




Urea poisoning in cattle: A brief review and diagnostic approach¹

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ABSTRACT.- Gimelli A., Pupin R.C., Guizelini C.C., Gomes D.C., Franco G.L., Vedovatto M., Gaspar A.O. & Lemos R.A.A. 2023. **Urea poisoning in cattle: A brief review and diagnostic approach.** *Pesquisa Veterinária Brasileira* 43:e07228, 2023. Laboratório de Anatomia Patológica da Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Av. Senador Felinto Muller 2443, Campo Grande, MS 79070-900, Brazil. E-mail: amanda_gimelli@hotmail.com

Urea is an organic compound characterized as a white, solid, and hygroscopic substance. It is recognized as a source of non-protein nitrogen (NPN) and is widely used as a partial replacement for protein in cattle diets due to the ability of the ruminal microbiota to convert it into microbial protein. Despite the advantages of using urea, it also has limitations, particularly the proximity between metabolizable and toxic or fatal doses. Furthermore, for safe use, a period of adaptation is necessary for the animals. Poisoning is characterized by rapid and generally fatal development, which is frequent in non-adapted animals but can also occur in those with previous adaptations. The aim of this study was to characterize the clinical, epidemiological, and pathological aspects of urea poisoning through a brief review and a retrospective study. In addition, interviews were conducted with veterinarians who frequently send diagnostic material to the Laboratory of Anatomic Pathology of the “Faculdade de Medicina Veterinária e Zootecnia” (LAP-FAMEZ) to assess their perception of the outbreaks of urea poisoning. The objective was to obtain a comparative scenario between published cases and those received by the laboratory while considering the real situation of this condition in the field. During this retrospective study, only four outbreaks were investigated; in one, the diagnosis was possible through experimental reproduction. Of 35 interviewees, 88.9% said they had seen more than one case compatible with urea poisoning, but 87.5% did not perform a necropsy and/or send material to confirm the diagnosis. The results show that the reality of urea poisoning may be very distant from that reported in previous studies due to the difficulty often observed in the diagnostic approach, so we developed a flowchart aiming to provide a useful guide for field veterinarians.

INDEX TERMS: Ammonia, cattle disease, diagnostic, non-protein nitrogen, outbreaks.

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RESUMO.- [Intoxicação por ureia em bovinos: breve revisão e abordagem diagnóstica.] A ureia é um composto orgânico, que se apresenta como uma substância branca, sólida e higroscópica, e é reconhecida como fonte de nitrogênio não proteico (NPN), sendo amplamente utilizada como fertilizante e também como substituto parcial de proteína em bovinos devido à capacidade da microbiota ruminal de convertê-la em proteína microbiana. Apesar das vantagens que envolvem o uso da ureia, ela também apresenta limitações, a principal delas é a proximidade entre doses metabolizáveis e tóxicas ou fatais, e para que seja utilizada com segurança é necessário

um período de adaptação dos animais. A intoxicação é caracterizada por evolução rápida e geralmente fatal, sendo frequente em animais não adaptados, mas pode ocorrer naqueles com adaptação prévia. O objetivo deste estudo é caracterizar os aspectos clínicos, epidemiológicos e patológicos da intoxicação por ureia por meio de uma breve revisão e um estudo retrospectivo. Adicionalmente foram realizadas entrevistas com médicos veterinários que frequentemente enviam material para diagnóstico no Laboratório de Anatomia Patológica da Faculdade de Medicina Veterinária e Zootecnia (LAP-FAMEZ) com o objetivo de avaliar a percepção dos mesmos em relação aos surtos de intoxicação por ureia, na expectativa de se obter um quadro comparativo entre os casos recebidos pelo laboratório e levantamentos publicados e a real situação desta condição a campo. No período do estudo retrospectivo, foram acompanhados apenas quatro surtos, sendo que em um deles foi possível diagnosticar por reprodução experimental. Dos 35 entrevistados, 88,9% afirmaram ter atendido mais de um caso compatível com intoxicação por ureia, mas 87,5% deles não realizaram necropsia e/ou envio de material para confirmação do diagnóstico. Foi possível observar que a realidade da intoxicação por ureia pode estar muito distante daquela relatada em estudos anteriores, e devido à dificuldade muitas vezes observada na abordagem diagnóstica, desenvolvemos um fluxograma na expectativa de que ele constitua um guia útil para veterinários de campo.

TERMOS DE INDEXAÇÃO: Amônia, nitrogênio não-proteico, doenças de bovinos, diagnóstico, surtos.

INTRODUCTION

Urea is an organic compound that is solid, white, hygroscopic, and soluble in water. Due to its low cost, it is used as a source of non-protein nitrogen (NPN) to replace, partially the protein in cattle diets. It is given to cattle in blocks and mixed forms with concentrate, minerals, or molasses. Once in the rumen, urea is converted to ammonia, the main nitrogen source (N) for many prokaryotes, fungi, and plants, including the rumen microbial population. This promotes the growth of microorganisms and the synthesis of microbial protein that is used by the host. It is generally recognized that urea poisoning is the same as ammonia poisoning, the true toxic compound (Whitehair 1989, Gonçalves et al. 2011).

The utilization of urea for livestock might generate interest from an economic view because 100g of urea can be transformed into up to 287g of equivalent protein and can be used to replace up to 35% of dietary protein. In addition, urea supplementation has other advantages, such as easy accessibility and implementation at a lower cost, especially during the dry season. Despite the benefits, the substance has low acceptability by animals (if given in a pure form), it separates when mixed with other substances, and it has a small range between metabolizable doses and toxic or fatal doses, causing animal losses due to inappropriate consumption (Whitehair 1989, Kitamura et al. 2002, Shaikat et al. 2012, Sharma et al. 2016).

Poisoning is characterized by sudden and commonly fatal clinical conditions. Considering that animals adapted to consumption become less susceptible to poisoning, this occurs mainly when non-adapted cattle ingest toxic amounts. However, adapted animals can also present poisoning and

death under certain circumstances (Antonelli 2003, Gonçalves et al. 2011, Sharma et al. 2016).

Despite urea being cited as a common cause of accidental cattle poisoning (Parkes & Shilton 2019), in Brazil, reports are scarce, and most are not very detailed. The poisonings in previous studies represent 0.42 to 1.18% of the total cases (Schild et al. 2013, Souza et al. 2015, Queiroz et al. 2018, Pupin et al. 2019).

However, these data may not reflect the reality of the problem since data are obtained from surveys of animal pathology laboratories based on necropsies and necropsy samples. Urea poisoning often does not allow such a procedure due to its acute evolution and because the animals are often found dead suddenly, commonly in advanced autolysis. In addition, the methodology used for conclusive diagnosis is difficult to apply in practice, especially for field practitioners (Alden et al. 1976, Davidovich et al. 1977, Sharma et al. 2016), since conclusive diagnosis requires complementary tests such as ammonia levels in the blood or the suspected material, in addition to the determination of rumen pH and biochemical alterations (Clark et al. 1951, Alden et al. 1976).

The objective of this study was to briefly review urea poisoning. Furthermore, based on laboratory routine, a diagnostic approach is proposed to assist field veterinarians and reinforce their joint work with the veterinary diagnostic laboratory. Additionally, we demonstrate the picture of this condition in the field and its importance through interviews with practitioners.

MATERIALS AND METHODS

Animal ethics. The experiment was approved by the Animal Ethics Committee (CEUA) of “Universidade Federal de Mato Grosso do Sul” (UFMS), protocol number 1.0372019.

Review. A brief review of urea poisoning was conducted using different bibliographic databases. Case reports and experimental studies involving the topic were included. The keywords used for the search were “urea,” “ammonia,” “poisoning,” “bovine,” and “diagnosis.” Data surveys carried out by veterinary diagnostic laboratories (mainly pathology laboratories) on causes of mortality in cattle in Brazil were also included (keywords: “retrospective,” “study,” “toxic,” “diseases,” “epidemiology,” and “diseases of cattle”). Based on these searches, a brief review of the history, pathogenesis, clinical aspects, and diagnostic criteria was carried out.

Retrospective study. The records of cattle necropsies performed from January 2015 to December 2021 evaluated at the Laboratory of Anatomic Pathology of the “Faculdade de Medicina Veterinária e Zootecnia” (LAP-FAMEZ) at the UFMS were reviewed. The records consisted of necropsies performed by the LAP-FAMEZ staff or field veterinarians who later submitted the material for histopathological evaluation. Files were selected if they contained epidemiological, clinical, and anatomopathological information that allowed the diagnosis of urea poisoning. All information and complementary procedures were collected from the records of each outbreak. In one outbreak (2), an experimental reproduction was made using two sheep to confirm the source of the toxic compound.

Epidemiological investigation. Questionnaires about cases with aspects compatible with urea poisoning were sent to thirty-five veterinarians in the state of Mato Grosso do Sul. These veterinarians were chosen because they usually send samples or cattle for necropsies at LAP-FAMEZ.

RESULTS

Review

History. Although previously known, the use of urea in ruminant diet intensified during the First World War (1914-1918) in Germany due to the scarcity experienced at that time. But years later, it attracted growing interest from producers beyond Germany. Subsequently, a significant advantage of urea supplementation was first proven in dairy animals. In 1940, the use of urea in the ruminant diet was approved by the Association of American Feed Control Officials (Bartlett & Cotton 1938, Knodt et al. 1951, Santos et al. 2001). After reports of deaths caused by feeding urea, however, the first experiments on poisoning in sheep and cattle were carried out (Dinning et al. 1948).

In Brazil, although the use of urea in cattle diet is widely used and recommended (Santos et al. 2001, Kitamura et al. 2002), poisoning cases are not often described, making it difficult to establish an epidemiological profile. In a retrospective study in the state of Mato Grosso do Sul, over a period of 24 years, urea poisoning represented only 0.84% of the diagnoses of toxic diseases, which is equivalent to three outbreaks, and there is no additional information (Pupin et al. 2019). In another study encompassing some of the same cases, a morbidity rate of 0.6 to 10% and a lethality rate of 100% were described. In one of these reports, the authors described the deaths of animals 50 minutes after consuming rice straw and bran with a mixture of urea, and the cattle were found dead near the feeding trough, but no other information was given (Souza et al. 2015). No described cases of spontaneous poisoning in cattle in Brazil were found.

Epidemiological factors and pathophysiology. Poisoning in cattle can occur due to an error in the homogenization or when urea is not mixed, when it is offered on top of the feed, or even after rains due to its dilution and ingestion of greater amounts when ingested in water (Whitehair 1989). There are also cases of accidental or mistaken consumption when feeding the product unknowingly (Alden et al. 1976, Shaikat et al. 2012). Another observed form occurs when the supply is given intermittently or discontinuously, leading to adaptation loss (Huber & Kung Jr. 1981).

After consumption, once the urea reaches the rumen, it is rapidly hydrolyzed into ammonia compounds by the action of the enzyme urease, which is produced by the ruminal microbiota. The concentrations and absorption of ammonia compounds in the rumen lumen depend on factors such as the pH and rumen temperature (Visek 1984). The decomposition of urea to ammonia by urease is up to four times faster than its use by ruminal microorganisms.

The production and absorption of ammonia are promoted by diets that produce a more alkaline pH in the rumen environment, such as those rich in fiber and low in non-structural carbohydrates (NSCs) (starch and sugar), or even fasting (Mahadevan et al. 1976, Davidovich et al. 1977, Whitehair 1989). Once the ability of microorganisms to use this N source is exhausted, the remaining N is free in the lumen to be absorbed by the rumen walls and transported by the portal circulation to the liver. The ammonia is reconverted through the urea cycle for excretion, mainly renal. However, with an exacerbated increase in the production and absorption of ammonia, about one to two hours later, hepatocytes are overloaded, leading to persistently high values in the blood (Mahadevan et al. 1976, Davidovich et al. 1977, Shaikat et al. 2012).

Once inside the cells, ammonia blocks the Krebs cycle by saturating the glutamine-synthetase system, inhibiting cellular respiration, anaerobic glycolysis, and excessive production of lactic acid. This leads to metabolic acidosis. In neurons, ammonia causes destabilization of the passage of the nervous stimulus with the formation of false neurotransmitters, which causes neurological alterations and convulsive conditions. The high concentration of ammonia still interferes with glucose metabolism, causing hyperglycemia due to the stimulation of gluconeogenesis and hepatic glycogenolysis by the discharge of adrenaline. Finally, high concentrations of H⁺ (acidosis) cause an increase in potassium due to its movement to the extracellular fluid, leading to death due to cardiac arrest (Fraser 1963, Davidovich et al. 1977, Visek 1984, Kitamura et al. 2002, Antonelli et al. 2009, Gonçalves et al. 2011).

The clinical course is acute and usually fatal when the treatment is late. It is characterized by behavior changes with excitement or aggression, apathy, drooling, severe abdominal pain, incoordination, weakness, rapid and difficult breathing, ruminal atony, bloat, loud mooing, bruxism, stiffening of the limbs, and muscle tremors, which are initially located in the eyelids, lips, and neck and are later generalized. Finally, convulsions occur, which lead to coma and death (Fraser 1963, Kitamura et al. 2002, Antonelli 2003, Niles 2017).

Diagnosis. Diagnosis is often based on a history of ingestion of a source of NPN, followed by the abrupt onset of clinical signs or animals found dead near the site of consumption. Conclusive diagnosis requires complementary tests such as ammonia levels in the blood or the suspected material, in addition to determining rumen pH and biochemical alterations (Clark et al. 1951, Alden et al. 1976). Some authors describe the rumen ammonia concentration as a conclusive diagnosis criterion. However, it may not be useful since intoxicated and non-intoxicated animals often have the same ruminal concentration of ammonia (Bartley et al. 1976, Kitamura et al. 2002). Among the differential diagnoses for urea poisoning, the main ones are poisoning by nitrate and nitrite, hydrocyanic acid, lead, organophosphates, and ionophore antibiotics. Metabolic disorders such as hypomagnesemia can also be considered (Smith 2009, Thompson 2017). Table 1 presents additional data and changes in ammonia serum concentration from previous studies.

Prevention. In order to use urea in ruminant supplementation without causing poisoning, it is necessary to have a period of adaptation for the animals with a constant and increasing supply of amounts, which can vary from 45 to 113g/animal/day. It is recommended that the amount supplied not exceed 2-3% of the feed concentrate and that it be limited to 1% of the total volume of dry matter supplied. The adaptation of animals is necessary to increase the efficiency of the enzymes responsible for the urea cycle and for the adaptation of the ruminal microbiota to use NPN sources. Acclimatization can take days to weeks (three weeks on average), but it can be lost quickly (Huber & Kung Jr. 1981, Mel Scott Forestry Services 2008, Gonçalves et al. 2011, Thompson 2017, Parkes & Shilton 2019).

Retrospective study

During the period from January 2015 to December 2021, four outbreaks of urea poisoning were evaluated. The data are presented in Table 2 in chronological order of the outbreaks. In Outbreaks 1, 3, and 4, the animals showed clinical signs after

feeding on sources containing urea. In all cases, the animals were fed in troughs on pasture. In Outbreak 2, the cows invaded a maize paddock the day before, where mineral residues containing urea were discarded. In addition, it was raining, and multiple water reservoirs formed on this paddock. The veterinarian reported the possibility that the intoxicated animals had ingested this water.

Clinical and anatomopathological findings. The clinical signs observed were aggressiveness, incoordination, diffuse tremors, bloating, and green liquid in the mouth or nostrils observed approximately 30 minutes to 3.5 hours after consumption. The death occurred between 40 minutes and 2.5 hours later (except in Outbreak 1, where the time to death was not reported). Most of the animals were found dead, with no clinical signs observed. During the necropsy, the main findings were mild pulmonary edema and congestion, tympanic rumen, and ruminal contents in the airways. During the necropsy of the two cows in Outbreak 2, the rumen was filled with light green content that had a caustic odor and slightly alkaline pH (pH 8.0)

Diagnostic approach. Outbreaks 1, 3, and 4 diagnoses were based on epidemiology, compatible clinical signs, an absence of significant macro and microscopic lesions, and the exclusion of diseases that could be confused with urea poisoning. For the conclusive diagnosis of Outbreak 2, an experimental reproduction was carried out using two rumen-cannulated sheep. The first sheep (sheep 01) received two liters of the possibly contaminated water collected on the property, and the second one (sheep 02) received the same amount of water plus 500ml of vinegar. Sheep 01 showed clinical signs of poisoning 30 minutes after administration, was treated, and presented a resolution of the condition. Sheep 02 showed no clinical signs. It should be noted that in none of the outbreaks was

urea poisoning the initial suspicion. In Outbreak 1, the initial suspicion was poisoning by a contaminant in the feed, although urea was not mentioned specifically. In Outbreak 2, poisoning by *Aspergillus clavatus* was the main suspect due to the cows' feed being based on brewery waste (Bezerra Jr. et al. 2008). In Outbreak 3, there were suspicions of abamectin poisoning because the cattle had been treated with an abamectin-based dewormer one day before the appearance of clinical signs. In Outbreak 4, the first suspicion was botulism, due to the observation of clinical neurological signs, with the absence of macroscopic lesions. Based on the analysis of the information collected from the necropsy records, a flowchart was created to synthesize an adequate diagnostic approach in cases of presumptive urea poisoning (Fig.1).

Epidemiological investigation. The data obtained were grouped and are presented in Figure 2. Of the 35 interviewees, 32 said they had already followed cases compatible with urea poisoning, most more than once, resulting in 49 presumptive cases. Figure 2 presents the answers concerning the circumstances of the occurrence. In 87.5% of the interviews, the veterinarians reported not having performed a necropsy and/or sent material to confirm the diagnosis. The reasons considered for this are shown in Figure 3.

Regarding the number of cases observed in the last five years, the majority (52.9%) reported having followed one to two outbreaks. Four interviewees (11.8%) reported three to five cases, and the same number reported having followed up more than five cases in the same period. Most interviewees (81.2%) mentioned that up to 10 animals were affected, and the others reported 11 to 30 intoxicated animals in outbreaks.

Table 1. Complementary tests of previous studies in animals with experimental or spontaneous ammonia poisoning

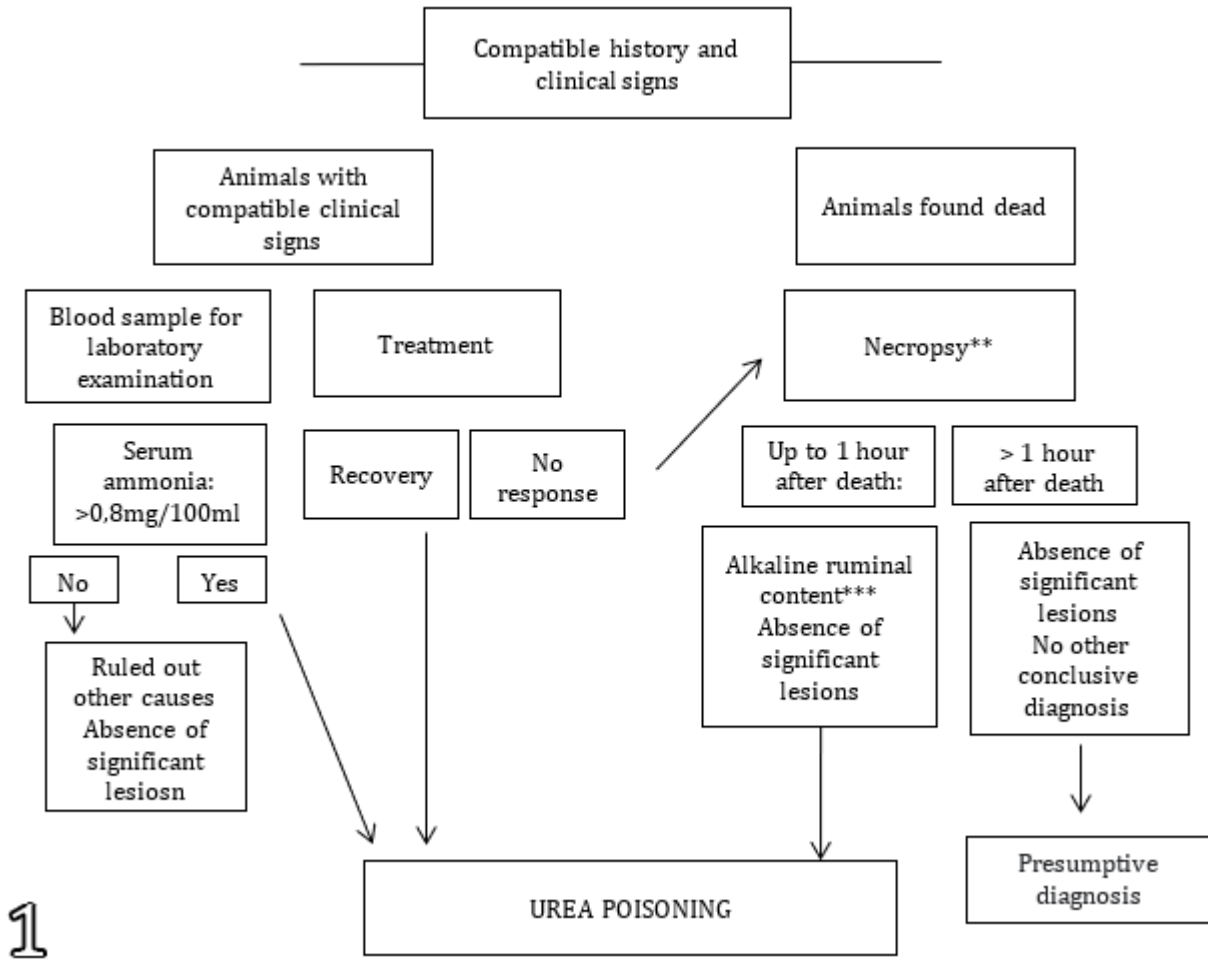
References	Ammonia serum concentration		Time for clinical signs	Ruminal pH*
	Onset of clinical signs	At the time of death		
Dinning et al. (1948)	2.5mg/100ml	4mg/100ml	20'	NI
Bartley et al. (1976)	0.8mg/100ml	NI	52.8'	7.41
Davidovich et al. (1977)	0.95mg/100ml	All survived	21'	7.9
Whitehair (1989)	1.0-2.0mg/100ml	2.0-3.0mg/100ml**	10 to 15'	NI
Antonelli et al. (2009)	782±140µmol/l	All survived	60±25' 79±26'***	NI
Sharma et al. (2016)	NI	NI	45'	8.7
Thompson (2017)	NI	NI	20-60'	>7.5
Parkes & Shilton (2019)	NI	NI	NI	7.5-8.0

NI = not indicated; * Measurement made at different times of toxic condition in each case, ** the lowest value was 1.2mg/100ml under field conditions, *** feeding granulated and extruded urea, respectively.

Table 2. Epidemiology of urea poisoning outbreaks monitored by LAP-FAMEZ from January 2015 to December 2021

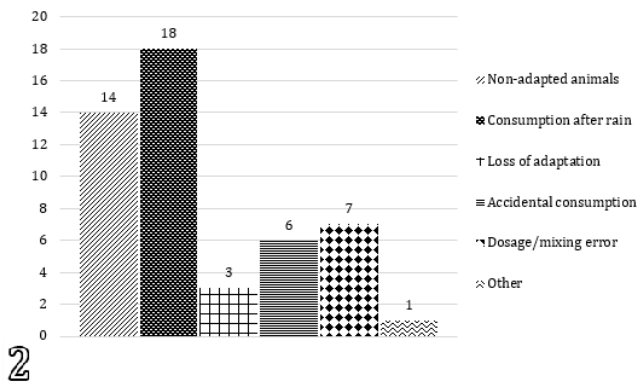
	Outbreak no. 1	Outbreak no. 2	Outbreak no. 3	Outbreak no. 4
Source	Mineral salt (10% urea)	Environmental waste	Protein salt made on the property (10% urea)	Commercial protein salt (6% urea)
Frequency	Once a week	Do not apply	NI	First time
Category	Calved cows	Lactating cows	Steer	Calves, steers and cows
Month	September	January	September	March
Population at risk	70 cows*	45 cows	459 steers	73 bovines**
Sick animals	19	2	9	15
Deaths	17	2	6	15
Necropsies	3	2	1	2

NI = not indicated; * Cows kept with the calves; due to the trough height, the calves did not have access to feed containing urea, ** 72 of the animals received the feed provided in the trough, and in one of them, the consumption was accidental.



1

Fig.1. Diagnostic approach for suspected cases of urea poisoning. Lower toxic value observed (Bartley et al. 1976) (*). Collected fragments from all organs, including the central nervous system (preserved in 10% formal and refrigerated as recommended by Barros et al. 2006) (**). pH test strips may be useful in field conditions (***)

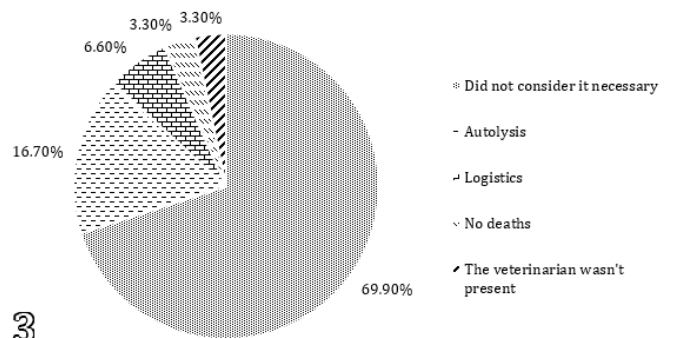


2

Fig.2. Urea poisoning in cattle. Epidemiological investigation. Number of presumptive cases of urea poisoning followed by field veterinarians and conditions associated with the poisoning.

DISCUSSION

During the retrospective study, four outbreaks were monitored by the LAP-FAMEZ staff, demonstrating a low frequency of



3

Fig.3. Reasons for not performing necropsy and/or sending samples to diagnosis.

diagnoses of urea poisoning in cattle from Mato Grosso do Sul. The same situation was observed in a 24-year study, which described three occurrences (Pupin et al. 2019), but the epidemiology of poisoning was not detailed. One of the reported outbreaks was also addressed in the present study. These results are similar to those described in research from other states where the disease was not present or showed a

that reach the diagnostic laboratories is far below the reality since 88.9% of the interviewees reported having followed-up outbreaks, but 87.5% of them did not perform a necropsy and/or send material to the laboratory. It is noteworthy that none of the cases described in the present retrospective study were referred by the veterinarians who answered the questionnaires and were selected because of their frequent partnership with LAP-FAMEZ.

The main reason described by the respondents for not sending material for diagnosis was that they did not consider it necessary. The second most mentioned reason was that the animals were found in an advanced state of autolysis, which makes it difficult to carry out tests and laboratory examinations. This finding differs from what was previously observed in other countries, where logistics and the number of dead animals were among the main determining factors reported (Watson et al. 2008).

Consuming feed or mineral salt containing urea after rains was the main factor of urea poisoning cited by the interviewees. In the retrospective study, the only outbreak related to rain did not occur due to the wetting or dilution of the mixture, as is commonly observed, but due to environmental contamination (accumulation of water at the disposal site of a mineral mixture containing urea). A lack of animal adaptation was the second most reported cause of poisoning (43.8%) in the interviews, and lack or loss of adaptation and excessive consumption occurred in three cases in the retrospective study.

Loss of adaptation as a cause of poisoning was reported by 9.4% of the interviewees and was not observed in the retrospective study. However, intermittent supply was the presumptive cause in Outbreak 1. These conditions are considered risk factors for poisoning in the same way as failures in the homogenization of the supplied mixture (Barros et al. 2006, Riet-Correa 2007). The need to adapt animals with a gradual increase in supply over a few weeks has long been recognized and widely recommended (Dinning et al. 1948, Gonçalves et al. 2011). However, we can observe that this practice is still neglected.

No reports were found in the literature or interviews with conditions identical to those in Outbreak 2 of the retrospective study. However, there are reports of high morbidity and mortality after using liquid fertilizer tanks to transport water to animals, in which 2.5% urea content was later detected (Alden et al. 1976). Although less common, poisoning due to water contamination by residues should always be considered.

The diagnosis of urea poisoning in Outbreaks 1, 3, and 4 were based on epidemiology and clinical evolution. Outbreak 2 was also based on an experimental reproduction in sheep, which showed improvement after treatment. It was not possible to specify the amount of urea ingested in any of the cases described. However, urea is considered highly toxic, especially when present in water, and the toxic dose for cattle varies from 0.3 to 0.5g/kg of body weight. The presence of 100 to 200g would be enough to poison an adult bovine (Whitehair 1989, Radostits et al. 2007).

The evidence of toxic levels in the material, as in Outbreak 2, may be of diagnostic value since the confirmation of urea poisoning is mainly based on the measurement of toxic concentrations of serum ammonia and rumen pH aided by other biochemical changes, which are considered criteria for the conclusive diagnosis of poisoning. However, this practice is

difficult to apply in field situations, as observed in all outbreaks of the retrospective study and most of the interviews. The difficulty is due to the need for samples to be collected and forwarded as soon as the animal dies. Most are usually found hours after death when most levels no longer have diagnostic value (Clark et al. 1951, Alden et al. 1976, Bartley et al. 1976, Davidovich et al. 1977, Antonelli et al. 2009).

Although most of the veterinarians interviewed mention that they did not forward tissue samples for laboratory tests when they suspected urea poisoning, as they did not consider this conduct necessary to confirm the diagnosis, in the present retrospective study, in all cases followed up, the first suspicion wasn't urea poisoning due to the epidemiological and clinical conditions being compatible with other differential diagnoses, such as botulism (Guizelini et al., 2019), *Aspergillus clavatus* poisoning (Bezerra Jr. et al. 2008) and abamectin (Guizelini et al. 2020). In another scenario, there are situations where urea poisoning is an important differential diagnosis for other conditions, such as hypothermia due to thermal inversion (Santos et al. 2012), since both share similar epidemiological conditions. One of the epidemiological factors in both conditions is rain, which precedes the sudden drop in ambient temperature leading to potential cases of hypothermia that can facilitate cattle ingesting larger amounts of urea, facilitating poisoning.

The development of a protocol with all the necessary steps to establish a definitive diagnosis of urea poisoning, applied to the Brazilian reality, as shown in Figure 1, is an important contribution to improving the efficiency of the diagnosis under field conditions. Since the disease has a fast clinical course and deaths are usually sudden, a faster diagnosis probably will result in lower deaths. The outbreaks followed in the present study demonstrate the need for rapid diagnosis for decision-making, enabling treatment and the adoption of control measures.

The onset of clinical signs agrees with previous reports of poisoning and generally occurs between 10 and 45 minutes after ingestion (Fraser 1963, Sharma et al. 2016). The complete evolution, from consumption to the moment of death, ranges from a few minutes to four hours, which may be influenced by the amount consumed or a delay in perception. The evolution of poisoning also depends on the speed at which ammonia is released into the rumen lumen, which is affected by the consumption of sources containing urease, such as soybean meal. It also depends on the change in ruminal pH in animals consuming low-carbohydrate diets or fasting. None of these were recognized in the presented cases (Fraser 1963, Radostits et al. 2007, Shaikat et al. 2012, Sharma et al. 2016). Due to the rapid evolution, affected animals are often found dead and sometimes close to the feeding troughs, as observed in several cases, which makes it impossible to observe clinical signs (Nakazato & Brum 1998).

In the cases presented, only in Outbreak 2 was it possible to provide a necropsy soon after the death of the second animal due to the quick contact between the veterinarian and the laboratory and the fact that the property was located nearby. In this case, it was possible to measure the animal's ruminal pH, which was above the physiological value of 6.2 to 7.2 for the species (Radostits et al. 2007). The observed ruminal alkalosis is an important finding for diagnosis. Unlike the concentration of ammonia in the rumen, the ruminal pH is directly correlated with the concentration of

serum ammonia and is considered one of the main findings of diagnostic value (Bartley et al. 1976, Radostits et al. 2007, Parkes & Shilton 2019, Niles 2017, Sharma et al. 2016). It is important to emphasize that the evaluation of ruminal pH only has diagnostic value when performed soon after death, as it is normal for the pH to rise and remain above six shortly after. In addition, the values can also change rapidly when the content is exposed to air during the necropsy procedure (Strafuss 1987). Ruminal pH values are also not considered to be of diagnostic value in animals that consume extruded urea (Antonelli 2003).

In the experimental poisoning, the sheep that presented clinical signs showed a positive response to treatment with vinegar. In this case, it was used for diagnostic purposes. This was supported by sheep 02, which received water plus 500ml of vinegar and did not become sick. Experimentally intoxicated sheep did not show changes in serum protein or hematocrit, which is expected in cases where the intervention is performed early (at the beginning of clinical signs), and the animal does not develop the characteristic dehydration that accompanies acute pulmonary edema (Davidovich et al. 1977).

In the outbreaks observed in the retrospective study, morbidity varied from 1.96% to 27.14%, and the lethality of poisonings was between 66.6% and 100%. These data are similar to those observed in previous reports, which demonstrate a high variation in morbidity (between 0.6% and 100%) with high lethality, which reaches 100% in most of the cases (89.5%-100%) (Alden et al. 1976, Mawhinney et al. 2009, Shaikat et al. 2012, Souza et al. 2015). Variable morbidity is expected as the intake of toxic or fatal urea levels is directly linked to the amount consumed by each animal and its weight. In this scenario, animals that consume a greater amount of contaminated feed or water, whether due to dominance or vigorous appetite, tend to quickly consume enough to cause poisoning and sometimes death (Alden et al. 1976, Whitehair 1989).

The necropsy findings were nonspecific, and the occurrence of pulmonary edema, frequently described in urea poisoning, stands out. Other alterations described in the literature were not observed, including gastroenteritis lesions, diffuse congestion, and hemorrhages (Barros et al. 2006). It is noteworthy that several authors have described the absence of significant lesions observed (Radostits et al. 2007, Shaikat et al. 2012, Sharma et al. 2016). In all cases of the present study, no significant histopathological findings were observed, which is considered a characteristic of the condition and supports the diagnosis of urea poisoning (Barros et al. 2006).

The main challenges for establishing the diagnosis in cases of urea poisoning are the rapid evolution, which makes it difficult to observe clinical signs and collect materials for laboratory tests, the absence of characteristic macro and microscopic lesions, and the similarity of clinical signs to those of other diseases (Fraser 1963). For the diagnosis to be reinforced, especially in cases where complementary tests are impracticable, it is important to carry out differential diagnoses for diseases with a similar course, such as poisoning by nitrate and nitrite, hydrocyanic acid, organophosphates, and carbamate, as well as ruminal acidosis, mycotoxicosis, and even hypomagnesemia (Fraser 1963, Radostits et al. 2007, Niles 2017). Without the complete history, the list of differentials is long due to the similarity of the evolution.

CONCLUSIONS

The occurrence of urea poisoning in cattle due to ingestion of water contaminated with waste products reinforces the importance of the correct destination of different residues in rural properties and the epidemiological approach in diagnostic consultations.

Urea poisoning is probably much more common than has been reported. Although field veterinarians often adopt a presumptive diagnosis without proof through complementary exams, it is important that they follow some reference to establish this diagnosis using simple tools that can help, even in field conditions. Based on the observed difficulty, we suggest an approach for field diagnosis of the disease in Figure 1.

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