominantly of macrophages and neutrophils. H&E. 400×.

described herein, processed with the same procedures but with the primary antibody omitted. Immunohistochemical staining for *H. somni* in heart was positive in all 3 steers. Numerous myocardial capillaries, venules, and small veins were filled with positively stained bacteria (Fig. 2C). *H. somni* formed aggregates apposed to vascular endothelium.

Samples of heart, lung, medulla oblongata, and cerebral cortex were inoculated onto blood agar and MacConkey agar plates. Plates were incubated both aerobically and under microaerophilic conditions at 37ºC for 48 h. Bacterial cultures from 3 hearts and lungs, and 1 brain yielded pure or almost-pure growth of *H. somni*–like bacteria. Isolates were identified as *H. somni* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Microflex LT; Bruker Daltonics, Billerica, MA). Based on biochemical tests and MALDI-TOF MS findings, isolates were identified as *H. somni*.

PCR amplification of the 16S rRNA of an isolate from case 3 was performed as described previously.[13] The PCR product was sequenced (Big Dye Terminator v.3.1; ABI PRISM 3100; Thermo Fisher Scientific, Waltham, MA). The sequence obtained was assembled, manually corrected, and compared to sequences in GenBank, using the BLAST algorithm of the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequence of this *H. somni* isolate was deposited in GenBank (MH379634). The sequence obtained showed 99% identity with sequences corresponding to the 16S rRNA gene of *H. somni*, type strain ATCC 43625 (GenBank accession AF549387).

Lung samples were homogenized in Eagle minimal essential medium supplemented with 10% fetal bovine serum before inoculation onto Madin–Darby bovine kidney cells and incubated at 37°C in 5% CO_2 for 5 d. Cells were observed daily. After 4 blind passages, cultures were tested for bovine viral diarrhea virus by indirect fluorescent antibody test,[6] with negative results.

A diagnosis of cardiac histophilosis was established for the 3 steers, based on gross and microscopic findings, and microbiology. Embolic pyogenic or opportunistic bacterial pathogens such as *Trueperella pyogenes, Listeria monocytogenes, Escherichia coli, Pseudomonas* spp., or *Streptococcus* spp. can also cause myocarditis in cattle, but such lesions tend to be randomly distributed, and outbreaks are rare.[16,18] *Clostridium chauvoei* also causes myocarditis,[3] but the distinctive gross lesions and paucity of inflammatory infiltrates are different from changes found in our cases. Most reports of cardiac histophilosis describe discrete lesions no larger than 3 cm,[7,16] such as those found in cases 1 and 2. Cardiac lesions in case 3 were larger and more widespread, resulting in extensive subendocardial necrotizing myocarditis.

Myocarditis caused by *H. somni* often affects one or both papillary muscles of the left ventricle.[7,16,18,25] In a study in which the hearts of 10 steers with spontaneous cardiac histophilosis were evaluated grossly,[18] 6 hearts had only 1 papillary muscle affected, and 2 each had both papillary muscles and interventricular septum affected. In our study, both papillary muscles were affected in 2 animals, with interventricular septum affected in the third. Susceptibility of the left ventricle papillary muscles to infarction is well known in humans; papillary muscles and adjacent left ventricle myocardium are particularly susceptible to ischemia and infarction, which may lead to contractile dysfunction.[26] *H. somni* infection may be associated with biofilm formation and thrombosis in small arteries.[17] Therefore, it is reasonable that *H somni* infection causes disturbance of blood perfusion and affects the papillary muscles. Histologically, the myocarditis in cases 1 and 3 was similar to previous reports of spontaneous and experimental cardiac histophilosis in cattle.[7,16-18,21,25]

In various reports[16,17] as well as in our study, large biofilm-like aggregates formed on endothelial surfaces of small venules and arteries in myocardium. Biofilm-like aggregates also occur in cases of TME and endocarditis.[17] The classical brain lesion of TME includes fibrinopurulent leptomeningitis, fibrin thrombi and intravascular proliferation of bacteria at sites of thrombosis, hemorrhage, necrosis, and microabscesses.[3,17,18] In our study, case 3 had fibrinopurulent leptomeningitis only and TME was absent. This form of nervous lesion has been referred to as atypical TME.[14] Purulent or fibrinopurulent meningitis in cattle may be caused by other bacteria, such as *T. pyogenes, Salmonella* spp., or *E. coli*.[3] In cases in which only leptomeningitis is found in the absence of multifocal hemorrhagic lesions, *H. somni* should be considered as a possible cause. Extensive biofilm-like aggregates were found in the leptomeninges of case 3.

There is scant information published about cardiac histophilosis in South America. Most reports of cardiac histophilosis come from Canada[8,20,22,24] and to a lesser extent the United States[6,16-18] and UK.[25] Myocardial disease was responsible for 42% of all deaths in one feedlot from Wyoming.[16] *H. somni* was the principal cause of myocardial disease in feedlot cattle in western Canada (70 of 92; 76%) in one study.[8] We therefore contacted 16 diagnosticians in 7 South American countries (Argentina, Brazil, Chile, Colombia, Ecuador, Uruguay, and Venezuela) to establish whether they had diagnosed HSDC and if they had recognized the cardiac form (Table 2). Diagnosticians in Argentina, Uruguay, and Brazil diagnosed histophilosis, primarily as a cause of TME,[4,5,12,19] pneumonia,[10,15] and sporadic abortion.[2,9,11] None had diagnosed cardiac histophilosis. We tried to establish contact with diagnosticians from other countries, including Peru and Bolivia, but did not receive a response. It is likely that histophilosis is widespread in South America. Explanations for the apparent rarity of cardiac histophilosis in the region may be that cases are not reported, the papillary muscles of the left ventricle are not routinely examined at autopsy, the slower emergence of intensive feedlot systems in most countries of the continent, and/or different endemic strains of the bacterium in this part of the world. The latter seems unlikely, given that our strain had 99% homology to the GenBank reference strain.

Table 2. Occurrence of bovine histophilosis in 7 South American countries based on personal communications and published literature.

Country	Laboratory-confirmed histophilosis	Typical manifestation	Frequency	Season	Production system	Reference and/or diagnostician (pers. comm.)
Argentina	Yes	Bronchopneumonia (4)	Frequent	Spring (1), fall (1), winter (2),	Feedlot (2) and fattening pasture (2)	*, †, 15
		TME (3)	Rare	Summer (1), spring (1), fall (1).	Feedlot (1) and fattening pasture (2)	‡, 4, 19
		Abortion (1)	Rare	NI	Dairy farm	1
Brazil	Yes	Bronchopneumonia (1)		Winter (1)	Feedlots	10
		TME (5)	Rare	Winter (3), spring (2)	Fattening pasture (5)	12
		Abortion (9)	Rare	NI	Dairy farm	9, 11
Chile	No	–	–	–	–	Federico Cifuentes, Enrique Paredes (pers. comm.)
Colombia	No	–	–	–	–	Francisco Pedraza, Héctor Cardenas (pers. comm.)
Ecuador	No	–		–	–	Gabriela Toro (pers. comm.)
Uruguay	Yes	Bronchopneumonia (1)	NI	Fall (1),	Dairy farm	Giannitti Federico (pers. comm.)
		TME (1)	Rare	Spring (1)	Fattening pasture	5
Venezuela	Yes	Bronchopneumonia	Frequent	NI	NI	Victor M. Bermudez (pers. comm.)
		TME	Rare	NI	NI	
		Abortion	Rare	NI	NI	

NI = not included; TME = thrombotic meningoencephalitis. Number of occurrences in parentheses.
* Giraudo et al. Pneumonia in calves due to *Haemophilus somnus*. II Meeting AAVLD; Dec 1987; Buenos Aires, Argentina.
† Lucca et al. *Histophilus somni*: outbreak in calves of establishment breeding and fattening. XV Conference JDTC; Sept 2014; Santa Fe, Argentina.
‡ Canton et al. Nervous histophilosis in heifers: report of a case and application of molecular diagnosis and immunohistochemistry. XXII Meeting AAVLD; Nov 2018; Cordoba, Argentina.

The time of occurrence of cardiac histophilosis observed in some studies (50–60 d in feedlots)[6,8] suggests that immunizing calves on arrival may allow time for most to develop protective immunity before natural challenge.[6] In our case, deaths occurred 33–102 d after admission, despite double immunization with commercial *H. somni* bacterins shortly after arrival. We may not have observed mortality rates comparable to those reported in other outbreaks in the United States[16] and Canada[8] given routine vaccination against histophilosis. In experimental infections, vaccination with bacterins reduces the severity of clinical respiratory disease after challenge and confers immunity against TEM. The capacity of commercial products to reduce losses as a result of TEM is one possible explanation for the apparent shift in clinical expression of histophilosis from the neurologic form (1960–late 1980s) to respiratory and cardiac disease (late 1980s to date).[18,20] A 2018 study[23] of strains of *H. somni* using major outer membrane protein (MOMP) gene sequence and pulsed-field gel electrophoresis (PFGE) showed that TME outbreaks may be associated with particular MOMP genetic clades and PFGE groups. This may explain the apparent decline of TME cases over time and the "emergence" or increased recognition of other forms of HSDC.[18,20]

Cardiac histophilosis should be considered as a differential diagnosis when feedlot cattle die acutely. Suggestive gross lesions are cardiac infarcts and/or discrete areas of myocardial necrosis with minimal suppurative exudate.

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