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Tremorgenic mycotoxicosis in cattle, caused by *Claviceps paspali*

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Abstract: *Claviceps paspali* is a fungus that mainly parasitises *Paspalum dilatatum*, generating a structure denominated sclerotium, in which indole-diterpenoid alkaloids are isolated. Its action mechanism is related to the inhibition of the gamma-aminobutyric acid receptor. It basically affects bovines, triggering the tremorgenic syndrome, the prevalence of this intoxication being relatively low in Europe. This work describes a clinical case on a cattle farm in Seville (southern Spain), composed of 91 bovines, 60% of which were principally affected with nervous clinical signs. The diagnosis was based on a clinical inspection of the animals, as well as of the presence of paspalum seed heads containing the sclerotia of *Claviceps paspali* in the plants present in the pastures. The causal agent was identified as being *Claviceps paspali*, which had parasitised numerous examples of *Paspalum paspaloides*. The indole-diterpenoid alkaloids produced by *Claviceps paspali* were identified using ultraviolet-visible spectrophotometry and mass spectrometry. At present, no effective aetiological treatment has been described for poisoning caused by this mycotoxin, so a supportive treatment was administered, and different handling methods were applied, resulting in the complete recovery of the animals. Finally, it was concluded that unusually high humidity and temperature levels for the region triggered the development of the sclerotium generated by this fungus.

Keywords: bovine; mycotoxins; *Paspalum*; sclerotium; tremor

List of abbreviations

CK = creatine kinase; **CNS** = central nervous system; **GABA** = gamma-aminobutyric acid; **LDH** = lactate dehydrogenase; **MS** = mass spectrometry; **T** = aspartate aminotransferase; **UV/VIS** = ultraviolet-visible spectrophotometry

Claviceps paspali invades the reproductive organ of plants, mainly of the genus *Paspalum*, and produces a sclerotium, in which are isolated indole-diterpenoid alkaloids, chiefly paspalitrem A, B and C, and paspaline. The plant species most commonly parasitised is *Paspalum dilatatum*, although other species can be affected like *Paspalum notatum*, *Paspalum distichum*, *Paspalum paspaloides*, *Paspalum vaginatum*, *Paspalum scrobiculatum* and *Paspalum urvillei* (Riet-Correa et al. 2013; Oberti et al. 2020). When the animals consume that sclerotium they suffer from the so-called tremorgenic syndrome, characterised by a nervous type of clinical sign, consisting of tremors, ataxia, abnormal head movements and hyperexcitability. Most of the cases described with this syndrome have

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been located in South America and South Africa, whereas cases in Europe are rare (Moyano et al. 2010). Cattle are more often affected than sheep or horses, primarily because they are more likely to consume the seed heads in which the toxin is located. Sheep seem to be less sensitive than cattle, but take longer to recover from the "staggering" episodes (Plumlee and Galey 1994).

Case description

This describes a clinical case in the western area of the province of Seville (southern Spain) on a farm containing 91 bovines. The estate has 14 hectares divided into two well-differentiated areas due to the land's arrangement. The predominant vegetation in the field, in which the animals grazed, when showing clinical signs was mainly made up of *Cynodon dactylon* (common grass), *Trifolium repens* (white clover), and *Trifolium fragiferum* (strawberry clover). The cases evolved during the month of October, in a warm climate and with humidity due to previous rainfall, the temperature being excessively high for that time of the year (32 °C ± 1).

In the animals' clinical examination, it was determined that approximately 30–40% of the herd was affected, with this percentage increasing up to 60% as time went by. The afflicted animals were all bovines, with two Limousine breed yearling calves in serious condition, together with Retinta breed heifers and milking cows.

The clinical signs exhibited were fundamentally of a nervous type, with unusual head movements, hyperexcitability, ataxia, hypermetria of the forelegs when walking, and tremors in different muscle groups. At the moment of handling the animals to examine them, which represented a stressful situation for them, the clinical signs became worse, and they displayed aggressiveness and sudden falling over (Figure 1). Their mean body temperature was 37.5 °C, showing tachycardia in all the cases.

Faced with these clear clinical signs, the differential diagnosis included: (1) Possible intoxication from their drinking water, (2) consumption of toxic

Figure 1. Video pictures of the affected animals with nervous type clinical signs (abnormal head movements, hyperexcitability, ataxia, and hypermetria of the forelegs and tremors in various muscle groups)

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Table 1. CK, AST and LDH values obtained in the blood biochemistry performed on the affected animals

Enzyme parameters	Reference value	Animal No. 1	Animal No. 2	Animal No. 3	Animal No. 4
CK (μkat/l)	0–5.83	8.27	10.05	6.17	30.07
AST (μkat/l)	1–2.08	2.48	2.52	2.58	5.53
LDH (μkat/l)	5.15–15.63	21.57	23.53	19.98	9.82

AST = aspartate aminotransferase; CK = creatine kinase; LDH = lactate dehydrogenase

plant species, (3) consumption of a toxic fungal source, and (4) an unknown infectious origin.

However, in view of the case characteristics, particularly the number of animals affected and reaction to their handling, the diagnosis was focused on the possible poisoning from the consumption of toxic species of plants or fungi.

First, blood specimens for biochemistry were taken from four animals that presented with the most severe clinical signs. There was a general rise in the enzymes creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) in the sampled animals (Table 1).

Second, the plant species present in the field was visually identified and, from its morphological characteristics, it was confirmed as being the grass *Paspalum paspaloides.* During inspection of the plant samples, it was observed that it had a similar structure to a sclerotium compatible with *Claviceps paspali* (Figure 2). Thus, the diagnosis of paspalum staggers was based on the clinical signs, the animals' history, and the presence of paspalum seed heads containing the sclerotia of *Claviceps paspali* (Botha et al. 1996). Laboratory confirmation was confirmed through ultraviolet-visible spectrophotometry (UV-VIS) and mass spectrometry (MS). Both for the extraction of the *Claviceps paspali* sclerotia and for the analysis of the ergot alkaloids, the procedure described by Uhlig et al. (2009) was followed. These techniques possess high sensitivity, which permitted the identification of the indole-diterpenoid alkaloids produced by *Claviceps paspali* (paspalitrem A, B and C and paspaline) (Uhlig et al. 2009; Hodgson 2010; Comte et al. 2017).

These results enabled the identification of the fungus *Claviceps paspali*, as the causal agent of the described clinical situation, which had parasitised the *Paspalum paspaloides* samples found on the farm.

The first preventive action, without having reached a definitive diagnosis, consisted of removing the cattle from the pasture where they displayed the clinical signs. A supportive treatment based on hepatic protectors, a glucosaline 5% solution, vitamin E, selenium, and 74% sulfur orally was applied. As a handling measure, the animals were stabled in yards in which they had to remain until they showed notable improvement.

Five days later, most of the animals had recovered, with no mortalities. Once the diagnosis was confirmed, the animals were relocated in an area of the field with little vegetation, and they were supplied with good quality, abundant hay. As a preventive measure, the farmer was advised to keep up high grazing pressure during the summer months to reduce the infection rate in the following year, as well as controlling the field by mowing the grass to eliminate the plant inflorescence.

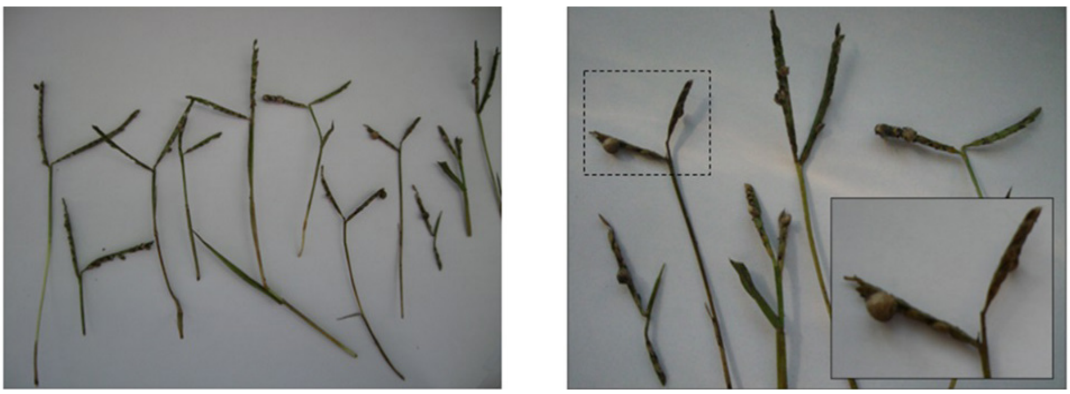

Figure 2. *Paspalum paspaloides* plant samples received in the laboratory, and details of a sclerotium compatible with *Claviceps paspali*

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DISCUSSION AND CONCLUSIONS

Claviceps paspali infects plants, producing a sclerotium containing indole-dipertenoid alkaloids, mostly paspalitrem A, B and C, and paspaline. This is enhanced by a warm, humid environment, and it is in the autumn when the inflorescences of the grass *Paspalum* spp., the principal plant species infected by it, develop a larger number of sclerotia (D'Esposito and Lopez 2001; Cawdell-Smith et al. 2010).

The case described evolved at a time of the year when the temperatures were excessively high with respect to the normal temperatures in this region, and in which there was high humidity due to the frequent rain events during previous days. Specifically, a maximum temperature of 31 °C was recorded and precipitation of 135.4 l/m^2 occurred in the month of October. Both conditions were favourable for fungal growth (D'Esposito and Lopez 2001; Odriozola 2013).

The toxins of *Claviceps paspali* act specifically on the Purkinje cells present in the cerebellum, altering the latter's modulatory action on the brain's nerve impulses. To be more precise, the *Claviceps paspali* mechanism of action is related to the inhibition of the gamma aminobutyric acid (GABA) receptor, release of neurotransmitters from the synapsis of the central nervous system (CNS) and from the peripheral nerves of the neuromuscular junction. It is a competitive-type inhibition induced by a flux of chloride ions (Plumlee and Galey 1994; D'Esposito and Lopez 2001; Cawdell-Smith et al. 2010). When the GABA receptor is unoccupied, it is inactive, and the coupled chlorine channel remains closed. The binding of the GABA inhibitory neurotransmitter to the receptor provokes the opening of the chlorine ion channel, which leads to the hyperpolarisation of the cell (Evans and Gupta 2018).

The animals affected were bovines, a species which tends towards having a greater prevalence and sensitivity to *Claviceps paspali* poisoning. Although age has not been established as being a determinant factor, younger animals are inclined to have increased susceptibility to toxicity caused by the mycotoxins generated by this fungus (Cawdell-Smith et al. 2010; Evans and Gupta 2018), which can be linked to the fact that the young animals on this farm exhibited the most severe clinical signs in the described case. The clinical signs were mainly neurological, so that both the clinical situation and the fact that it worsened when submitting the animals to stress, coincide with what was reported by Odriozola (2013) for the tremorgenic syndrome produced by *Claviceps paspali.*

As there were no deaths, it was not possible to determine if there were any lesions in the CNS. In other similar cases, the most significant anatomopathological findings were: diffuse petechiae in the brain parenchyma; neuronal degeneration; satellitosis; neurophagia; gliosis and a moderate degeneration in the neuropil of the peripheric areas of the brain (Moyano et al. 2010). However, as described by other authors, there was no mortality in the described case, another factor coinciding with this type of mycotoxicosis (D'Esposito and Lopez 2001).

With regard to the clinical diagnosis, the increase generally observed in the blood biochemistry of the enzymes creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) gave the most significant evidence. An increase in CK is indicative of acute, both skeletal and cardiac, muscle damage (Bonino et al. 2013). The enzymes CK, AST and LDH are physiologically found inside the muscle fibres and are released after their necrosis, or due to changes in the permeability of the sarcolemma membrane caused by hypoxia. It has also been verified that, under stressful situations and after intense physical exercise, the concentration of these enzymes considerably increases (Mas et al. 2010). Therefore, the increment is related to the damage produced in the muscle cells as a result of the severe tremorgenic activity.

It is not currently known what dose of sclerotia or *Claviceps paspali* is required to elicit staggers in cattle. It is, therefore, not possible to establish a correlation between the *Claviceps paspali* concentration and the observed clinical signs, the severity of which may have been a result of the types of toxins that were present and/or the dose that was consumed (Cawdell-Smith et al. 2010).

Another fundamental part of the diagnosis was based on the presence of a sclerotium showing a morphology compatible with that of *Claviceps paspali*, localised in a host grass. When classifying the different species in accordance with the characteristics of their sclerotium, *Claviceps paspali* is included with *Claviceps queenslandica*, *Claviceps hirtella* and *Claviceps orthocladae* as a species with a sclerotium of a subglobular-to-elongated shape, and pale in colour. The specific characteristics that a *Claviceps paspali* sclerotium should have for its identification

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are to be spherical in shape, of a hard consistency, light brown in colour, with a rough surface, and a diameter of 2–5 mm (D'Esposito and Lopez 2001; Kren and Cvak 2006). From its morphological characteristics, it was concluded that the plant species was *Paspalum paspaloides*, which constituted the principal host of this fungus species. Nevertheless, the main plant species infected by *Claviceps paspali* is *Paspalum dilatatum* (Cawdell-Smith et al. 2010). With respect to the plant, *Paspalum paspaloides* is considered to be an invasive species in several countries, including Spain. For its control, the most effective prevention action is the conservation of riparian vegetation and wetlands in good condition. When an invasion occurs, any action to remove it, either mechanically or chemically, is very limited (Sanz-Elorza et al. 2004).

The analysis techniques performed for the identification of the indole-terpenoid alkaloids produced by *Claviceps paspali*, as well as for the confirmation of the fungus species causing the clinical presentation, coincide with those reported by different authors (Uhlig et al. 2009; Comte et al. 2017). However, other authors describe other commonly employed techniques, such as: high performance liquid chromatography (HPLC), which is a technique with high sensitivity; capillary electrophoresis (CE); or tandem mass spectrometry (MS-MS) (Krska et al. 2008; Tiwary 2009; Crews 2015).

As there was no effective aetiological treatment, the treatment given to the animals was based on supportive therapy (Riet-Correa et al. 2013). Similar to the case reported by Cawdell-Smith et al. (2010) in horses poisoned by *Claviceps paspali*, the animals were kept well fed. These supportive actions were implemented to control the mycotoxicosis. The actions consisted of administering fluid therapy, together with different vitamin complexes, but not with antibiotics or anti-inflammatory medication.

In this type of poisoning, effective handling measures should be decisive, for example, removing the animals from the infected area, isolating them in a yard and keeping them well fed. All the above measures constitute general actions to apply in order to control the mycotoxicosis (Riet-Correa et al. 2013). The recovery rate, described at 5 days after doing so, coincides with that given for *Claviceps paspali* poisoning, in which the clinical signs are estimated as being in remission around a week after the elimination of the source of intoxication (Evans and Gupta 2018). There are some cases in which the recovery occurred even earlier, like those in the work of Cawdell-Smith et al. (2010), in which the recovery of some affected horses was achieved after only two days. However, the results were not favourable in all the cases, and some studies have reported that, despite removing the animals from the affected area, they continued to display clinical signs, thus the total recovery was not possible, so that they finally died. On other occasions, the clinical signs became so serious that the humane euthanasia of the animals was necessary, as has been described by other authors (Cawdell-Smith et al. 2010; Moyano et al. 2010).

For prevention, care should be taken to avoid the presence of mouldy feedstuff in areas where animals have unsupervised or unrestricted access, and to remove any potentially contaminative material. Animals should not be free to roam in areas where discarded foodstuff or refuse is stored, or in the vicinity of compost piles. Livestock should not be fed overtly mould-contaminated forage or concentrates, and care should be taken when feeding food or beverage manufacturing by-products (Burrows and Tyrl 2013; Evans and Gupta 2018).

Finally, it was concluded that, despite the low prevalence of the mycotoxicosis caused by *Claviceps paspali* in the region described in the study case, the unusually high humidity and temperature levels for the region triggered the development of the sclerotium generated by this fungus. Secondly, the analytical diagnosis proved to be fundamental for confirming that *Claviceps paspali* was the causal agent of the described clinical picture, as well as for identifying the principal indole-terpenoid alkaloids produced by that species. Lastly, as there is no effective aetiological treatment for this *Claviceps paspali* poisoning, the application of adequate prevention measures constitutes the most efficacious method in the control of this type of mycotoxicosis.

Conflict of interest

The authors declare no conflict of interest.

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