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Case Report - Veterinary Medicine

Organophosphate poisoning in sheep – case report

[*Intoxicação por organofosforados em ovinos – relato de caso*]

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ABSTRACT

This report describes an outbreak of exogenous organophosphate poisoning in a flock of Lacaune sheep. Forty sheep had signs of delayed poisoning after accidental exposure to an organophosphate-based insecticide (Diclorvol 1000 CE®, Diclorvós 82,5%, ChemoNE, Brazil). The clinical signs were bradycardia, bronchoconstriction, diarrhea, salivation, nasal discharge, apathy, prostration, motor incoordination, head pressing against objects, neck stiffness, paresis, flaccid paralysis of the limbs, permanent decubitus, pedaling movements in lateral decubitus, and death. After the onset of the clinical signs, sheep died in the flock over a period of sixty days. For the diagnosis, four animals were necropsied and samples of their brains, kidneys, and lungs were taken for toxicological analysis by thin layer chromatography; blood samples were also taken for biochemical analysis. The following was detected: liver alterations in serum biochemistry; pneumonia, edema, and congestion in macroscopic pathological analysis; hepatic microsteatosis, congestion, edema, and hemorrhage in lung, kidney, and liver histology; and organophosphates in the pool of samples from the sheep that were necropsied. The association between the epidemiological clinical findings and the laboratory results showed a clinical picture of organophosphate-induced delayed polyneuropathy, highly lethal in sheep, which alerts to the risk of improper use of these substances in animal facilities.

Keywords: anticholinesterase, Dichlorvos, delayed poisoning, Lacaune, nervous system

RESUMO

Neste relato é descrito um surto de intoxicação exógena por organofosforados em rebanho de ovinos da raça Lacaune. Quarenta ovinos apresentaram sinais de intoxicação tardia após exposição acidental a inseticida à base de compostos organofosforados (Diclorvol 1000 CE®, Diclorvós 82,5%, ChemoNE, Brasil). Os sinais clínicos observados foram bradicardia, broncoconstrição, diarreia, salivação, secreção nasal, apatia, prostração, incoordenação motora, pressão da cabeça contra objetos, rigidez do pescoço, paresia, paralisia flácida dos membros, decúbito permanente, movimentos de pedalar em decúbito lateral e morte. Após início dos sinais clínicos, ocorreram mortes de ovinos no rebanho durante 60 dias. Para o diagnóstico, foram necropsiados quatro animais, colhidas amostras de cérebro, rins e pulmão, para pesquisa toxicológica por cromatografia em camada delgada, e de sangue, para análises bioquímicas. Observaram-se alterações hepáticas na bioquímica sérica; pneumonia, edema e congestão na análise patológica macroscópica; e microesteatose hepática, congestão, edema e hemorragia na histologia de pulmão, rins e fígado; além da detecção de substância organofosforada no pool *de amostras dos ovinos necropsiados. A associação entre os achados clínicos epidemiológicos e o diagnóstico laboratorial evidenciou um quadro clínico de polineuropatia tardia induzida por compostos organofosforados, de alta letalidade em ovinos, resultado que alerta para o risco do uso sem orientação desses tipos de substâncias em instalações animais.*

Palavras-chave: anticolinesterásico, Diclorvós, intoxicação tardia, Lacaune, sistema nervoso

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INTRODUCTION

The use of pesticides in agricultural activities generates substantial controversy because, although chemical pest control increases agricultural production and reduces the burden of disease in animals, its intensive and/or incorrect use often causes harmful effects (Santos *et al*., 2014; Vale and Lotti, 2015) and, eventually, outbreaks of poisoning among domestic animals (Santos *et al*., 2021).

Organophosphorus compounds such as dichlorvos (2,2-dichlorovinyl dimethyl phosphate) are toxic mainly by inhibiting the activity of cholinesterases, especially the acetylcholinesterase present in cholinergic synapses, thereby preventing the hydrolysis of acetylcholine, which leads to its accumulation in the synaptic cleft of the central and peripheral nervous system, as well as in the postganglionic and autonomic neuromuscular junctions, and results in cholinergic hyperstimulation (Mukherjee and Gupta, 2020), with the eventual collapse of the central nervous system and acute death (Lopes *et al*., 2014).

Sometimes, animals exposed to these chemical agents show signs of toxicity for several days, which culminate in chronic neurotoxicity. This chronic or delayed neurotoxicity is called organophosphate-induced delayed polyneuropathy (OPIDP) and is an unusual consequence of acute toxicity (Mukherjee and Gupta, 2020). Caused by agents such as chlorpyrifos and dichlorvos (Grecco *et al*., 2009), OPIDP develops due to the phosphorylation and aging of an enzyme specific to peripheral nerves, called neurotoxic esterase (NTE) (Vale and Lotti, 2015), that result in axonal degeneration and subsequent demyelination of nerve fibers (Vale and Lotti, 2015).

In ruminants, the few cases of delayed organophosphate poisoning have been reported in buffalo (Grecco *et al*., 2009; Santos *et al*., 2021), because most cases of poisoning have an acute clinical picture of up to 24 hours, as described in cattle (Castro *et al*., 2007; Mendonça *et al*., 2010; Oliveira-Filho *et al*., 2010; Santos *et al*., 2014; Bertoni, *et al*., 2017) and goats (Lopes *et al*., 2014).

Because of the scarcity of scientific reports and knowledge of the behavior of sheep with this poisoning, and to the detriment of the use of this group of chemical substances in animal facilities, the aim of this report was to describe the clinical, anatomopathological, and toxicological findings of exogenous intoxication by organophosphorus compounds in Lacaune sheep raised in northeastern Brazil.

CASUISTRY

In April 2021, four sheep aged between four and 18 months were sent to the Large Animal Clinic (LAC) of the Department of Veterinary Medicine (DVM) of the Federal Rural University of Pernambuco (UFRPE), Recife, Brazil, for necroscopic examination from a flock of Lacaune sheep bred in Pernambuco, Brazil. Animals between three and 36 months old were showing signs of neurological changes and dying.

During anamnesis, it was known that there had already been two other deaths on the farm and that the cleaning protocol of the facilities had been modified twenty days before these first cases due to an increase in flies and cockroaches. In this modification, Diclorvol 1000 CE® (Diclorvós 82,5%, ChemoNE, Brazil) and Barrage® (Cipermetrina, Zoetis, Brazil) were used to sanitize the stalls and external pens every two weeks; however, the corridor to the stalls was cleaned every day. After a change in the cleaning protocol, six animals showed weakness, inappetence, dyspnea, bilateral mucous nasal secretions, and were lying down. They were treated by the owner with oxytetracycline hydrochloride 20 g (Oxitrat LA®, Vallée, Brazil) at a dose of 20 mg/kg (IM) due to the suspicion of pneumonia, but without success. Two died on the property and the other four were sent for necropsy. Before the death of these four animals, blood was collected for subsequent biochemical analysis. After this episode, two other animals began to show neurological changes such as motor incoordination, flaccid paralysis of the limbs, decubitus, frothy salivation, and finally died. After these deaths, the sheep began to exhibit compulsive behaviors, gnawing on the wood of the slatted floor of the stalls and eating each other's wool, especially off their backs.

The flock was composed of 150 Lacaune sheep, raised in a semi-intensive system for milk production and milked mechanically once a day. Of these, around 100 adult animals were raised in a pasture area with two elevated slatted pens and an external paddock of 1,000m^2, and the remaining sheep were raised 500 meters from these facilities, in ten 12m^2 stalls built and initially used for raising horses, which had wooden slatted floors and 1.5m doors, 4m high cement walls, external troughs, and clay-tile covers. It held lambs and adult sheep. There was ventilation at three points: at the point of access to the external troughs, at the top of the wall where the troughs were located, through a ventilation window near the cover, and at the upper part of the door.

The animals in the stalls and external pens were released onto separate elephant grass pastures and returned to the facilities in the late afternoon. The feed consisted of chopped elephant grass and balanced feed for the species and was given twice daily in troughs. In addition, the sheep received a mineral mix and water ad libitum. Occasionally, wet brewery waste was offered.

The sheep were necropsied in the Animal Pathology Laboratory of the DVM/UFRPE and tissue samples were taken for histopathological and toxicological analyses. The anatomopathological findings in the four necropsied animals were mainly in the brain, with congestion of the leptomeninges vessels and hemorrhages in their circumvolutions (Fig. 1A to D), and in the lungs, with pneumonia, congestion, and edema (Fig. 2A and B). The liver had a nutmeg appearance (Fig. 2E) and subepicardial hemorrhage was found in one sheep and pericardial edema in another one (Fig. 2C, D and G).

In histopathological analysis with optical microscopy samples of the heart, lung, kidney, liver, spleen, rumen, reticulum, omasum, abomasum, intestine, and central nervous system were collected and fixed in a 10% buffered formalin solution and processed using the paraffin impregnation technique with hematoxylin-eosin staining, it was observed in liver; congestion, hepatitis, cholestasis, and microsteatosis, lungs; congestion, edema, and hemorrhage, kidneys; congestion and central nervous system; congestion, perivascular edema, and glial cells (Fig.3).

Samples of liver, kidney, and brain tissue from the four sheep were collected and stored at -20 ºC and sent to the Laboratory Pró Ambiente Análises Químicas e Toxicológicas Ltda in Porto Alegre, Rio Grande do Sul, Brazil, for qualitative toxicological analysis of organophosphates and pyrethroids by thin layer chromatography using a pool of organ samples from the necropsied sheep. The tissue samples submitted to selective extraction and analyzed by thin layer chromatography showed that the pool of organ samples was positive for organophosphate and negative for pyrethroid.

After referral of these sheep for necropsy and with the presumptive diagnosis of organophosphorus compound poisoning, the owner began to administer 1% atropine sulfate (0.5mg/kg, IV) and antitoxin (Mercepton®, Bravet, 10 to 30 ml/animal/day, IM) to the symptomatic animals in an attempt to minimize the effects, however cases of death of sheep with neurological signs didn't not cease in the flock.

During the processing of samples for histopathological and toxicological analysis, a team of veterinarians from LAC/UFRPE visited the farm for epidemiological investigation of the outbreak, clinical examination and collection of blood samples from the animals.

On the day of the technical visit, four animals had ataxia, muscle spasms, pedaling movements, dyspnea with foam in the nostrils, and rumen reflux. The other exposed animals showed varied clinical signs such as bradycardia, bronchoconstriction, congested mucous membranes, diarrhea, salivation, apathy, paresis, head tremors, and in some cases motor incoordination, head pressing against objects, neck stiffness, flaccid paralysis of the limbs, permanent decubitus, and pedaling movements in lateral decubitus. In addition, they showed the compulsive behaviors of gnawing on the wood of the stall's slated floor and eating each other's wool.

Thirty-eight blood samples were collected, four of which were taken immediately after the death of the necropsied animals and 34 were taken from other sheep in the flock that had been

exposed to the products and were symptomatic at the time of the visit. Sample collection was performed by jugular venipuncture in vacuum tubes (Vacutainer®) without anticoagulant, which were stored in a recyclable/reusable Styrofoam box with ice and transported to the Buiatrics Laboratory at DVM/UFRPE for centrifugation at 3000 rpm for ten minutes. The obtained aliquots were stored in Eppendorf® microcentrifuge tubes, labelled, and frozen at -20 ºC until analysis.

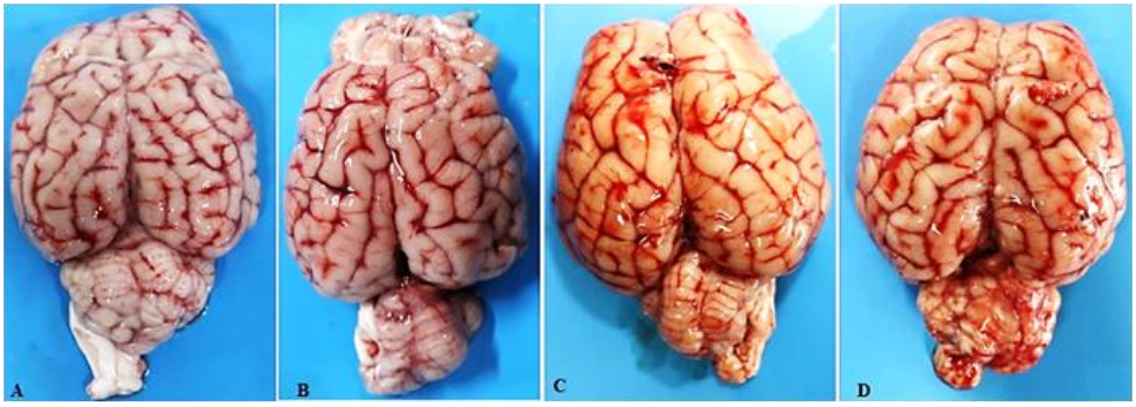

Figure 1. Brains of four sheep intoxicated by organophosphates. (A-D) Presence of congestion of the leptomeninges and hemorrhage in the cerebral circumvolutions.

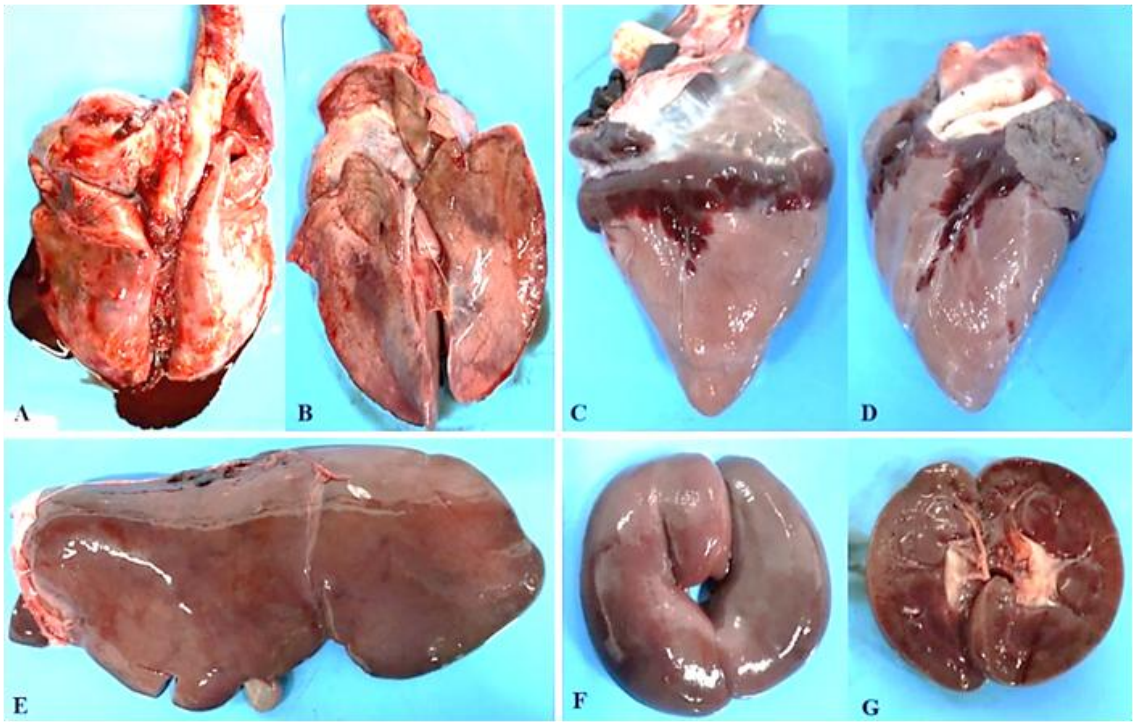

Figure 2. Macroscopic changes in the organs of four sheep intoxicated by organophosphates. (A) Dorsal and (B) ventral views of lungs showing edema and congestion, (C and D) heart with extensive hemorrhagic areas throughout the paraconal sulcus, at the base of the ventricles and atrium, (E) liver with a nutmeg appearance, and (F) kidneys diffusely darkened and (G) with an area of hemorrhage in the renal pelvis.

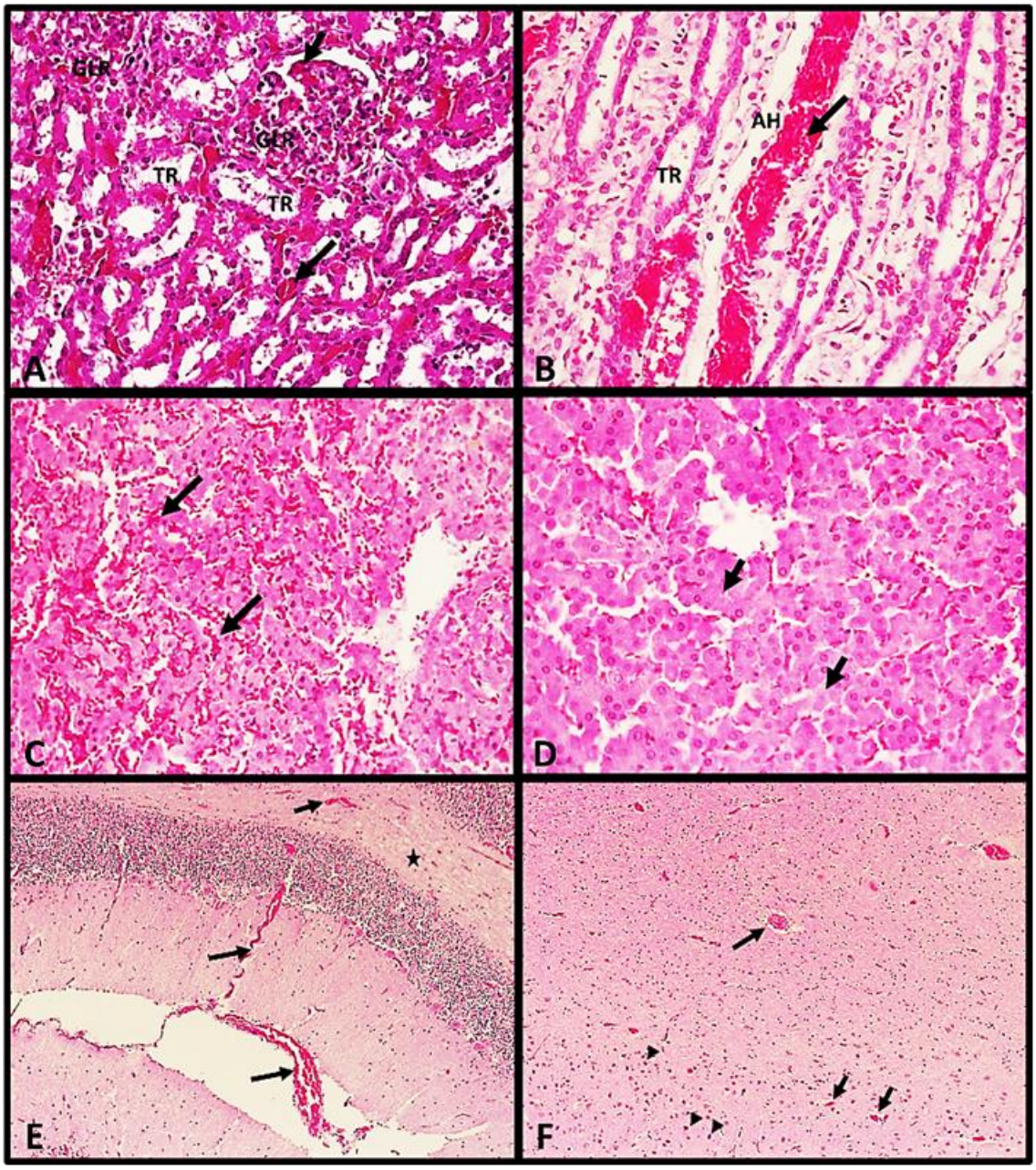

Figure 3. Histopathological analysis of organ samples from sheep intoxicated by organophosphates. (A) Kidney showing renal glomerulus (RGL) with reduced subcapsular space and congested capillaries (arrow). Renal tubules (RT) with loss of cell polarity, cell debris in the tubular lumen, and congested peritubular capillaries in the interstitium (long arrow). (B) Medullary region of the kidney with thick portion of the loop of Henle (RT) and thin portion of the loop of Henle (AH) in longitudinal section permeated by intense congestion of the peritubular straight vessels (long arrow). (C and D) Liver containing dilated sinusoid capillaries with intense congestion (arrow) and hyperplastic hepatocytes in the process of coagulative necrosis (short arrow). (E) Cerebellum with cerebellar cortex with intense congestion of vessels in the leptomeninges (arrow) and white matter with congested vessels (short arrow) and slight vacuolation (star). (F) Brain with numerous red neurons (arrowhead) and congested cortical vessels (arrow). White matter with congested vessels, increased perivascular space (long arrow), and glial cell edema (oligodendrocyte vacuolation).

The serum samples were subjected to biochemical analysis for gamma glutamyl transferase (GGT), aspartate aminotransferase (AST), alkaline phosphatase (AP), cholesterol, creatinine, urea, total plasma protein (TPP), albumin, globulin, calcium (Ca), phosphorus (P), triglycerides, and plasma fibrinogen (PF) using reagent kits in an automatic biochemical analyzer, model LABMAX 240®, both from Labtest. Urea levels of the necropsied animals were not measured, nor the levels of cholesterol, triglycerides, and fibrinogen in the 34 samples of the exposed sheep. Was used to analyze the data obtained from the biochemical analyses, after checking the normality of the residuals using the Shapiro-Wilk test and the homogeneity of the variance using Levene's test.

The biochemical analysis of the four necropsied sheep and 34 sheep exposed to organophosphate compounds showed liver damage with high levels of the enzymes GGT and AST, low protein and Ca levels, and decreased creatinine. The necropsied sheep also had hypercholesterolemia, hyperfibrinogenemia, and hypophosphatemia (Table 1).

Table 1. Biochemical analysis of four sheep necropsied and 34 sheep exposed to organophosphate compounds

Biochemical (*Unit of measurement*)	Sheep						Reference values
	Necropsied (n=4)				Exposed (n=34)		
	1	2	3	4	Mean e SEM	SD	
GGT (*U/L*)	47$^{\uparrow}$	137$^{\uparrow}$	52$^{\uparrow}$	43$^{\uparrow}$	52,09±2,29$^{\uparrow}$	13,38	<32
AST (*U/L*)	870$^{\uparrow}$	240$^{\uparrow}$	124$^{\uparrow}$	123$^{\uparrow}$	120,85±11,34$^{\uparrow}$	66,15	<90
Alkaline Phosphatase (*U/L*)	311	1.025$^{\uparrow}$	132	115	250,76±28,49	166,11	<387
Cholesterol (*mg/dL*)	146$^{\uparrow}$	101$^{\uparrow}$	131$^{\uparrow}$	97$^{\uparrow}$	NR	NR	52 a 76
Creatinine (*mg/dL*)	1,24	3,74$^{\uparrow}$	0,79$^{\downarrow}$	0,95$^{\downarrow}$	0,96±0,03$^{\downarrow}$	0,17	1,2 a 1,9
Urea (mg/dL)	NR	NR	NR	NR	35,73±3,11	17,05	17 a 43
Plasma protein (*g/L*)	3,82$^{\downarrow}$	5,01$^{\downarrow}$	6,08	4,64$^{\downarrow}$	5,48±0,19$^{\downarrow}$	1,11	6 a 7,9
Albumin (*g/L*)	1,23$^{\downarrow}$	2,94	2,32$^{\downarrow}$	2,23$^{\downarrow}$	2,81±0,09	0,51	2,6 a 4,2
Globulin (*g/L*)	2,59$^{\downarrow}$	2,07$^{\downarrow}$	3,76	2,41$^{\downarrow}$	2,66±0,21$^{\downarrow}$	1,22	3,5 a 5,7
Calcium (*mg/dL*)	5,62$^{\downarrow}$	5,49$^{\downarrow}$	7,69$^{\downarrow}$	7,31$^{\downarrow}$	7,21±0,31$^{\downarrow}$	1,81	11,5 a 12,8
Phosphorus (*mg/dL*)	2,63$^{\downarrow}$	4,47$^{\downarrow}$	4,55$^{\downarrow}$	6,71	6,56±0,36	2,08	5 a 7,3
Triglycerides (*mg/dL*)	54,3	136,57$^{\uparrow}$	69,57$^{\uparrow}$	99,79$^{\uparrow}$	NA	NA	15 a 57
Fibrinogen (*mg/dL*)	800$^{\uparrow}$	1500$^{\uparrow}$	700$^{\uparrow}$	800$^{\uparrow}$	NA	NA	100 a 500

Legend: SEM = Standard Error of the Mean, SD = Standard Deviation, NA = not analyzed, $^{\uparrow}$= above the reference value and $^{\downarrow}$= below the reference value

To decontaminate the facilities, solid waste was removed, and the sites washed with soap and water, and subsequently sprayed with a 5% sodium hypochlorite solution (1:20) to inactivate the toxic compound molecules. Despite this procedure, some animals died, totaling forty deaths in an interval of approximately sixty days, between April and May 2021. Considering the time interval between the use of the chemical compound and the last deaths, the outbreak was characterized as delayed poisoning.

It was estimated that 47 sheep of different categories (young, lactating females, and males) were exposed to the chemical compounds as they were housed in the stalls sprayed with the insecticides. Of these, forty animals showed nervous system symptoms and died. Considering the exposed sheep, the morbidity and mortality rates were 85% (40/47) and 100%, respectively.

DISCUSSION

This outbreak highlighted the importance of carrying out clinical examination combined with epidemiological analysis, because organophosphate poisoning develops with non-specific clinical signs, with an initial presentation of cholinergic syndrome with muscarinic alterations that is followed by nicotinic and neurological changes. Chronologically, the clinical signs presented by 23 adult mixed-breed cattle intoxicated 24 hours after being sprayed with a diazinon-based insecticide were dyspnea, sialorrhea, diarrhea, and decubitus, which are consistent with muscarinic alterations; then, nicotinic symptoms appeared, namely the inability to remain upright and flaccid paresis (Castro *et al*., 2007). However, in severely intoxicated animals, this order of clinical

presentation may not be observed, as it depends on factors related to the form and degree of exposure (Vale and Lotti, 2015).

Reports of organophosphate poisoning in ruminants are associated with the improper application of the chemical compound using pour-ons (Castro *et al*., 2007; Grecco *et al*., 2009; Mendonça *et al*., 2010; Oliveira-Filho *et al*., 2010; Santos *et al*., 2014; Bertoni *et al*., 2017; Santos *et al*., 2021), the sensitivity of the species to the recommended dose (Lopes *et al*., 2014), and the accidental ingestion of contaminated water (Oliveira-Filho *et al*., 2010). However, in the present outbreak, it was not possible to determine the exact route of intoxication; however, the digestive and/or cutaneous route is suspected, as well as the inhalation route, because the insecticide was sprayed on the concrete walls and the slatted wooden floors of the stalls.

It is suspected that the sheep lying in lateral decubitus on the wooden floor that was impregnated with the product favored not only absorption via the skin but also impregnation into the wool. Thus, they may also have ingested residues of the chemical compound by eating each other's wool and gnawing on the wooden slats of the stalls, a behavior that was observed during a visit to the farm. Another contributing factor to the contamination of the animals in the stalls may have been structure of the latter, because they were originally built for horses and had a poor ventilation system that only allowed the wind to pass through above the door and through the openings in one of the walls, which were located near the cover and at the points of access to the troughs, which were located on the outside.

This condition is possible because while these chemical compounds remain in the environment, their molecular structure undergoes a transformation and their permanence or degradation in the environment depends on the interaction between physical and chemical processes that allow biotransformation. Thus, the greater the quantity of the insecticide and the smaller the quantity of degrading agents in the environment, the less biodegradation occurs and the longer the product persists in the environment (Mukherjee and Gupta, 2020).

As a result, factors linked to the physical structure of the facilities may be associated with the high morbidity (85%) and mortality (100%) rates observed in this outbreak. The morbidity rates in the literature range from 22.8% (Grecco *et al*., 2009) to 85% (Bertoni *et al*., 2017). This variation results from factors such as the route of exposure (Grecco *et al*., 2009; Oliveira-Filho *et al*., 2010; Bertoni *et al*., 2017), the dose used (Lopes *et al*., 2014), the route of administration (Santos *et al*., 2014), the species (Lopes *et al*., 2014; Santos *et al*., 2021), and the breeding management system (Castro *et al*., 2007).

The high liposolubility of organophosphorus compounds makes it easier for the body to absorb them, so the cholinergic syndrome resulting from their toxicodynamics will present with clinical signs that depend on the site of action. Acute cholinergic syndrome is the main cause of death in animals (Lopes *et al*., 2014; Bertoni *et al*., 2017). In this type of poisoning, the onset of clinical signs can vary between fifteen minutes and twelve hours (Vale and Lotti, 2015).

Unusually, the cholinergic syndrome that resulted from the present outbreak was characterized as delayed occurrence, since it developed over up to sixty days. Similar situations have been reported regarding outbreaks in cattle herds, with the clinical course varying between seven and 45 days (Castro *et al*., 2007; Santos *et al*., 2021). This delay in the appearance and progression of clinical signs is strictly related to the chemical compound involved and to the speed and degree of drop in acetylcholinesterase and in esterase phosphorylation (Vale and Lotti, 2015).

The clinical findings in this study corroborate both the reports suggestive of OPIDP (Grecco *et al*., 2009; Santos *et al*., 2021) and those of acute outbreaks (Castro *et al*., 2007; Mendonça *et al*., 2010; Oliveira-Filho *et al*., 2010; Santos *et al*., 2014; Bertoni *et al*., 2017), because they resulted from mixed cholinergic stimulation. Sometimes, cases of delayed toxicity may or may not show clinical signs of acetylcholinesterase inhibition because, in this condition, peripheral nerve damage from esterase phosphorylation is more relevant (Vale and Lotti, 2015).

Although measuring acetylcholinesterase activity is very useful in diagnosing organophosphate poisoning, it was not possible to evaluate it in these sheep. However, the reduction in the percentage of cholinesterase in the blood varies depending on the biochemical targets and cannot be used as a presumptive diagnosis for delayed neurotoxicity induced by organophosphate ester exposure, due to its variation according to the quantity of product absorbed and duration of exposure (Santos *et al*., 2021).

Because of the non-specificity of the clinical signs in anticholinesterase toxicosis, poisoning in ruminants can be confirmed by toxicological analysis (Mendonça *et al*., 2010). The analytical methods generally used to detect these pesticides are thin layer chromatography and gas chromatography, which measure quantity and exposure to the toxic substance, respectively. In the present study, thin-layer chromatography was chosen because it is a low-cost and easy-to-perform technique compared to gas chromatography (Oliveira-Filho *et al*., 2010).

The rapid progression of toxicosis makes it difficult to characterize it through necroscopic examination, because it is not always possible to observe alterations (Santos *et al*., 2014). When present, they consist of congestion, hemorrhage, and edema in various organs (Grecco *et al*., 2009; Mendonça *et al*., 2010). In general, microscopic lesions are not observed (Castro *et al*., 2007; Grecco *et al*., 2009; Oliveira-Filho *et al*., 2010), especially in acute cases (Mendonça *et al*., 2010), and when they are detected in late cases they consist specifically in axonal degeneration in the myelinated nerve fibers of the central and peripheral nervous system (Mendonça *et al*., 2010; Santos *et al*., 2021), which is in line with the present findings.

Exposure to high concentrations of organophosphate, for a prolonged period or not, overloads the liver, which can no longer be detoxified, resulting in the development of serious pathological conditions. Thus, the histological lesions and biochemical changes observed in this outbreak indicate that there was an imbalance between the uptake, synthesis, and oxidation of metabolites that led to metabolic decompensation and the establishment of an inflammatory condition. Chronic poisoning by pesticides can cause liver damage and, consequently, changes in transaminases and the AP enzyme, whose level was altered in only one of the necropsied sheep (Vale and Lotti, 2015).

Given how quickly the clinical condition progresses, both treatment and forms of control must be implemented immediately. Atropine sulfate, associated or not with an oxime, is the drug of first choice for this toxicosis and should be administered within 24 to 48 hours of the onset of symptoms (Lopes *et al*., 2014). Dalto *et al*. (2011) were successful when using a therapeutic dose of 0.5 mg/kg of atropine sulfate 1% intravenously (IV), combined with supportive fluid therapy, on an average of 24 hours after intoxication of calves by ethion 15%, unlike Castro *et al*. (2007) who reported that the cattle died the day after receiving a single dose (0.5 mg/kg, IV) of atropine sulfate. Lopes *et al*. (2014) used a therapeutic dose of 1% atropine sulfate (0.5 mg/kg, IV) and reversed the muscarinic clinical signs in 62.5% (5/8) of goats poisoned with trichlorfon.

The treatment with atropine sulfate after the suspected poisoning of the sheep was not effective in preventing the deaths of the animals, similar to what occurred in other outbreaks affecting cattle, buffalo, and goats (Castro *et al*., 2007, Grecco *et al*., 2009, Lopes *et al*., 2014). Some animals do not respond to treatment with atropine (Lopes *et al*., 2014) because it blocks the effects of acetylcholine on muscarinic nerve terminals, making it ineffective in the management of manifestations mediated by nicotinic receptors (Grecco *et al*., 2009), because recovery from axonal injury and/or degeneration is irreversible (Vale and Lotti, 2015). This is in line with the histopathological findings in the present outbreak and further explains the fact that deaths continued to occur even after the chemical decontamination of the facilities.

Sodium hypochlorite 5% (1/20) was used under the guidance of the manufacturer of the organophosphorus compound, with the aim of inactivating the toxic molecules in the environment, as dichlorvos degenerate rapidly through biotic and abiotic processes. Hypochlorite has oxidizing properties against organophosphorus compound molecules in aqueous media and is used in water treatment plants. It is used to eliminate pesticides in surface water because chlorination is a

viable option for removing (in water) organophosphorus pesticides with a phosphorothioate group, such as diazinon and chlorpyrifos, during oxidation and disinfection procedures; however, it is not viable for eliminating pesticides with a phosphate group such as dichlorvos (Hassaan and Nemr, 2020).

CONCLUSION

The diagnosis of exogenous organophosphate poisoning in the sheep flock was made based on clinical, epidemiological, laboratory, and toxicological findings, thereby suggesting the occurrence of induced delayed polyneuropathy and highlighting the need to use these compounds cautiously and under guidance in animal facilities due to their high lethality. Moreover, further scientific reports are needed to establish the behavioral pattern of animals with delayed poisoning.

REFERENCES

BERTONI, E.S.; BIONDO, A.W.; MINOZZO, C.D. *et al*. Intoxicação aguda por organofosforado em bovinos do Paraná, Brasil. *Arch. Vet. Sci*., v.22, p.62-66, 2017.

CASTRO, M.B.; MOSCARDINI, A.R.C.; REIS JÚNIOR, J.L. *et al*. Intoxicação aguda por diazinon em bovinos. *Ciênc. Rural*, v.37, p.1498-1501, 2007.

DALTO, A.G.C.; ALBORNOZ, L.; GONZÁLEZ, P.C.S. *et al.* Intoxicação por organofosforados em bezerros no Uruguai. *Acta Sci. Vet*., v.39, p.983, 2011.

GRECCO, F.B.; SCHILD, A.L.; SOARES, M.P. *et al.* Intoxicação por organofosforados em búfalos (*Bubalus bubalis*) no Rio Grande do Sul. *Pesqui. Vet. Bras*., v.3, p.211-214, 2009.

HASSAAN, M.A.; NEMR, A.E. Pesticides pollution: classifications, human health impact, extraction and treatment techniques. *Egypt. J. Aquat. Res*., v.46, p.207-220, 2020.

LOPES, W.D.Z.; CARVALHO, R.S.; GRACIOLI, D.P. *et al*. Intoxicação aguda por triclorfon em caprinos tratados com a dose terapêutica. *Pesq. Vet. Bras*., v.34, p.114-118, 2014.

MENDONÇA, F.S.; FREITAS, S.H.; DÓRIA, R.G.S. *et al*. Intoxicação por diclorvós e cipermetrina em bovinos em Mato Grosso: relato de caso. *Cienc. Anim. Bras*., v.11, p.743-749, 2010.

MUKHERJEE, S.; GUPTA, R.D. Organophosphorus nerve agents: types, toxicity, and treatments. *J. Toxicol*., v.20, p.3007984, 2020.

OLIVEIRA-FILHO, J.C.; CARMO, P.M.S.; PIEREZAN, F. *et al*. Intoxicação por organofosforado em bovinos no Rio Grande do Sul. *Pesq. Vet. Bras*., v.30, p.803-806, 2010.

SANTOS, B.L.; MARCOLONGO-PEREIRA, C.; STIGGER, A.L. *et al*. Uso inadequado de organofosforados: uma prática de risco para bovinos no Sul do Rio Grande do Sul. *Pesq. Vet. Bras*., v.34, p.655-658, 2014.

SANTOS, I.R.; CLEBER, C.L.; DAL PONT, T.P. Outbreak of organophosphorus compound-induced delayed neurotoxicity in water buffaloes. *Ciênc. Rural*, v.51, p.1-5, 2021.

VALE, A.; LOTTI, M. Organophosphorus and carbamate insecticide poisoning. *Handb. Clin. Neurol*., v.131, p.149-168, 2015.