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PATHOLOGY

Outbreak of organophosphorus compound-induced delayed neurotoxicity in water buffaloes

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ABSTRACT: *Forty 1-2-y-old water buffaloes were simultaneously treated with trichlorfon and chlorpyrifos products in the recommended dose for cattle. After a week, 19 animals started presenting clinical signs characterized by apathy, diarrhea, aggressiveness, dehydration, and motor incoordination, followed by flaccid paralysis and permanent lateral recumbency. All affected buffaloes died after a clinical course of 1–4 days. Reduction of serum cholinesterase activity in three cases was indicative of significant exposure to organophosphorus compounds (OPs). Pathological examination of three buffaloes revealed no gross and histological lesions. By thin layer chromatography, chlorpyrifos residues and trace of trichlorfon residues were detected in fresh tissue samples. The epidemiological, clinical, pathological, and toxicological findings were highly compatible with OPs-induced delayed neurotoxicity, a neurological manifestation rarely described in domestic animals.*
Key words: *buffaloes, chlorpyrifos, poisoning, trichlorfon.*

Surto de neuropatia tardia induzida por organofosforados em búfalos

RESUMO: *Quarenta búfalos foram simultaneamente tratados com clorpirifós e triclorfom na dose recomendada para bovinos. Após uma semana, 19 animais apresentaram sinais clínicos caracterizados por apatia, diarreia, agressividade, desidratação e incoordenação motora, seguidos por paralisia flácida e decúbito lateral permanente. Todos os búfalos afetados morreram após um curso clínico de 1–4 dias. Redução da atividade da colinesterase sérica em três casos foi indicativa de exposição significativa a organofosforados (OPs). O exame patológico de três búfalos não revelou lesões macroscópicas e histológicas. Por cromatografia em camada delgada, resíduos de clorpirifós e traços de resíduos de triclorfon foram detectados em amostras de tecidos frescos. Os achados epidemiológicos, clínicos, patológicos e toxicológicos foram compatíveis com neuropatia tardia induzida por OPs, uma manifestação neurológica raramente descrita em animais domésticos.*
Palavras-chave: *búfalos, clorpirifós, intoxicação, triclorfon.*

Organophosphorus compounds (OPs) are organic derivates of phosphorus. Since the first OPs synthesis in 1837, they have been widely used for different purposes (e.g., nerve chemical warfare agents, insecticides, acaricides, fungicides, and herbicides) (BOWLS, 2003). Currently, a wide range of OPs products are applied worldwide in agriculture and veterinary medicine. Accidental and intentional high-level exposure to OPs in humans causes acute poisoning (MORRIS et al., 2014); and occupational exposure and delayed complications of acute poisoning to some OPs in humans induces chronic toxicity, including OPs-induced delayed neurotoxicity (OPIDN) (ETEMAD et al., 2014). Similar accidental acute poisoning or chronic toxicity by OPs may affect all farm animals (CONSTABLE et al., 2017); although natural poisoning by OPs in buffaloes has been rarely described in the literature (GRECCO et al., 2009). Herein, we reported an outbreak of OPIDN in a herd of domestic water buffaloes (*Bubalus bubalis*).

In August 2020, the owner of a farm located in Montenegro, Rio Grande do Sul, Brazil (29°41′19″ S, 51°27′40″W), reported an outbreak of a neurological disease leading to death of domestic water buffaloes. The farm was visited to collect epidemiological and clinical data and to perform postmortem examinations. The described herd was composed of 85 1-2-y-old buffaloes (female and male; estimated body weight of 200–300 kg) and was kept in native

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pastures (grassland). Animals used to be treated with antiparasitic drugs aiming to control endoparasites and ectoparasites. All buffaloes were subjected to intramuscular administration of a trichlorfon antiparasitic drug (10–12.5 mg/Kg). On the same day, 40 buffaloes also received a chlorpyrifos pour-on ectoparasiticide application (4.5–7 mg/Kg). After 7 days, 19 animals exposed to both products presented clinical signs of apathy, diarrhea, aggressiveness, dehydration, and motor incoordination, followed by flaccid paralysis, permanent lateral recumbency, and death. Considering only the affected buffaloes, the morbidity and lethality rates were 47.5% and 100%, respectively. The disease progression between the onset of clinical signs and natural death was 1–4 days.

During the on-site visit, the herd was observed and several animals showed lateral recumbency (Figure 1) or dead. Blood samples of three live animals (cases 1–3) were collected by jugular puncture and subsequently centrifuged. Serum cholinesterase (ChE) activity was determined by a colorimetric method (DIETZ et al., 1973). All analyzed sera presented a reduction of 60.8–64.6% in ChE activity (Table 1). Three animals were submitted to a systematic postmortem examination after euthanasia due to poor prognosis (cases 1 and 2) or natural death (case 4). Samples of organs from the thoracic and abdominal cavities, nervous system (brain, spinal cord, and mid-level sciatic nerve), and forelimb and hindlimb skeletal muscles were collected, fixed in 10% neutral-buffered formalin, processed routinely for histopathology, embedded in paraffin wax, and stained with hematoxylin and eosin. Sections of all levels of the nervous system were also stained by Luxol Fast Blue. However, no significant gross or histological lesions were observed in the evaluated animals. In addition, during the post-mortem examination, fresh samples of liver, kidney, and central nervous system were collected and stored frozen (-20 °C) for further toxicological tests.

A presumptive diagnosis of OPs poisoning was made based on the history, number of affected animals, clinical findings, laboratory tests, and lack of significant gross or histological lesions. Therefore, serum and selected fresh sample tissues (liver, kidney, and central nervous system) were analyzed for trichlorfon and chlorpyrifos residues by thin layer chromatography (TLC). After extraction and clean-up procedures by adapted QuECHERS method (OLIVEIRA et al., 2018) the extracts were applied on the silica gel plate for analysis in the trough chamber. Spots were visualized under ultraviolet light and

Figure 1 - Organophosphorus compound-induced delayed neurotoxicity in water buffaloes. Three animals showed lateral recumbency.

using specific chemical reactions. The qualitative evaluations were made via the retardation factor values (TORRES et al., 2017). Cases 1, 2, and 4 presented chlorpyrifos residues in at least one fresh sample tissue, and case 4 showed trace of trichlorfon residues in the kidney; whilst all analyzed serum were negative for trichlorfon and chlorpyrifos residues (Table 1). Therefore, the herd presumptive diagnosis of OPs poisoning was confirmed by the detection of trichlorfon and chlorpyrifos residues in the evaluated organs.

Animal and environmental risk factors associated with poisoning by OPs in farm animals include the use of high concentrations of a drug in target animal species and the application of products in non-target animal species (CONSTABLE et al., 2017). The main risk factor associated with the only natural poisoning by OPs in buffaloes described in the literature was the use of chlorpyrifos in the recommended dose for cattle (12 mg/Kg) (GRECCO et al., 2009). Similarly, in this outbreak, both chlorpyrifos and trichlorfon products were applied based on the recommendations for cattle (resulting in a total dose of OPs of 14.5–19.5 mg/Kg). In addition, none of the commercial products used had label recommendations for buffaloes (non-target species usage). Although chlorpyrifos and trichlorfon sensitivity is unknown in buffaloes, these findings reinforce that this species is more susceptible to OPs intoxication compared to cattle, suggesting that the doses recommended for cattle cannot be safely used in buffaloes (RAINA et al., 1990; GRECCO et al., 2009). Interestingly, the buffaloes exposed only to the trichlorfon product showed no clinical signs.

The clinical signs, lack of acute cholinergic syndrome, and the time elapsed between OPs exposure and onset of neurological signs in the buffaloes were highly compatible with chronic toxicity by OPs, specifically OPIDN. OPIDN is a symmetrical sensory-motor axonopathy that occurs 1–4 weeks after a single exposure of certain OPs, including chlorpyrifos and trichlorfon (SATOH & JOKANOVI´C, 2014). OPIDN has been reported in humans (WIJEYESAKERE & RICHARDSON, 2010); although natural cases of OPIDN are rarely described in domestic animals (BECK et al., 1977; THOMPSON et al., 1993). Generally, the clinical signs develop as a result of progressive distal degeneration of sensory and motor axons (SATOH & JOKANOVI´C, 2014). All clinical findings observed in this outbreak report were compatible with OPIDN in domestic ruminants, including probable death due to severe dehydration (CONSTABLE et al., 2017). In contrast, a cholinergic syndrome is described in an outbreak of chronic poisoning by OPs in buffaloes (suggestive of OPIDN by the authors) (GRECCO et al., 2009).

Several mechanisms of action are associated with the pathogenesis of chronic toxicity of OPs. Unlike cholinergic toxicity, the irreversible inhibition of neuropathy target esterase (NTE; formerly called neurotoxic esterase) is the likely cause of OPIDN (FUNK et al., 1994). The exact mechanism of NTE inhibition leading to OPIDN is

Table 1 - Serum cholinesterase (ChE) activity and thin layer chromatography (TLC) identification of trichlorfon and chlorpyrifos residues in tissues.

Case	Sex	Tissue	ChE activity*	----------TCL----------	
				Trichlorfon	Chlorpyrifos
1	Female	Serum	93 U/L	–	–
		Nervous system		–	+
		Liver		–	+
		Kidney		–	+
2	Female	Serum	88 U/L	–	–
		Nervous system		–	+
		Liver		–	+
		Kidney		–	–
3**	Female	Serum	90 U/L	–	–
4	Male	Nervous system		–	–
		Liver		–	–
		Kidney		Trace	+

* Reference range = 224.6–262.84 U/L (SINGH et al., 2016); ** Not submitted to postmortem examination.

unknown; however, previous studies have conjectured that a negatively charged phosphoryl residue in the inhibited NTE results in the chemical cut of the axon (SATOH & JOKANOVI´C, 2014). NTE activity might be determined in blood samples (specifically evaluating circulating lymphocytes) or nervous system samples to verify exposure to neuropathic OPs in humans (WIJEYESAKERE & RICHARDSON, 2010). Unfortunately, NTE activity detection tests are not available in laboratories in our region; therefore, these exams were not performed as a complementary exam in this study. Additionally, investigations of NTE activity in healthy buffaloes and buffaloes exposed to OPs have not been described.

In cases of OPIDN, the postmortem examination shows absent or nonspecific gross lesions (BECK et al., 1977). Histological findings are mainly characterized by axonal degeneration in the myelinated nerve fibers of the longer tracts in both central and peripheral nervous systems (EHRICH & JORTNER, 2010). The lesion distribution varies greatly according to dosage, affected species, and relative potency of the OPs (JOHNSON & HENSCHLER, 1975). Generally, the lesions occur in long peripheral nerves, ascending tracts at cervical spinal cord, cerebellar, and medullary levels, and descending tracts at lumbosacral spinal cord level (ABOU-DONIA et al., 1986). These histological lesions can remain minimal in the first weeks after exposure to OPs (FUNK et al., 1994), which corroborates with the lack of histological lesions in this outbreak.

During the herd diagnostic investigation, acute OPs poisoning was initially considered as the main differential diagnosis due to the epidemiological and laboratory features (history of OPs exposure, number of affected animals, and reduction of the serum ChE activity). However, acetylcholinesterase inhibition induces a cholinergic syndrome few minutes to several hours after acute exposure to OPs (MORRIS et al., 2014), which was not observed in our cases. Additionally, only ChE activity <25% of the normal values indicates severe anticholinesterase poisoning (MEERDINK, 1989), in contrast to the changes described herein. Based only on clinical signs and geographic region studied, other secondary differential diagnoses included several toxic and infectious diseases (e.g., ionophore toxicity, botulism, and rabies). These differential diagnoses were excluded based on epidemiological, clinical, pathological, and toxicological findings.

The identification of OPs residues in tissue samples of poisoned buffaloes was essential in the diagnostic approach. In cases suspected of pesticide poisoning, tissue samples from target organs are usually collected during the postmortem examination of animals. In this study, the detection of chlorpyrifos residues showed different positive tissue samples among cases; and the negative results of trichlorfon residues in tissue samples likely occurred due to the different routes of administration of the OPs products (intramuscular and pour-on). Moreover, probably, the time between the exposure to OPs and blood sampling caused the negative results of both chlorpyrifos and trichlorfon residues in serum samples. While TLC procedure is an important chromatography method in the determination of pesticide residues, this technique provides only qualitative and semiquantitative analysis (SHERMA et al., 2017). Therefore, the quantitative analyzes of chlorpyrifos and trichlorfon were not performed in our cases.

The present study demonstrated that OPIDN should be considered in the pathological differential diagnosis of neurological diseases in buffaloes. Additionally, the use of OPs products in non-target animal species was the most important epidemiological risk factor for the poisoning.

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BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

We authors of the article entitled “Outbreak of organophosphorus compound-induced delayed neurotoxicity in water buffaloes” declared, for all due purposes, the project that gave rise to the present data of the same has not been submitted for evaluation of the Ethics Committee of the Universidade Federal do Rio Grande do Sul. However, we are aware of the content of the Brazilian resolutions of the Conselho Nacional de Controle de Experimentação Animal (CONCEA) if it involves animals. Thus, the authors assumed full responsibility for the presented data and are available for possible questions, should they be required by the competent authorities.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; and in the decision to publish the results.

AUTHORS’ CONTRIBUTIONS

The authors contributed equally to the manuscript.

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