Title

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Journal

Journal of Veterinary Diagnostic Investigation, 36(1)

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Publication Date

2024

DOI

10.1177/10406387231215756

Peer reviewed

Journal of Veterinary Diagnostic Investigation
2024, Vol. 36(1) 95–102

DOI: 10.1177/10406387231215756
jvdi.sagepub.com

Pollen beetle (*Astylus atromaculatus*)-associated gastroenteric disease in cattle: report of 6 natural outbreaks

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Abstract. *Astylus atromaculatus* is a pollen beetle native to South America, commonly found in crop flowers. Experimental intoxication of sheep and guinea pigs by this beetle resulting in fibrinonecrotizing enteritis has been reported. We describe here 6 natural outbreaks of intoxication in cattle associated with consumption of alfalfa (5 of 6) and mixed native (1 of 6) pastures heavily contaminated with *A. atromaculatus*. The outbreaks occurred during the summer (January–February) of 2023 in Argentina ($n = 4$) and Uruguay ($n = 2$), in beef cattle under extensive or semi-extensive rearing systems, with overall cumulative incidence and mortality of 22.3% and 17.8%, respectively. The main clinical signs included acute onset of anorexia, lethargy, hyperthermia, hindlimb weakness, reluctance to move, and diarrhea, for up to 15 d. In 2 outbreaks, sudden death was observed. Eight Hereford, Angus, and/or crossbreed heifers, cows, steers, and/or calves were autopsied. Gross and microscopic findings included multifocal necrosis with fibrinous pseudomembranes in the forestomachs and/or small and large intestines. Fragments or whole specimens of *A. atromaculatus* were identified in the ruminal content of all animals. Testing for multiple gastroenteric pathogens was negative as was testing of *A. atromaculatus* for cantharidin and batrachotoxin. GC-MS and LC-MS/MS performed on the beetles did not identify any known toxic compounds. Based on the exposure to *A. atromaculatus*–contaminated pasture, gross and microscopic lesions, and negative results of all testing for multiple gastroenteric pathogens, a diagnosis of intoxication by *A. atromaculatus* is proposed. Disease caused by *A. atromaculatus* consumption has not been reported previously in cattle, to our knowledge.

Keywords: *Astylus atromaculatus*; cattle; coleoptera; enteritis; pollen beetle; rumenitis.

Astylus atromaculatus Blanchard (*Melyridae*) is a pollen beetle native to South America, where it is commonly found in crops such as sunflower and maize. This beetle is commonly named the spotted maize beetle, astilo moteado, or 7 de oro.[4,12,21] Flowers of the crops are necessary for the survival of *A. atromaculatus*. Adult stages are 7–9 mm long and are identified by yellow wing coverts (elytra) with symmetrical black spots, and black thorax, legs, and antennae.[8,19] This beetle has been introduced from South America to South Africa and North America.[8]

Although it has been postulated that ingestion of *A. atromaculatus* is toxic to animals, experimental intoxication in guinea pigs and sheep with this beetle has been reported only once.[8] In both species, the intoxication was experimentally reproduced by administering pollen beetles blended in distilled water by stomach tube. In both animal species, the intoxication resulted in pseudomembranous enteritis. However, the toxic principle of this beetle could not be identified.[8] Recently, severe and acute gastroenteric disease and death were experimentally produced in cattle dosed orally with large numbers of *A. atromaculatus* (http://www.inia.uy/investigación-e-innovación/plataformas/Plataforma-de-Salud-Animal-/Estudio-realizado-en-INIA-confirma-que-el-escarabajo-Siete-de-Oro-es-toxico-para-bovinos; Spanish). In addition, presumptive natural outbreaks of intoxication by this insect occurred in sheep during the 2023 summer in

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Argentina and Uruguay (https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/comunicacion/noticias/escarabajo-siete-oro-uniradd-alerta-mortandad-rumiantes; Spanish).

We retrieved no cases of *A. atromaculatus* spontaneous intoxication in cattle in a search of Google, PubMed, CAB Direct, Web of Science, and Scopus, suggesting that no descriptions of this condition have been reported in cattle. We describe here 6 natural outbreaks of disease and mortality in beef cattle associated with consumption of pastures heavily contaminated with *A. atromaculatus*.

Materials and methods

Epidemiologic data were collected, including geographic location, affected cattle category, incidence and mortality, and source of *A. atromaculatus* consumption. In addition, clinical findings as reported by field veterinarians were reviewed, and 2 affected animals were clinically examined by 2 of the authors (J.A. García, J.M. Livio). The pastures on which the animals grazed were examined, and insects associated with all 6 outbreaks were collected for identification.

Autopsies were performed on 8 animals from the 6 outbreaks. Two autopsies were performed on each one of outbreaks 1 and 2, and an autopsy was performed on one animal from each of outbreaks 3–6.

Histopathology and several other ancillary tests were performed following standard operating procedures of the National Institute of Agricultural Technology (Argentina), DILAVE "Miguel C. Rubino" Laboratory (Uruguay), and the California Animal Health and Food Safety Laboratory–UCDavis (USA). Samples of liver, kidney, spleen, lung, skeletal muscle, small and large intestines, stomach, forestomachs, adrenal gland, lymph node, esophagus, urinary bladder, heart, and brain were collected, fixed by immersion in 10% neutral-buffered formalin (pH 7.2) for 48 h and embedded in paraffin. Four-micrometer sections were prepared routinely and stained with H&E. Sections of small intestine, rumen, and reticulum were also processed for immunohistochemistry for *Listeria* spp., bovine viral diarrhea virus 1 (BVDV-1; *Pestivirus bovis*), bovine coronavirus (BCoV; *Betacoronavirus 1*), and bovine herpesvirus 1 (BoHV-1; *Varicellovirus bovinealpha 1*). Other tests included aerobic and anaerobic culture; *Salmonella* spp. PCR of spleen, mesenteric lymph nodes, and small intestinal content; direct examination of wet mounts of colonic and rectal mucosa and feces; and PCR for BVDV on mesenteric lymph node and ear skin.

Beetles collected from the alfalfa pasture on which animals of outbreak 1 had been grazing were dried at 37°C for 48 h and ground for toxicologic studies. Insect material was homogenized in 0.1% formic acid in water, and then extracted into 0.1% formic acid in acetonitrile. The resulting supernatant was cleaned up using QuEChERS, followed by lipid removal by dispersive solid-phase extraction. The resulting product was then split into 2 equal portions and evaporated under nitrogen, with 1 portion redissolved for liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis and the other for gas chromatography–mass spectrometry (GC-MS) analysis.

LC-MS/MS analysis was conducted (Q Exactive Orbitrap mass spectrometer; Thermo Fisher) with electrospray ionization in positive mode. An Eclipse Plus C18 column (100 × 2.1 mm; Agilent) was used with 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B), and a gradient from 1–98% mobile phase B. Data were searched for any positive identifications using an in-house library of > 200 compounds, including pesticides (e.g., organophosphates, carbamates, pyrethroids), environmental toxicants, drugs, alkaloids, and other natural products. A separate LC-MS/MS run was also done using parallel-reaction monitoring for batrachotoxin, and comparisons were made to a reference standard.

Targeted GC-MS analysis of cantharidin was conducted (7890 B GC, 5977A MSD, and DB-5MS column; Agilent). Selected ion monitoring (SIM) was used for cantharidin detection, and comparisons were made to a reference standard.

An additional extraction was conducted for non-targeted GC-MS screening, by adding ammonium hydroxide and extracting into ethyl acetate. The resulting supernatant was analyzed by GC-MS as described for cantharidin, with the exception that it was run scanning the entire range of 40–700 *m/z*, rather than using SIM. Data were searched for any positive identifications using commercial libraries.[1,11]

Results

Epidemiology and clinical signs

The outbreaks occurred during the summer of 2023 (January–February) in Argentina ($n = 4$; Pergamino and General Villegas departments of Buenos Aires province, and San Francisco and Villa Constitución departments of Santa Fe province) and Uruguay ($n = 2$; Soriano department; Table 1). Briefly, all outbreaks occurred in beef cattle under extensive or semi-extensive rearing conditions and included Aberdeen Angus, Hereford, and/or crossbreed animals, with a 22.3% mean incidence across farms. The highest mortality (19.2–27.1%) occurred in outbreaks 1 and 2, on animals fed freshly cut green alfalfa that was heavily infested with *A. atromaculatus*. These plants had been harvested and chopped early in the morning before feeding to the animals at mid-morning. In outbreaks 3–6, in which animals had been grazing directly on pastures heavily infested with *A. atromaculatus*, the mortality was 0.5–7.5%. The average case fatality rate was 80.1% (25–100%; Table 1).

Clinical signs were first observed 1–5 d after exposure to the pastures infested with *A. atromaculatus* or feeding plants

Table 1. Epidemiologic data of 6 outbreaks of natural intoxication by *Astylus atromaculatus* in beef cattle farms of Argentina and Uruguay.

Outbreak	Location	Category affected	Incidence, % (affected/total)	Mortality rate, % (no. dead/total)	Case fatality rate, %	Source of *A. atromaculatus*
1	General Villegas, Buenos Aires, Argentina	Heifers; bulls	20.5 (80/390)	19.2 (75/390)	93.8	Alfalfa chaff
2	Pergamino, Buenos Aires, Argentina	Steers; heifers	35.3 (300/850)	27.1 (230/850)	76.7	Alfalfa chaff
3	San Francisco, Santa Fe, Argentina	Cows	2.2 (4/185)	0.5 (1/185)	25	Alfalfa pasture
4	Villa Constitución, Santa Fe, Argentina	Cows	5.8 (7/121)	5.8 (7/121)	100	Alfalfa pasture
5	Soriano, Uruguay	Cows; calves	1.5 (3/200)	1.5 (3/200)	100	Alfalfa pasture
6	Soriano, Uruguay	Heifers	10 (4/40)	7.5 (3/40)	75	Native pasture*
Average	NA	NA	22.3%	17.8%	80.1	NA

NA = not applicable.
* Native pasture consisted mostly of *Paspalum* sp.

Table 2. Clinical signs and gross findings in 6 outbreaks of natural intoxication of cattle with *Astylus atromaculatus*.

Outbreak*	Clinical signs	Main gross findings†	Anatomic region affected (*n*)
1	Anorexia, diarrhea (occasionally with blood), depression, reluctance to move, sudden death	Multifocal hemorrhagic and fibrinonecrotizing enterocolitis; focally extensive ulceration with reddened ruminal mucosa	Jejunum (2), ileum (2), colon (1), ileocecal valve (2), rectum (1), rumen (1), reticulum (1)
2	Anorexia, diarrhea (occasionally with blood), depression, reluctance to move, sudden death	Multifocal hemorrhagic and fibrinonecrotizing enteritis; ruminal, reticular, and omasal multifocal-coalescing ulceration	Jejunum (2), ileum (2), ileocecal valve (1), rumen (1), reticulum (2), omasum (1)
3	Depression, diarrhea, and sudden death	Diffuse necrohemorrhagic enteritis; focally extensive reddened ruminal mucosa	Jejunum, ileum, ileocecal valve, colon, rumen
4	Depression and diarrhea	Diffuse necrohemorrhagic enteritis	Jejunum, ileum, rumen
5	Diarrhea	Multifocal hemorrhagic and fibrinonecrotizing enteritis	Jejunum
6	Diarrhea, bloat, and sudden death	Multifocal hemorrhagic and fibrinonecrotizing enteritis	Jejunum

* In all cases, entire or parts of *A. atromaculatus* were found in ruminal content.
† Two autopsies were performed in each outbreak 1 and 2; 1 autopsy was performed in each of the remaining outbreaks, 3–6.

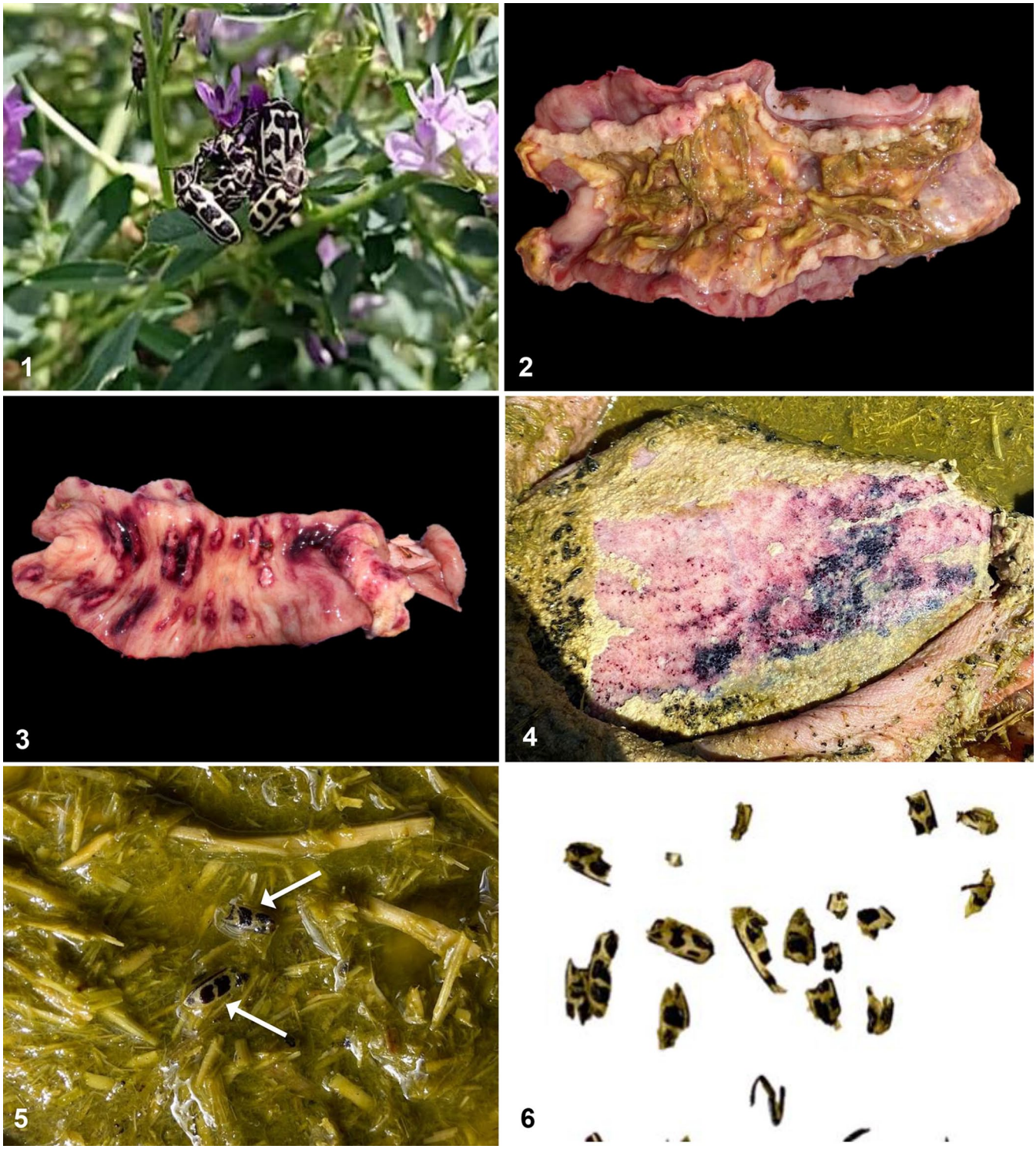
harvested from those pastures, were similar in all outbreaks, and included anorexia, lethargy, hyperthermia, hindlimb weakness, reluctance to move, recumbency, and diarrhea, which was occasionally bloody (Table 2). Sudden death was common mainly in outbreaks 1 and 2. In animals that survived for up to 15 d, tucked-up abdomens were observed. In one animal from outbreak 6, ruminal bloat occurred. Most affected animals died within 5 d of the first exposure to insects, although in a few cases in outbreaks 1 and 2, the clinical course extended for 15 d after initial consumption of the beetles. The 2 animals examined clinically were found isolated from the rest of the herd, in sternal recumbency with reluctance to stand and severe apathy. The 2 animals had rectal temperatures of 41°C and 42°C, dyspnea, and ruminal atony.

Pasture examination

Beetles collected from the pastures were identified as *A. atromaculatus* in all 6 outbreaks (Fig. 1). In outbreaks 1–5, the pastures consisted of alfalfa with 80–90% of the plants in the flowering stage. The number of insects varied from a few to ~80 individuals per plant. The insects were more active after sunrise when the temperatures increased, but remained sedentary in clusters during the night. In outbreak 6, animals were grazing on native pasture consisting mostly of *Paspalum* spp., of which the great majority of the plants were flowering, with 10–20 insects per plant.

Gross findings

Briefly, the 8 animals autopsied had fibrinonecrotizing enteritis or enterocolitis (Table 2; Fig. 2). In all cases ($n = 8$), the jejunum was affected, followed by the ileum ($n = 6$), colon ($n = 2$), and rectum ($n = 1$). The intestinal lesions were characterized by extensive segmental necrohemorrhagic areas, occasionally covered with fibrinous pseudomembranes. Occasionally ($n = 3$), the intestinal mucosa had multifocal-to-coalescing oval, 1–2-cm ulcers with dark-red centers,



Figures 1–6. *Astylus atromaculatus* and gross findings in cattle naturally intoxicated with this insect. **Figure 1.** *A. atromaculatus* pollinating alfalfa flowers. Note characteristic yellow elytra with bilaterally symmetric black spots. **Figure 2.** Diffuse necrotic jejunal enteritis covered with fibrinous pseudomembranes. **Figure 3.** Multifocal-to-coalescing ulcerations throughout the jejunal mucosa. **Figure 4.** Focally extensive detachment of ruminal mucosa with necrohemorrhagic surface. **Figure 5.** Ruminal content with elytra fragments (arrows) from *A. atromaculatus*. **Figure 6.** Fragments of *A. atromaculatus* collected from ruminal content, mainly elytra and legs.

surrounded by a hemorrhagic ring, which were covered by fibrin (Fig. 3). These lesions were also visible from the serosal side. In less-affected areas of the intestine, there was abundant yellow mucoid content, and the mucosa was diffusely hyperemic. In all cases, the content of the small intestine was liquid and occasionally hemorrhagic. In the mucosa of the large intestine, a few areas of hemorrhage and necrosis were observed. Colonic and cecal contents were liquid and hemorrhagic ($n=2$). The ileocecal valve was thickened, and the mucosa was necrohemorrhagic ($n=4$). In 2 animals, there was a moderate amount of clear liquid and fibrin strands adhered to the serosa of the jejunum.

The rumen and/or reticulum were affected in 4 cases; the omasum was affected in only 1 animal. Ruminal lesions consisted of extensive areas of reddened and detached mucosa ($n=3$; Fig. 4). Similar lesions were observed in the reticulum ($n=3$). In only 1 case, the omasum had small ulcers. The forestomachs and abomasum were filled with fibrous vegetal material mixed with large amounts of water and soil ($n=3$). The ruminal and abomasal content pH was ~7, and 3–4 in 4 animals in which these measurements were taken. In the ruminal content of all 8 cases, fragments of *A. atromaculatus* were found, consisting mostly of elytra and legs, and rarely entire beetles (Figs. 5, 6). The abomasal mucosa was diffusely red ($n=4$). The urinary bladder of 3 animals had mucoid content and multifocal-to-coalescing congested areas in the mucosa.

Microscopic findings

Microscopically, the main lesion was multifocal extensive fibrinonecrotizing inflammation in the jejunum and ileum, less frequently in the rumen and reticulum, and unusually in the colon, omasum, or rectum (Table 2). The jejunum was the most severely affected intestinal section, with necrosis of the upper third of the mucosa and, less frequently, areas of transmural necrosis and inflammation (Fig. 7). Intestinal necrosis was characterized by hypereosinophilic areas of the lamina propria with loss of villus and crypt epithelium and large amounts of fibrin. In these areas, diffuse mild-to-moderate inflammatory infiltrate was composed mainly of neutrophils and lymphocytes. Overlying the necrotic mucosa was a pseudomembrane of necrotic debris, fibrin, RBCs, and inflammatory cells. The areas of necrosis were delimited by a line of inflammatory infiltrate of degenerate and viable neutrophils. Multifocal ulceration of the superficial epithelium through which fibrin, viable and degenerate neutrophils, a few erythrocytes, and necrotic debris were exuded to the lumen was observed. Multifocal crypt dilation with cell debris and mucus in the lumen was observed (Fig. 8). In the submucosa, rarely, a few blood vessels had fibrinoid necrosis and were surrounded by a rim of degenerate and viable neutrophils, and fewer lymphocytes, plasma cells, and macrophages (Fig. 9).

The ileum, colon, and rectum had lesions similar to those described for the jejunum. In addition, in the ileum of 3 cases, crypts of Lieberkühn had herniated into the submucosa, where they effaced lymphoid follicles in Peyer patches. In the reticulum and rumen, the mucosa had multifocal areas of necrosis and ulceration with fibrinous exudate and hemorrhage, mostly with detachment of the epithelium. The areas of necrosis were separated from the deep, more-normal areas by a band of degenerate neutrophils (Fig. 10). The submucosa was multifocally expanded by fibrin, hemorrhage, edema, neutrophilic and lymphocytic infiltrate, and numerous blood vessels with fibrinoid degeneration characterized by hyaline change. In the surrounding less-affected areas, there was degeneration and necrosis of keratinocytes in the stratum spinosum. A moderately diffuse lymphoplasmacytic infiltrate was evident in the mucosa and submucosa. In one case, focally extensive ulceration was present in the omasum. In 2 cases, mild-to-moderate aggregates of subepithelial lymphocytes with occasional macrophages, mainly perivascular, were observed. The urinary bladder had marked diffuse congestion in 3 animals.

Microbiology

Testing for BVDV, *Salmonella* spp., BCoV, BoHV-1, and *Listeria* spp. was negative in 6 of the 8 autopsied animals on which these tests were performed. No parasites or parasite eggs were observed on wet mucosal smears from any of the 8 animals.

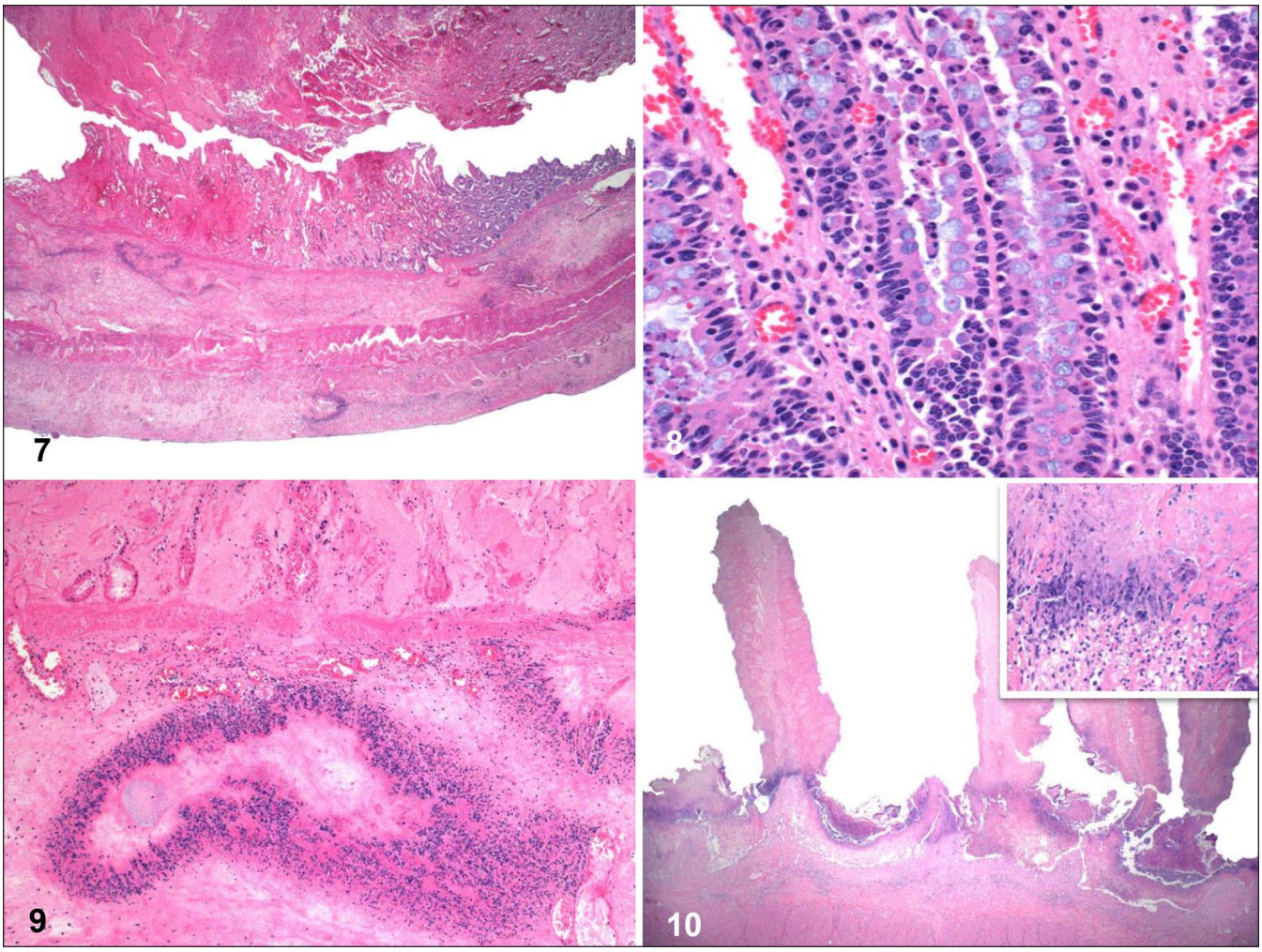
Toxicology

Neither batrachotoxin nor cantharidin were detected in the tested insect material using LC-MS/MS and GC-MS at reporting limits of 1 and 500 ppb, respectively. No other toxic substances were identified by non-targeted GC-MS and LC-MS screening methods.

Discussion

The epidemiologic evidence, clinical signs, anatomopathologic findings, including the presence of the beetles in the ruminal content, and the negative results of tests for several other gastrointestinal pathogens in all 6 outbreaks are highly suggestive of intoxication by *A. atromaculatus*. Intoxication by this beetle has been reproduced experimentally in sheep and guinea pigs resulting in fibrinonecrotizing enteritis,[8] and the disease was reproduced in cattle resulting in fibrinonecrotizing rumenitis and enteritis, and death (http://www.inia.uy/investigación-e-innovación/plataformas/Plataforma-de-Salud-Animal-/Estudio-realizado-en-INIA-confirma-que-el-escarabajo-Siete-de-Oro-es-toxico-para-bovinos; Spanish).

To our knowledge, natural intoxication with *A. atromaculatus* has not been reported previously in cattle. In 1972, outbreaks in cattle in South Africa were suspected, but no autopsies were performed on affected animals.[8] The most important epidemiologic feature of these 6 outbreaks is the association with pastures heavily contaminated with the bee-



Figures 7–10. Microscopic lesions in cattle naturally intoxicated with *Astylus atromaculatus*. **Figure 7.** Transmural jejunal necrosis and inflammation characterized by hypereosinophilic areas of the lamina propria, loss of villi and crypt epithelium, and large amounts of fibrin delimited by a line of inflammatory infiltrate of degenerate and viable neutrophils. Overlying the necrotic mucosa is a fibrinous pseudomembrane with necrotic debris. HE. **Figure 8.** Dilated crypts with dead cells and cell debris in the jejunal lumen. Neutrophils surround the affected crypts. H&E. **Figure 9.** Area of necrosis surrounded by a rim of degenerated neutrophils in the jejunal submucosa. H&E. **Figure 10.** Full-thickness necrosis of ruminal villi, which are separated from the deeper tissues by a band of degenerate neutrophils (inset). H&E.

tle, coupled with the acute clinical onset immediately after exposure to the beetles.

A. atromaculatus is a common pollen beetle in crops in South America, feeding mainly on maize, sunflower, and sorghum, among others.[4,7,12,21] The areas in which these outbreaks occurred are well known for their intensive agricultural activity. During the summer of 2023, severe drought was recorded in these areas (http://www.inia.uy/Publicaciones/Documentos%20compartidos/Informe%20agroclimatico%20INIA-GRAS%20Enero%20de%202023.pdf, Spanish; https://www.smn.gob.ar/sites/default/files/informe_sequia.pdf, Spanish) with consequent low crop production and flowering rate. It is speculated that these environmental conditions, in particular the lack of crop flowers, led the beetles to seek other pollen niches on which to feed (i.e., the alfalfa and mixed-species pastures, which were in full bloom). Anecdotal information also indicates that during the summer of 2023, outbreaks of diarrhea and mortality were reported in sheep, horses, dogs, and chickens that had access to the beetles; unfortunately, autopsies were performed only in sheep, in which gross and microscopic changes similar to those described in the cattle of this paper were found (J.A. García, G.J. Cantón, unpublished observations).

The highest mortality was observed in animals fed freshly cut green alfalfa, under semi-extensive rearing systems. In those cases, the alfalfa was harvested in the early morning, which is the period when the pollen beetle is least active and

is found in clusters over the flowers.[2,5] The lower mortality in the outbreaks associated with consumption of the beetle in natural pastures was probably associated with the fact that the animals were grazing during the day when the beetles are more active and could fly, which probably led to fewer insects clustered on the pasture flowers. Based on a previous study[8] and our own experience, it is likely that a critical mass of insects needs to be ingested for clinical signs to occur.

Other insects, such as blister beetles (*Meloidae*), are associated with gastrointestinal disease in horses and cattle after consumption of contaminated alfalfa.[3,9,13,16] Blister beetles produce cantharidin, a potent irritant that causes necrosis and degeneration of the intestinal mucosa.[16,20] Although the intestinal lesions observed in the animals of our study are similar, although more severe, to those associated with cantharidin-producing insects, we did not find cantharidin in *A. atromaculatus* in our study, nor in the previous study.[8] We did not find blister beetles in any of our 6 outbreaks, nor were significant urinary, esophageal, or cardiac lesions, characteristic of cantharidin intoxication, found in the affected animals.[13,16,18]

Intoxication in cattle affecting the gastrointestinal system has been reported by pederin toxin following accidental ingestion of tropical *Paederus fuscipes*[9]; we did not identify this insect in our study. Based on the chemical structure of pederin, it would have been detectable by our LC-MS/MS screen, but there was no evidence of its protonated or sodium adduct in the tested beetle sample.

Other conditions that may produce similar mucosal lesions in the alimentary tract, and that have been described in Argentina and Uruguay, include intoxication by *Ricinus communis*,[10] lead arsenate,[14] and *Baccharis corifidolia*.[15] No exposure to any of these toxicants was observed in the animals in our outbreaks. Ruminal acidosis was also ruled out based on normal ruminal pH coupled with the absence of typical ruminal lesions, and lack of history of exposure to diets rich in rapidly fermentable carbohydrates. Infectious agents, including BVDV, BCoV, BoHV-1, *Salmonella* spp., *Yersinia* spp., *Listeria* spp., and *Eimeria* spp., were also ruled out based on immunohistochemistry and/or bacterial culture results.

The leakage and fibrin observed in the intestinal mucosa of affected animals suggest vascular injury leading to vascular permeability, which, among other conditions, occurs in cases of cantharidin intoxication.[6,17,22] Fibrinoid vascular necrosis was observed in a few cases of outbreaks 1 and 2. It is possible that the putative toxic principle of *A. atromaculatus* has its primary action on blood vessels. It is also possible that the action of this agent starts on the enterocytes, and the vascular lesions observed in some animals were secondary.

We used a general untargeted approach looking for a wide variety of possible natural and synthetic toxicants by both GC-MS and LC-MS/MS. For the untargeted approach, our GC-MS libraries included thousands of potential compounds, and our LC-MS/MS library included >200 compounds. We also followed a targeted approach specific for batrachotoxin and cantharidin. A more exhaustive approach might include additional extraction and sample clean-up strategies, alternative chromatography methods, and additional libraries. Additionally, it might be possible to better characterize the chemical nature of potential toxicants (e.g., hydrophilic or hydrophobic) by reproducing the syndrome using different extracts of the beetles.

Acknowledgments

We thank Kevin Mcloughlin, Jorge Rusconi, Ernesto Sacconi, Gustavo Aunchayna, and Nicolás Mathe for allowing investigation of the farms involved.

Declaration of conflicting interests

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

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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