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Wischnewsky spots in cases of fatal hypothermia in cattle[1]

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ABSTRACT.- Souza L.L., Guizelini C.C., Almeida B.A., Silva T.X, Lima A.S., Carvalho V.F., Driemeier D. & Lemos R.A.A. 2024. **Wischnewsky spots in cases of fatal hypothermia in cattle**. *Pesquisa Veterinária Brasileira 44:e07408, 2024*. Laboratório de Anatomia Patológica, Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Av. Sen. Filinto Müler 2443, Campo Grande, MS 79070-900, Brazil. E-mail: larissa.lobeiro@ufms.br

Wischnewsky spots (WS) are hemorrhagic, non-erosive, and non-ulcerative lesions that affect the gastric mucosa. They are considered common in the stomachs of humans who have died from hypothermia and are rarely described in other animal species. This study describes the occurrence of WS in cattle that had died of hypothermia in two cities in the state of Mato Grosso do Sul. Two properties were visited, and three cattle were necropsied by the team from the Pathological Anatomy Laboratory of the Federal University of Mato Grosso do Sul (LAP-UFMS). The epidemiological conditions and clinical signs of the cattle examined on both properties were similar. The deaths occurred on days when there was a sudden drop in environmental temperature, with continuous rain for more than 24 hours. During the visits, the most affected live cattle were found in sternal decubitus, with their heads resting on their flanks, or laterally in a comatose state, with nystagmus and a rectal temperature below 32°C. Macroscopically, there were multifocal to coalescent red and black areas on the mucosal surface of the abomasums of the three cattle necropsied, mainly at the apex of the folds, oval or punctate in shape, measuring 0.1 to 1.0cm in diameter. Microscopically, these dark areas corresponded to areas of rarefaction of the mucosal epithelial cells associated with hemorrhage and the frequent deposition of golden-brown pigment (hematin) in the extracellular medium. The diagnosis of hypothermia in cattle was based on epidemiology, clinical-pathological examination, and the exclusion of differential diagnoses. The lesions found in the abomasum of cattle that had died from hypothermia were compatible with WS, highlighting the importance of recognizing them in cases of cattle deaths in which this condition is suspected.

INDEX TERMS: Wischnewsky spots, abomasum, cold, bovine diseases, hypothermia, cattle.

RESUMO.- [**Manchas de Wischnewsky em casos de hipotermia fatal em bovinos.**] As manchas de Wischnewsky (WS) são lesões hemorrágicas, não erosivas e não ulcerativas que acometem a mucosa gástrica. Eles são considerados comuns no estômago de humanos que morreram de hipotermia e raramente são descritos em outras espécies animais. Este estudo descreve a ocorrência de WS em bovinos que morreram por hipotermia em duas cidades do estado de Mato Grosso do Sul. Duas propriedades foram visitadas e três bovinos foram necropsiados pela equipe do Laboratório de Anatomia Patológica da Universidade Federal de Mato Grosso do Sul (LAP-UFMS). As condições epidemiológicas e os sinais clínicos dos bovinos examinados nas duas propriedades foram semelhantes. As mortes ocorreram em dias em que houve

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queda brusca da temperatura ambiente, com chuva contínua por mais de 24 horas. Os bovinos vivos mais acometidos no momento das visitas foram encontrados em decúbito esternal, com a cabeça apoiada nos flancos, ou lateralmente em estado de coma, com nistagmo e temperatura retal abaixo de 32°C. Macroscopicamente, havia áreas vermelhas e pretas multifocais a coalescentes na superfície mucosa dos abomasos dos três bovinos necropsiados, principalmente no ápice das pregas, de formato oval ou pontiforme, medindo de 0,1 a 1,0cm de diâmetro. Microscopicamente, essas áreas escuras correspondiam a áreas de rarefação das células epiteliais da mucosa associadas à hemorragia e à frequente deposição de pigmento marrom-dourado (hematina) no meio extracelular. O diagnóstico de hipotermia em bovinos foi baseado na epidemiologia, no exame clínico-patológico e na exclusão de diagnósticos diferenciais. As lesões encontradas no abomaso de bovinos que morreram por hipotermia foram compatíveis com WS, destacando a importância de reconhecê-las em casos de mortes de bovinos em que haja suspeita desta condição.

TERMOS DE INDEXAÇÃO: Manchas de Wischnewsky, abomaso, frio, doenças de bovinos, hipotermia, bovinos.

INTRODUCTION

Hypothermia is the reduction of body temperature below the normal physiological temperature, which occurs when heat loss is faster than its production by the body, causing functional injuries and animal deaths (Merck et al. 2013). In cattle, intense heat loss occurs when a sudden drop in environmental temperature is associated with wind and rain. Cattle are usually found dead the day after the sudden drop in temperature or are found alive in a sternal decubitus position, with their heads resting on their flanks, or laterally in a comatose state, with nystagmus and a rectal temperature below 37°C (Santos et al. 2012, Lemos & Riet-Correa 2022).

Deaths from hypothermia occur in cattle of different ages. However, they are more frequent in newborns when there is an association between low environmental temperature, low birth weight, and the calf's starvation, as reduced energy reserves result in heat production (Riet-Correa 2022).

A *post-mortem* finding observed in cases of cold stress is the occurrence of so-called "Wischnewsky spots" (WS), often described in autopsies of humans with hypothermia and characterized by red to dark brown spots on the stomach mucosa, which correspond to a superficial, non-erosive, and non-ulcerative hemorrhagic lesion of the mucosa (Tsokos et al. 2006, Bright et al. 2013a, Palmiere et al. 2014, Sacco et al. 2022). In other animal species, this finding is rarely described. However, it has already been documented in two dogs found dead in an extremely cold environment (Stern & Vieson 2017), in suspected cases of hypothermia in a free-living monkey and a domestic rabbit (Almeida et al. 2021), and in manatees that presented cold stress (Martony et al. 2019).

In forensic pathology, although the absence of WS does not exclude cases of fatal hypothermia, the presence of this finding is indicative. It helps in the investigation of the mortality of people and other animals exposed to low environmental temperature, especially where information such as body temperature and the exact circumstance of death is not available (Bright et al. 2013a, Palmiere et al. 2014).

Given the lack of reports of this finding in cattle with hypothermia and the limitation of important clinical and epidemiological information in potential cases of death from this condition, this study describes the anatomopathological findings of three cases of hypothermia in cattle monitored in the state of Mato Grosso do Sul, with emphasis on the characterization of WS in the necropsied animals.

MATERIALS AND METHODS

Animal Ethics. This study was not submitted to the Ethics Committee on the Use of Animals (CEUA) of the "Universidade Federal de Mato Grosso do Sul" (UFMS), because the cases studied were sick or dead cattle from laboratory routine and the legislation only provides for the use of live animals for experimentation.

On June 15th and 16th of 2023, a team from the "Laboratório de Anatomia Patológica" of the "Universidade Federal de Mato Grosso do Sul" (LAP-UFMS) visited two properties to attend to cases of bovine mortality, on the first day in Campo Grande (Property 1) and the other day in Nova Andradina (Property 2), both cities in the state of Mato Grosso do Sul.

Epidemiological data from the properties visited were obtained through interviews with the owners and epidemiological and clinical investigations by the LAP-UFMS team. The following parameters were assessed to characterize the epidemiology of the places where the cases had occurred: body condition score of the cattle necropsied, presence of natural or artificial shelters, breed and age of the cattle affected, distributions of deaths in the different pastures on the property, and location of the cadavers within the same pasture. The body condition score (ECC) was classified on a scale of 1 to 9, where the extremes correspond to very thin and very fat animals, respectively (Nicholson & Butterworth 1986).

Climate data, such as the minimum and maximum temperatures in the cities of the properties visited on the day and on days close to when the deaths had occurred, were obtained by consulting the AccuWeather website recorded, and the necropsies were carried out by the LAP team.

On Property 1, a 12-year-old cow found dead was necropsied. At the same time, clinical examination and body temperature measurement using a digital thermometer (G-tech®) were carried out on four other cattle that had resisted the sudden drop in temperature and were found alive.

On Property 2, two cattle were necropsied: an adult crossbreed cow and a 7-month-old Nelore calf. The cow was found dead during the visit, the calf and two other animals found alive in lateral decubitus were clinically assessed, and their rectal temperature was measured with the same thermometer used on Property 1. The calf and another adult bovine were submitted to blood sampling for a complete blood count and biochemical tests for the enzymes aspartate aminotransferase (AST), urea, and creatinine, as well as feces collection directly from the rectal ampulla for the egg count per gram of feces (OPG) test. Due to the calf's poor prognosis, it was euthanized and necropsied. Euthanasia was carried out in accordance with guidelines from the "Conselho Federal de Medicina Veterinária (CFMV)" from Brazil.

During necropsy, samples of all organs were collected, fixed in 10% formalin, processed routinely, and stained with hematoxylin and eosin (HE).

RESULTS

The epidemiological conditions and clinical signs of the cattle examined on the two properties visited were similar. On both properties, there was a sudden in environmental temperature in the week in which the animals were necropsied (Table 1) and constant rainfall for more than 24 hours in the periods leading up to the death of the cattle.

On both properties, the animals found dead, including the necropsied animals, were in groups, close to fences, or alone among small bushes and tree trunks in different paddocks, which had neither natural nor artificial shelters, making the area where the cattle stayed an open field. Deaths were rapid, varying between one and two days, and concentrated in the two days when the adverse environmental conditions were present. The most affected live cattle were found in lateral decubitus, unable to maintain themselves in sternal recumbency or stand up, even with assistance. They also had imperceptible and abdominal breathing, did not react to environmental stimuli, but sometimes tried to move their thoracic limbs, imitating the pedaling movement.

Property 1 used an extensive breeding system. In one paddock, located in the highest region of the farm, out of a total of 151 Nelore cows and 138 calves at food, 100 had died (95% of the dead animals were cows) between June 14th and 15th, with a higher concentration of deaths on June 14. During the visit, three adult cows and a calf were alive and showed similar clinical signs: head shaking and frequent mooing. The rectal temperature of these animals was below 32°C, below the minimum temperature detected by the digital thermometer. A 12-year-old cow found dead was necropsied.

Property 2 also used a breeding system with calves, heifers, cows, and bulls in a semi-extensive production system, sequestering animals in troughs and providing feed during dry periods. There were 1,556 cattle on the property, divided into eight lots of 100 to 150 animals. Up until the day of the visit, 72 deaths had been recorded, including cattle of all categories and from different paddocks, except for bulls. The euthanized calf and an adult Nelore cow showed the same clinical signs; they were found in lateral decubitus and showed depression, absence of corneal reflex, nystagmus, shallow and abdominal breathing, and did not react to environmental stimuli but sometimes tried to move their thoracic limbs, imitating the pedaling movement. The calf's rectal temperature was below the minimum mark on the digital thermometer (32°C), whereas the adult's rectal temperature was 32.4°C. In the biochemical profile tests, there was an elevation in the serum level of aspartate aminotransferase (AST) in the cow and calf of 391.7 and 421.6 U/L, respectively (reference interval, 54-135U/L - Cornell University 2017). No alterations were observed in the blood count of either animal. The OPG tests showed low counts of strongylid eggs in the calf (175 eggs) and in the cow (0 eggs).

The three cattle necropsied were macroscopically in fair body condition, with fat reserves that made it impossible to see the bony prominences. The ECC was classified as 6 (fair to good). In the abomasum, throughout the mucosal surface, especially at the apex of the folds, there were multifocal to coalescent, punctate to oval areas, which varied from red to black and measured between 0.1 to 1.0cm in diameter (Fig.1 and 2). The other organs showed no macroscopic alterations.

Histologically, the blackened focal areas observed macroscopically in the abomasum corresponded to regions of rarefaction of the mucosal epithelial cells associated with hemorrhage and the frequent deposition of a golden-brown pigment, compatible with hematin, abundant in the extracellular medium (Fig.3 and 4). Sometimes, the parietal cells in the middle of and adjacent to the pigment were pycnotic and karyolitic. In addition, a moderate lymphocytic infiltrate was observed in the lamina propria of two of the cattle evaluated.

Table 1. Minimum and maximum temperatures in the week in which deaths from hypothermia had occurred in cattle in the cities of the properties visited in Mato Grosso do Sul, on June of 2023

Property	Dates	Minimum temperature (°C)	Maximum temperature (°C)
1 (Campo Grande)	June 10 and 11, 2023	18	31
	June 14 and 15, 2023	6	14
2 (Nova Andradina)	June 10 and 11, 2023	16	31
	June 15 and 16, 2023	7	18

Source: AccuWeather.

Fig.1. Wischnewsky spots in hypothermia in cattle. Multiple black dots and spots, sometimes random red ones, on the mucosal surface of the abomasum.

Fig.2. Wischnewsky spots in hypothermia in cattle. Black, flat, coalescing foci on the mucosal surface of the abomasum, mainly at the apex of the folds.

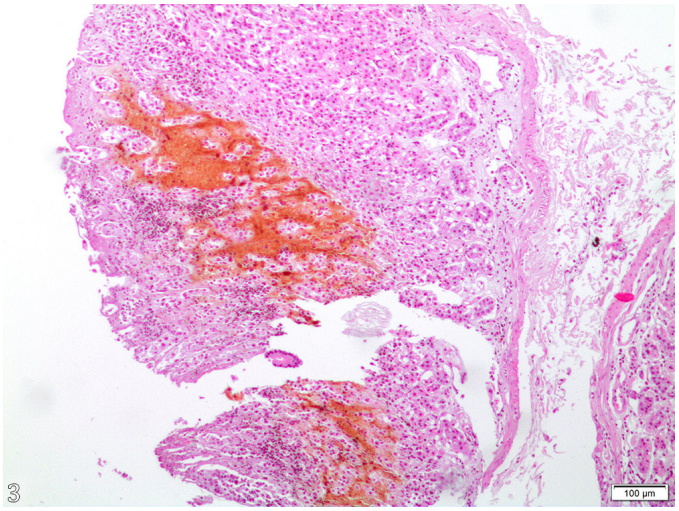

Fig.3. Wischnewsky spots in hypothermia in cattle. Abomasum. Two areas have marked deposition of golden-brown pigment (hematin) in the middle of the mucosal epithelial cells. HE, obj.10x.

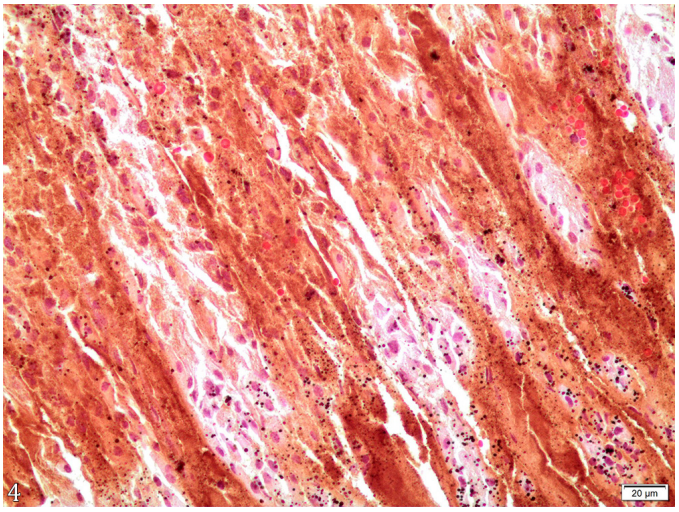

Fig.4. Wischnewsky spots in hypothermia in cattle. Abomasum. Marked deposition of hematin accompanied by areas of hemorrhage. The parietal cells are preserved. HE, obj.40x.

DISCUSSION

The diagnosis of hypothermia in cattle was based on epidemiological, clinical, and anatomopathological findings and the exclusion of differential diagnoses. Measuring the body temperature of the live cattle on the two properties, which was below 32°C, led to the conclusion that the clinical picture was hypothermia since this temperature is considerably below the body temperature considered normal for the species, which is 38.5°C (Constable et al. 2017).

According to world map productions using the Köppen-Geiger climate system, the predominant climate in the state of Mato Grosso do Sul is Aw - a megathermic climate with a dry winter and summer rainfall, in which the average temperature of the coldest month is above 18°C and rainfall is less than 60mm in the least rainy month (Peel et al. 2007, Beck et al. 2018). Outbreaks of bovine mortality due to hypothermia in the state were mostly associated with a sudden drop in temperature, associated with rain and wind, poor nutritional status of the animals, and the absence of natural shelters on the properties (Santos et al. 2012). The nutritional status of the necropsied cattle and other affected animals on the properties was fair to good, unlike in previous outbreaks. The absence of natural shelters in the paddocks where the deaths had occurred was a similar epidemiological characteristic in all cases and is increasingly common in the state due to changes in the production system, with Mato Grosso do Sul being the state with the largest area of adoption of crop-livestock-forest integration (CLFI) systems in Brazil (Skorupa & Manzatto 2019).

In cattle, urea poisoning and botulism are the main differential diagnoses of hypothermia since these diseases have similar clinical signs, no significant *post-mortem* lesions, and the death of several animals in a short period. In the case of urea intoxication, although it is related to rain on properties with uncovered troughs, the animals are usually found dead near the troughs (Gimelli et al. 2023), a situation that was not observed in the present cases.

The microscopic finding of hypereosinophilic neurons (red neurons) in the gray matter of the brain has already been described in cases of hypothermia in cattle (Colodel et al. 2006, Santos et al. 2012, Lemos & Riet-Correa 2022). However, validating this finding as evidence of neuronal degeneration or death can be erroneous and has a poor diagnostic character since it may be an artifact produced by handling the organ or by trauma to the brain tissue (Jortner 2006).

The red and black foci identified in the abomasum of affected cattle are macro and microscopically similar to the WS associated with fatal hypothermia in humans. This *post-mortem* finding is characterized by hemorrhagic, random, and multidimensional lesions of the gastric folds (Tsokos et al. 2006, Palmiere et al. 2014). An experimental study using rats showed that cold stimulates increased gastrin release and acidification of the gastric mucosa, causing small hemorrhages (Yang et al. 2020). Microscopically, these hemorrhagic lesions can be hematinized after the interaction of hemoglobin released in the autolysis of red blood cells and gastric secretion; they are therefore classified as peri-agonal circumscribed hemorrhages related to hypothermia (Tsokos et al. 2006). The presence of hemorrhages in the mucosa of the cases presented suggests that the golden-brown pigment is also hematin.

Notably, WS must be differentiated from abomasal ulcers and erosions, which occur in cattle and are incidental findings seen in animals of various ages that died from various causes. Ulcers and erosions are macroscopically characterized by linear to punctate, brown-to-black depressions on the mucosal surface (Uzal et al. 2016). Although they are usually multiple and occur randomly throughout the organ, they are characterized by tissue loss associated with inflammation, which is not the case in WS observed in cases of hypothermia (Tsokos et al. 2006).

The formation of WS reported in humans is complex as they are associated with various factors, such as decreased body temperature, physical and psychological stress, and other comorbidities (Bright et al. 2014, Sacco et al. 2022). In animals, stress factors have also been identified as being associated with cases of hypothermia, such as neoplasia and starvation in dogs (Stern & Vieson 2017), multiple fractures in

rabbits, and severe intestinal parasitism in monkeys (Almeida et al. 2021). In the cattle evaluated, none of these physical stress factors could be identified. However, as demonstrated in experiments with rats, psychological stress or the feeling of imminent death can be a significant effect modifier in the development of WS in cases of lethal hypothermia (Bright et al. 2013b).

CONCLUSIONS

The lesions found in the abomasum of cattle that had died from hypothermia are compatible with Wischnewsky spots (WS), which have been frequently identified in humans and described in other animal species exposed to cold.

The presence of WS associated with clinical and epidemiological findings may be suggestive of hypothermia in the absence of other diseases, highlighting the importance of recognizing them in cases of bovine deaths in which hypothermia is suspected.

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Conflict of interest statement.- The authors declare that there are no conflicts of interest.

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