Annual ryegrass toxicity in Thoroughbred horses in Ceres in the Western Cape Province, South Africa

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ABSTRACT
An outbreak of annual ryegrass toxicity occurred on a Thoroughbred stud in Ceres in the Western Cape Province of South Africa. This is the 1st report of annual ryegrass toxicity in horses in South Africa, although the condition has been reported in cattle and sheep populations in the past. Annual ryegrass toxicity is characterised by a variety of neurological signs including tremors, convulsions, recumbency and in many cases death. The description of the outbreak includes the history, clinical presentation and treatment protocol administered during the outbreak. Various epidemiological variables and their influence in the outbreak are also considered.

Keywords: annual ryegrass toxicity, horse, corynetoxins, neurological, magnesium sulphate.


INTRODUCTION
Annual ryegrass toxicity (ARGT) is an often fatal disease of livestock following ingestion of annual ryegrass (Lolium rigidum) seed heads which have been contaminated by corynetoxin-producing Rathayibacter toxicus through this bacterium’s association with the nematode Anguina funesta. The corynetoxins produced are tunicamyluracil antibiotics which are toxic due to their inhibition of protein glycosylation. The disease causes significant central nervous system-associated clinical signs that are often preceded by stressful activities. These signs are owing to cerebellar interstitial oedema caused by vascular leakage resulting from endothelial damage by the toxins. This disease is highly prevalent in certain areas of Australia where outbreaks occur regularly in sheep and cattle populations. ARGT has been described in South Africa, with reports of high mortalities in sheep near Caledon in 1979 and 3 more outbreaks in cattle in 1980, all in the Western Cape Province. One of the outbreaks in cattle was near Prince Albert Hamlet, which is within 10 km of Ceres.

The 1st reported cases of ARGT in horses occurred in Western Australia and were described in 1996. Three different farms near Perth were affected, 5 horses died or were euthanased and 2 affected horses survived following treatment. The authors also indicated that similar cases of ARGT in horses had occurred in South Australia. In addition, 11 horses died in an outbreak of flood plain staggerers during 1991 in New South Wales, Australia. This disease is produced by the same toxins that cause ARGT and occurs when stock eat blown grass (Lachnanthes filiformis, formerly Agrostis avenacea) infected with R. toxicus that is vectored by the nematode Anguina paludicola. Equine cases of ARGT have not been described in South Africa.

CASE HISTORY
The ARGT outbreak occurred on a Thoroughbred breeding stud farm on the outskirts of Ceres in the Western Cape Province in South Africa. This region has a winter rainfall period that gives rise to wild annual ryegrass throughout the region. The farm involved fed locally produced oaten hay as roughage to all mares throughout the summer months in 2008/2009. The outbreak commenced on 7 February 2009 and the last new case was seen on 11 February 2009. The morbidity of the disease decreased dramatically following removal of the oaten hay on 8 February 2009.

Six mares, 1 colt and 1 yearling filly were affected during the outbreak. The morbidity rate on the farm was 6.2% (8/120), with a mortality rate of 1.7% (2/120) and a case fatality rate of 25% (2/8). Seven of the 8 cases were in the same paddock (Paddock 19) on the farm, and there was a 29% (7/24) morbidity rate in this paddock. The only other case occurred in a paddock diagonally across from Paddock 19, and the morbidity rate in this camp was 6.7% (1/15).

Clinical signs
The index case was initially thought to be colic as the mare was found recumbent, very uncomfortable and seemingly in pain. She had a severe tachycardia, a lack of borborygmi and severely congested oral mucous membranes. When the mare displayed severe convulsions the stud manager opted for euthanasia. All other cases developed hypermetria and cerebellar ataxia, and 5 became recumbent. Some recumbent horses developed convulsions with paddling of the limbs. None of the cases had an elevated temperature. At least 2 horses’ clinical signs became apparent after they had been removed from their original paddocks and stabled. Both of the fatalities were mares that were euthanased due to severe clinical signs. Two horses relapsed within 6 days of the original clinical signs but on both occasions responded to treatment and recovered fully.

Time to clinical response after treatment was varied but, in general, the attending veterinarians felt that cases responded well. The shortest recorded period of clinical response after treatment was 20 min, and in this case the horse was recumbent at the time of treatment. Two other cases took between 1 and 1.5 h to show visible signs of improvement and 1 animal only stood up from recumbency 6 h after treatment. In the last case the mare only allowed her foal to suckle the day following treatment.

A post mortem was not performed on the index case in the outbreak; however, a full post mortem was performed on the mare that was euthanased on 8 February 2009. This mare presented with cerebellar...
atonia that included hypermetria and incoordination. She later collapsed and had muscle fasciculation while recumbent and had a seizure prior to sedation and eventual euthanasia. On post mortem a moderate pulmonary oedema was found with mild hyperpericardium and subendocardial haemorrhage in the left ventricle. A haemorrhagic ascites was also noted and the bladder had prolapsed through the vagina prior to euthanasia. Histopathological examination of representative coronal sections of the brain showed no specific lesions.

**Diagnosis**

The diagnosis was based on the presence of central nervous system associated clinical signs that are typical of ARG7 and the positive identification, by the Stellenbosch Provincial Veterinary Laboratory, of seeds contaminated with bacterial galls, found within bales of oaten hay being fed to the horses.

**Epidemiological considerations**

A 4.5 ha, contoured field of farmland was hired in Ceres by the stud for the purpose of planting oats and making oaten hay. The cutting of the crop was delayed for 2–3 weeks due to late seasonal rains and was baled soon after 26 November 2008. The affected oaten hay was fed for 9 weeks, from the time it was baled until the 2nd day of the outbreak. The timeline associated with this outbreak was consistent with a toxin exposure, as the cases showed a clear indication of a point epidemic. The mode of cases occurred on the same day as the index case on 7 February 2009. The 2nd day of the outbreak gave rise to 3 cases with a single case occurring on 11 February. The affected oaten hay was removed on 8 February.

The feeding pattern of the affected oaten hay was consistent for all paddocks. The hay was fed on the ground and always in the same position within each paddock. Annual ryegrass is a weed that is found within bales of oaten hay being fed to the horses.

**Treatment**

Horses showing clinical signs of ARG7 were immediately treated with magnesium sulphate (MgSO4 50 %, Kyron Laboratories) solution intravenously using a treatment regimen previously described for horses with annual ryegrass toxicity (ARGT). The initial dosage ranged from 60–200 mg/kg using 300 kg as an average weight for these horses. In general the dose used was approximately 100 mg/kg. The recommended route of administration of this drug is subcutaneous but no negative effects of intravenous administration were noted during the outbreak. The MgSO4 treatment was repeated as required if animals continued to show any central nervous signs. A dose was also given in the evening to affected animals as a prophylactic against convulsions during the night. Other drugs used included 1 mg/kg flunixin meglumine (Finadyne®, Shering-Plough AH) as well as approximately 0.08 mg/kg dexamethasone sodium phosphate (Calvasone, Norbrook®). Recumbent animals were treated with a regimen of intravenous fluids which included dimethyl sulphoxide and Norcal PMD (Norbrook®). The latter contains calcium borogluconate, dextrose and magnesium hypophosphite. In 2 cases which were severely affected and deemed to be in severe shock, a combination of 100 mg per horse of prednisolone sodium succinate (Solu-Delta-Cortef®, Pfizer AH) and 500 to 1000 m (per horse of hydroxyethyl starch (HAES-steril® 6 %, Fresenius Kabi) was administered intravenously. All cases received a standard vitamin and mineral supplement which included sodium glycercophosphate and vitamins B12, B6 and B5 (Kyrophos Metabolic-V, Kyron Laboratories).

**DISCUSSION**

This is the 1st description of ARG7 in horses in South Africa. The outbreak described has similarities to an outbreak of ARG7 in horses previously described in Western Australia 9. The clinical manifestations of the disease were similar and both were associated with a specific source of toxin and feeding of this source for a specific period. This period may range from several days to weeks in duration11 and depends on the level of contamination of the source and the quantity being fed. In the outbreak described here the affected oaten hay was fed for 9 weeks prior to the outbreak, and had been fed for a specific period to most of the horses on the farm.

The corynetoxins produced by *R. toxicus*22 are cumulative toxins in spite of having a short (4-hour) plasma half-life17–19. It seems an anomaly to have a point epidemic due to a cumulative toxin but the sudden onset of the outbreak of ARG7 described here is typical of many of the outbreaks of ARG7 seen in all species in Australia and is probably due to the disease requiring a stress-associated trigger16. In this outbreak environmental temperature was thought to be a likely trigger. In the current outbreak, only 1 clinical case occurred after the removal of the toxic feedstuff, indicating that a continual intake of the toxin is required for clinical signs to develop.

An important consideration in this outbreak is that cutting of the oat crop was delayed by 2–3 weeks. This additional period would have allowed the ryegrass plants to complete reproduction and the seed heads to reach full maturity. Toxin production within the bacterial galls commences during flowering, rapidly increases during seed set, and the maximum concentrations of toxin are reached at the time that the seeds become fully mature22. In addition, conditions for ryegrass growth within the oat crop may have varied due to contouring in the field, causing run-off water to concentrate in different sections. As the crop was cut sequentially and the bales taken to storage in the order that they were prepared, there may have been groups of bales in the storage facility that contained greater levels of ryegrass seed. These groups of bales may therefore have contained higher levels of toxin and could have been fed closer to the time of the outbreak.

All the horses on the farm were fed the contaminated hay, but ARG7 only became apparent in 2 paddocks. A possible explanation for this is that the wild ryegrass in these paddocks was also toxic. As this ryegrass was not examined for seeds contaminated with bacterial galls it is not known whether this was the case. However, it takes several years for the populations of the nematode and bacterium in annual ryegrass to reach levels that present a risk to livestock46. It was evident to the farm management and attending veterinarians during this outbreak that clinical signs were precipitated by stress, particularly during the afternoon. February is one of the hotter months of the year in the Western Cape Province and it is likely that the high temperatures, particularly in the afternoon, contributed to the stress that led to clinical manifestation of ARG7. Weather data from the nearest weather station (Worcester, 34.2 km away) revealed that 7 February 2009, the day the outbreak began, was the hottest day of the year to date, with a maximum of 37.3 °C and a minimum of 20.5 °C. The thermoneutral zone of horses falls between 5 and 25 °C, so in the prevailing environmental conditions these horses were under some degree of heat stress. Wind speed data were not available for Ceres.

Two horses relapsed within a week and the treatment regimen was reinstalled based on the severity of the clinical signs. This is a reasonably high proportion given that 2 of the 8 cases were euthanased due to poor prognosis and were
therefore not candidates for a relapse. This clinical feature must be taken into consideration in the event of other ARGt outbreaks. Of the 42 mares on the farm during the outbreak, 40 were pregnant, and while abortion was not seen in this outbreak it has occurred in ovine cases of ARGt in the past.19

No specific antitoxin or antidote against the corynetoxins produced by R. toxicus is available, so symptomatic treatment is all that can be attempted in cases of ARGt. In this outbreak MgSO4 was used to prevent muscle contractions associated with convulsions. This salt is used in human medicine to prevent the convulsions associated with eclampsia and it acts by preventing the release of acetylcholine at the neuromuscular junction (NMJ).15 During outbreaks of ARGt in sheep and cattle generally only clinically affected animals are treated with MgSO4. Since horses are more valuable and found in smaller numbers it is probably advisable to treat all horses potentially exposed to the toxin as it is inexpensive and could provide a form of prophylaxis to prevent development of the clinical signs. It is important not to use MgSO4 concurrently with calcium because this provides a greater source of Ca2+ at the NMJ, which decreases the effectiveness of Mg2+ in preventing muscle contraction.15 The attending veterinarians also recommend the use of an indwelling intravenous catheter for the MgSO4 treatment as it decreases the stress associated with repeated injections.

Flunixin meglumine has anecdotally been used to good effect in cattle affected by ARGt in Australia (JGA, pers. obs.) and South Africa (P le Roux, private veterinarian, pers. comm., 2009). Although no specific reason for this being effective was obvious, the anti-inflammatory properties of this drug may have limited the muscle damage caused by clonic and tonic contractions.

In the current outbreak, HES (hydroxyethyl starch) was used to treat horses with signs of shock. As starch increases the osmotic pressure in the blood, interstitial fluid is drawn into blood vessels, increasing blood volume and therefore increasing mean arterial blood pressure. In humans, there is evidence that HES does not cross damaged blood-CSF barriers but it is still not clear whether it crosses the blood brain barrier.26 If HES crossed the blood-brain barrier in horses with ARGt it might cause a greater osmotic pressure within the already compromised interstitial tissue. Studies in humans have, however, shown that the use of HES to improve cerebral blood pressure in patients with severe cranio-cerebral injury is safe.18 Nevertheless, use of HES in ARGt cases should be considered with caution until more is understood about the extent of damage to the cerebellar vasculature in this disease. If the damage is extensive enough to allow HES across the blood brain barrier the disease will be exacerbated, as more fluid will be drawn into the cerebellar interstitium.

CONCLUSION

ARGt is well known and described in Australia. It has been described to a lesser degree in South Africa, possibly owing to misdiagnosis or a lack of reporting. The circumstances leading to this outbreak were as follows: later than usual cutting and baling of the oaten hay led to maximum toxicity in the affected ryegrass within the hay; heat stress, associated with the very hot weather in February, provided a stress trigger; increased toxin ingestion just prior to the clinical outbreak may have occurred because specific bales contained higher concentrations of toxin. Once the disease was diagnosed and the toxic hay removed, the morbidity of the disease decreased rapidly, with only 1 new case and 2 relapses occurring. The treatment of ARGt was symptomatic, but it is important to consider treating all potentially in-contact horses with MgSO4, during an outbreak. Avoiding the use of calcium as part of the treatment regimen should also be considered. The use of anti-inflammatory drugs may be useful to reduce inflammation associated with muscle damage due to convulsions. Should the causative organisms of ARGt become more widespread, horse owners should ensure that the hay they feed and the ryegrass in paddocks are both free of toxic seed galls. Currently the Stellenbosch Provincial Veterinary Laboratory visually examines ryegrass seed for the toxic bacterial galls. In Australia an ELISA for the bacteria24 is used commercially and an ELISA for the corynetoxins25 has also been developed. It may be worth implementing these assays in South Africa.

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