RESUMO.- [Achados clínicos e toxicológicos na intoxicação por nitrato e nitrito em bovinos no Nordeste do Brasil.]

Relatamos dois surtos de intoxicação por nitrato e nitrito na Paraíba, Nordeste do Brasil. O primeiro por *Pennisetum purpureum* (capim-elefante), e o segundo por *P. purpureum* e *Brachiaria* spp. (capim braquiária); ambos ocorreram durante uma estiagem prolongada. No primeiro surto, a irrigação das pastagens com água poluída e esgoto contribuiu para o acúmulo de nitrato. O segundo surto ocorreu em pastagens cultivadas na borda de uma barragem, que havia sido alagada por muito tempo, acumulando grande quantidade de matéria orgânica na terra. Outros fatores prováveis de risco para o acúmulo de nitrato incluíram o uso de fertilizantes e herbicidas e queima do cerrado. No primeiro surto, quatro de oito bezerros e quatro de 42 vacas morreram, e no segundo surto, 49 de 243 bovinos, incluindo adultos, bezerros e um bezerro de dois dias, morreram. Os sintomas clínicos incluíram disfania, cianose, ataxia e queda, levando ao óbito. A presença de nitratos foi detectada nos dois surtos usando o teste de diphenylamina. Testes quantitativos no segundo surto foram realizados usando um medidor portátil de nitratos, e níveis altos de nitratos foram encontrados. A presença de nitratos e nitratos nos ruminantes do semiárido do Nordeste do Brasil é frequente devido à cultivadora de grama na borda de barragens que foram cobertas por água por um período de tempo ou em áreas irrigadas com esgoto e/ou água poluída. Além disso, o uso de um medidor portátil para medir nitratos é uma opção eficaz para a quantificação de nitratos em pastagens.

INDEX TERMS: Clinics, toxicology, nitrate poisoning, nitrite poisoning, cattle, Brazil, wastewater, diphenylamine test, portable meter, methylene blue.
Acid (2,4-D)-based herbicides and derivatives may induce extreme toxic forage (Al-Qudah et al. 2009). Fertilization with nitrogen fertilizers or organic matter of animal origin can affect nitrate accumulation (Mengel & Kirkby 1987). Plants can also accumulate high amounts of nitrates in the cells to be assimilated later (Andriolo 1999). Environmental factors, such as light and temperature, can affect nitrate accumulation (Mengel & Kirkby 1987). Plants can also accumulate high amounts of nitrates in the first rains after a dry period when they show rapid growth (Aiello & Mays 2001). Many ranchers have used untreated sewage directly to the soil represents one of the oldest systems of final disposal of sanitary waste (Mara & Cairncross 1989).

The accumulation of nitrates in plants occurs due to the imbalance between its absorption and assimilation, and excessive amounts of nitrates are stored in vacuoles in plant cells to be assimilated later (Andriolo 1999). Environmental factors, such as light radiation intensity and air temperature, can affect nitrate accumulation (Mengel & Kirkby 1987). Plants can also accumulate high amounts of nitrates in the first rains after a dry period when they show rapid growth (Aiello & Mays 2001). Many ranchers have used untreated wastewater for irrigation, transforming good quality forage into extremely toxic forage (Al-Qua deh et al. 2009). Fertilization with nitrogen fertilizers or organic matter of animal origin and treatment of pastures with dichlorophenoxyacetic acid (2,4-D)-based herbicides and derivatives may induce increased nitrate concentrations in plants (Radostits et al. 2007). Molybdenum deficiency causes nitrate accumulation in plants because this nutrient is a component of the nitrate reductase enzyme, thus contributing to the assimilation of nitrate by plants (Andriolo 1999).

In the physiological pathogenesis of nitrate and nitrite poisoning in ruminants, ingested nitrates are transformed into nitrites in the rumen and then into ammonia (Ko zlo ski 2009); however, depending on several factors, including the initial nitrate concentration, rumen flora, and diet, the conversion of nitrites into ammonia is inadequate and ingested nitrate can be reduced to nitrite in the digestive tract, resulting in an elevation of nitrite levels in the bloodstream (Riet-Alvariza 1993, Radostits et al. 2007). The nitrites present in the blood oxidize ferrous iron in hemoglobin to ferric iron, resulting in the formation of methemoglobin (Tokarnia et al. 2012). Methemoglobin is unable to transport oxygen for cellular respiration, resulting in cellular anoxia (Wright & Davison 1964, Van Zijnderveld et al. 2011).

The main clinical signs observed in nitrate and nitrite poisoning are sialorrhea, bruxism, tiredness, tachypnea or progressive dyspnea, ataxia, muscle tremors, abdominal contraction, unsteady walking, cyanotic mucous membranes, drowsiness, recumbence, reluctance to move, and seizures. Death usually occurs between 1-10 hours after the onset of clinical signs (Medeiro & et al. 2003).

This study investigated outbreaks of nitrate and nitrite poisoning in pastures of Pennisetum purpureum (elephant grass) and Brachiaria spp. (brachiaria grass) in the state of Paraíba, Northeastern Brazil.
RESULTS

The first outbreak of this study was recorded in May 2013 in the municipality of Patos/PB. Out of the total of 42 cattle, 20 were milk cows and 22 were calves. Two days after starting to ingest the grass, six calves became ill and four died approximately five minutes after the onset of clinical signs. Previously, the animals were fed on a native pasture, but due to the shortage of forage, feeding began with elephant grass (Pennisetum purpureum) cut from an area irrigated with municipal wastewater and sewage and fertilized with cattle and horse manure. The observed clinical signs included dyspnea, cyanosis, ataxia, falls, and death. Four calves were necropsied. Macroscopically, ocular and vaginal mucous membranes with brownish coloration, dark blood (chocolate color), and brownish skeletal musculature were observed. The brain and kidney surface appeared brownish in color. There was a large amount of green matter (elephant grass) in the rumen that exuded a strong odor, suggestive of nitrous gases. No changes were observed on microscopy. The diphenylamine test of the blood and ruminal content revealed a positive reaction with strong blush coloration in less than two minutes (Fig.2). Two poisoned calves were treated intravenously with 4mg/kg of 2-4% methylene blue and recovered.

In the outbreaks that occurred in the municipality of Cabaceiras/PB, from April to May 2015, out of a total of 243 dairy cattle, 49 died being 36 adults and 13 yearlings (Table 1) including a 2-day-old calf that was born weak and showed no appetite. The animals were distributed across seven farms, raised in a semi-intensive system that grazed on native pastures (“caatinga”) and received forage, composed predominantly of P. purpureum and Brachiaria spp. (brachiaria grass), mainly Brachiaria brizantha, cut on the banks of the dam in an area previously occupied by water, which, due to the drought, had been drained (Fig.3 and 4). The dam is a large water reservoir named Epitácio Pessoa Dam, popularly known as Boqueirão. It is in the municipality of Boqueirão, in the Brazilian state of Paraíba. Its basin extends through the municipalities of Boqueirão, Cabaceiras and São Miguel de Taipu and supplies the cities of Campina Grande, Boqueirão, Queimadas, Pocinhos, Catunité, Riacho de Santo Antônio and Barra de São Miguel in Paraíba. In the semi-arid region of Northeastern Brazil large dams are used as water sources for the cities. During the dry season as the dams decrease the volume of water, farmers use the areas previously covered by water in the border of the dam to cultivate pastures and other types of crops and vegetables. This practice is known as ebb agriculture (“agricultura de vazante”).

Cattle of different ages were fed grass with high nitrate content, and many animals were affected. On the first visit to the farm where the first outbreak occurred, seven cattle had died out of a total of 19. The owner had fed the cattle, for the first time, with brachiaria grass (Brachiaria spp.) and elephant grass (P. purpureum) cut and supplied as forage in the morning and late afternoon. The next day, 4-12h after eating the grass, the animals were found dead with distended abdomen with no observation of previous clinical signs. Necropsy of one cow was performed five hours after the animal’s death, and no gross or microscopic changes were observed.

In several farms in Cabaceiras/PB, cattle were feed with green and lush P. purpureum and Brachiaria spp. cultivated around the Epitácio Pessoa Dam and irrigated with water from nearby artesian wells. The farmers reported the occurrence of death in cattle, and the epidemiological and clinical picture that suggested nitrate and nitrite poisoning was verified. The main epidemiological factors identified as triggers or facilitators of nitrate accumulation in the forage included the use of previously submerged areas, some of which had remained covered by water for nearly 11 years, leaving the soil with large amounts of decaying organic matter and residues of grass and other plants. In some of these areas the organic matter had been burned before being cultivated. These areas were cultivated with purple varieties of P. purpureum and brachiaria grass (Brachiaria spp.) (Fig.3 and 4).

Table 1. Outbreak of nitrate and nitrite poisoning in cattle in the municipality of Cabaceiras/PB, number of farms, dead animals, and total herd size

<table>
<thead>
<tr>
<th>Farm</th>
<th>Number of dead animals</th>
<th>Total no. of animals per herd</th>
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<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Adults</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>2</td>
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<tr>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>36</td>
</tr>
</tbody>
</table>
The farmers reported and it was verified that tomato (*Solanum lycopersicum*), chili (*Capsicum annuum*) and beans (*Phaseolus vulgaris*) were often cultivated in areas close to where the grass was planted (Fig. 5 and 6), and fertilizers and pesticides had been commonly used in the soil for at least 20 years, without following any fertility program or soil analysis.

The diphenylamine test of the pastures in Cabaceiras/PB showed a positive reaction with dark blue color in less than 30 s (Fig. 2). High levels of nitrate were detected in *Brachiaria* spp. grass (6600 ppm) on Farm 2 and elephant grass (*P. purpureum*), both in the purple (ranging 7300-9800 ppm) and green variety (ranging 4700-5200 ppm) using a portable nitrate meter.

In Patos/PB, the nitrate level increased with irrigation and decreased with plant maturity and without irrigation. The diphenylamine test in the forage of the 2013 outbreak tested positive. In 2014, in the same area without irrigation and fertilization, the test was negative. In 2014, the area recently irrigated with wastewater yielding a positive reaction. The Patos/PB outbreak occurred in May 2013, but portable meter tests were conducted in June 2014. In the outbreak area, the nitrate level ranged 1600-2500 ppm and there had been no irrigation with wastewater since the outbreak. The newly planted grasslands area on the same farm was irrigated with untreated residual water (sewage) and presented nitrate levels of 4100 ppm and 4500 ppm at 30 and 40 days after planting.

In Paraíba, rainfall data showed the occurrence of drought during outbreaks of nitrate and nitrite poisoning in cattle. In Cabaceiras/PB in 2015, rainfall was low in the rainy season. In Patos/PB, during 2013, precipitation was higher than that observed in Cabaceiras/PB (Table 2) but below the mean level (AESA 2016).

The result of the wastewater analysis from the municipality of Patos/PB that irrigated the pasture in 2014 revealed a high amount of ammoniacal nitrogen (45 ppm of nitrogen) and nitrate levels were 199 ppm. In the municipality of Cabaceiras/PB, the wastewater analysis with a portable meter revealed a nitrate level of 7 ppm, which was considered negative.

The farmers were informed that high nitrate levels in the forage caused poisoning, which was proven by plant analysis along with clinical and pathological findings. They were instructed on the intravenous treatment protocol for nitrate and nitrite poisoning with 4 mg/kg of 2-4% methylene blue in animals with signs of poisoning. They were also advised in the form of avoid nitrate accumulation in new pastures and

Fig. 3-6. (3) Cultures of *Brachiaria brizantha* (bottom of the figure) and *Pennisetum purpureum* (middle of the figure). (4) *Pennisetum purpureum*. Note the tubulation for irrigation. (5) Different cultures in the border of the dam. (6) Irrigated culture of bean (*Phaseolus vulgaris*).
to test the toxicity of the plants before grazing or harvesting. Silage was mentioned as an alternative to decrease in 40-60% nitrate concentrations in forage (Robson 2003).

**DISCUSSION**

The diagnosis of nitrate and nitrite cattle poisoning in outbreaks in Paraíba was based on the analysis of epidemiological data, clinical signs, identification and quantification of nitrates in forage, absence of microscopic lesions, and response to treatment with methylene blue. All of these are fundamental tools for the diagnosis of nitrate and nitrite poisoning in ruminants (Tokarnia et al. 2012).

In the outbreak in Patos, necropsy of one bovine was performed more than five hours after death when methemoglobin reversal to hemoglobin may have occurred, leading to the disappearance of the dark color of the blood, carcass and musculature, which hinders macroscopic diagnosis of nitrate and nitrite poisoning (Tokarnia et al. 2012). Brown to blackened blood at the time of necropsy is often seen in nitrite poisoning if the necropsy is performed soon after death (Tokarnia et al. 2012). During histopathological assessment, absence of microscopic alterations in poisoned cattle were similar to those found by other authors (Medeiros et al. 2003, Radostits et al. 2007).

The case of a birth of a debilitated calf that died two days after delivery suggests that the pregnant cow had developed chronic poisoning. Abortions or stillborns occur when cows feed on sublethal doses of nitrates due to the increased affinity of fetal blood for nitrites (Rosenberger 1975, Radostits et al. 2007).

In previous studies in Northeastern Brazil nitrate and nitrite poisoning was associated Pennisetum purpureum and Echinochloa polystachya in cattle (Medeiros et al. 2003) and by Portulaca elatior in goats and sheep (Simões et al. 2018). In the outbreaks reported herein the disease was caused by P. purpureum and Brachiaria spp, mainly Brachiaria brizantha. Species of Brachiaria have not been previously reported as causing nitrate and nitrites poisoning; however, Brachiaria radicans may contain high nitrate concentrations, as reported by Tokarnia et al. (2012).

As shown in Table 1, the outbreaks of nitrate and nitrite poisoning in cattle reported in this study occurred during a prolonged dry season, in contrast to the description by Medeiros et al. (2003), who reported that poisoning occurs after the first rains following a long drought.

The irrigation of pastures with wastewater and fertilization with cattle and equine manure can contribute to nitrate accumulation in forage. The analysis of wastewater in Patos/PB revealed a high amount of ammoniacal nitrogen, while in Cabaceiras/PB, the result obtained using a portable meter was negative. According to Antileo et al. (1997), ammonia can be oxidized by Nitrosomonas bacteria, a process called nitrification, into nitrates and later into nitrates by Nitrobacter, Nitrocystis, and Nitrospina bacteria. Under Ordinance 518/04 of the Ministry of Health, the maximum content of nitrates allowed in drinking water is 10mg/L (Brasil 2004). According to Al-Qudah et al. (2009), irrigation with wastewater without the necessary monitoring is commonly used in countries that have water scarcity.

In Cabaceiras/PB, the poisoning occurred with grass planted in the bed of a large dam where the soil was rich in organic matter since it has been submerged for over 10 years. Similar situation was reported in an outbreak in the state of Ceará in a pond used to supply the city of Quixeramobim (Riet-Correa 2007). Other environmental and anthropic factors may contribute to poisoning episodes including drought and use of chemical fertilizers and herbicides. The use of herbicides and chemical fertilizers was also reported by Wright & Davison (1964) to be a predisposing factor for nitrate accumulation in plants. Other factors probably influencing the high concentration of nitrates in plants were the fertilization of the soil with high amounts of organic matter and the usage of fires that generated ash, also reported by Whittier (2011).

The diphenylamine test of the forage provided to cattle yielded a positive result. Nitrate levels above 5000ppm in forage used in cattle feeding are not safe (Haskell 2008); however, according to Karch (2007), in addition to assessing nitrate levels, the diphenylamine test can detect hypochlorite, chlorate, bromate, iodate, chromate, dichromate, permanganate, vanadate, lead, and manganese. The portable nitrate meter can be used to monitor nitrate levels immediately, providing swift results, and can accurately determine the concentration at the time of measurement. The results demonstrate the importance that this tool presents in monitoring the nitrates concentrations in plants (Guimarães 1998).

In the outbreaks in Patos/PB, the suspension of irrigation with wastewater at different stages of plant development resulted in a reduction in the forage nitrate content. The use of wastewater in newly implanted grasslands may have contributed to the increasing nitrate levels in plants, as mentioned by Al-Qudah et al. (2009).

Nitrate and nitrite poisoning in ruminants in the semiarid region of Northeastern Brazil is an important condition due to the diversity of risk factors identified in pasture management, especially during the dry season. The occurrence of new outbreaks every year highlights the importance of farmers and technicians knowing the diagnosis and treatment of the disease, in order to avoid the economic losses resulting from it.

**CONCLUSIONS**

In the semiarid region of Northeastern Brazil large dams are used as water sources for the cities. During the dry season as the dam decreases the volume of water, farmers use these

### Table 2. Rainfall in the municipalities of Patos/PB in 2013 and Cabaceiras/PB in 2015

<table>
<thead>
<tr>
<th>City/Year</th>
<th>Monthly rainfall (mm³)</th>
<th>Total rainfall (mm³)</th>
<th>Normal rainfall (mm³)</th>
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<tbody>
<tr>
<td>Patos/PB in 2013</td>
<td>January: 20.6</td>
<td>196.1</td>
<td>548.54*</td>
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<td></td>
<td>February: 13.1</td>
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<tr>
<td></td>
<td>March: 75.4</td>
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<tr>
<td></td>
<td>April: 60.4</td>
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<tr>
<td></td>
<td>May: 20.6</td>
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</tr>
<tr>
<td>Cabaceiras/PB in 2015</td>
<td>0</td>
<td>41.3</td>
<td>176.2**</td>
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<td>12.2</td>
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<td>1.5</td>
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<td>20.8</td>
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areas previously covered by water to cultivate pastures and other types of crops and vegetables. Pastures produced in these conditions contain high nitrate concentration causing poisoning.

Another common practice causing nitrate and nitrite poisoning is the irrigation of pastures around the cities with wastewater and sewage, sometimes fertilized with cattle and horse manure.

Conflict of interest statement.- The authors declared no conflicts of interest.

REFERENCES


