Mortality supposedly due to intoxication by pyrrolizidine alkaloids from *heliotropium indicum* in a horse population in costa rica: A case report

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MORTALITY SUPPOSEDLY DUE TO INTOXICATION BY PYRROLIZIDINE ALKALOIDS FROM *HELIOTROPIUM INDICUM* IN A HORSE POPULATION IN COSTA RICA: A CASE REPORT

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SUMMARY

This article describes a case of massive mortality among horses which was probably due to intoxication by pyrrolizidine alkaloids from *Heliotropium indicum*. Over 4 years more than 75% of a population of about 110 horses on a farm in Costa Rica died after showing nervous neurological symptoms. Two clinical manifestations were encountered, an acute and a chronic one, both with a fatal outcome. Pathological findings in 2 horses coincided with those reported in the literature for intoxication by pyrrolizidine alkaloids and were not specific for VEE. However Venezuelan equine encephalitis (VEE) was the main differential diagnosis and could not completely be excluded because this disease was endemic in the region and VEE titres were found to be high. Taxonomic and toxicological investigations implicated *Heliotropium indicum* as the most probable principal cause of the intoxication.

Keywords: Horse, hepatoencephalopathy, pyrrolizidine alkaloids, neurological signs, VEE.

INTRODUCTION

Neurological disorders in equine patients often present serious problems to the attending veterinarian due to the wide variety of signs and the complexity of the disease processes involved (9,10). If a large part of the population is affected, individual ailments, such as trauma, tumour, or the extension of a septic process elsewhere in the head into the cerebral cavity, can be discarded from the list of possible diagnoses, which facilitates the final diagnosis to some extent. However, a vast number of possibilities still remain even though at times a final diagnosis may be needed urgently when important economic interests are involved.

The agent of Venezuelan equine encephalitis (VEE), an arthropod-borne virus belonging to the group of Alphaviruses, is endemic in large parts of Central America, and outbreaks of the epizootic form of this virus have been reported to cause

massive equine mortality characterized by neurological signs (4). Outbreaks of Eastern equine encephalitis (EEE), caused by a virus from the same group, have been detected sporadically but are characterized by a relatively low mortality (4,8,13). Other possible causes of neurological disorders in equine populations in the region include hepatoencephalopathy due to poisoning by toxic plants, babesiosis, protozoal myeloencephalitis, verminous encephalitis, rabies, and leukoencephalomalacia (1,4,15).

This article reports on the high death rate among horses on a farm situated in the Nicoya peninsula of Guanacaste province, Costa Rica. The animals exhibited neurological signs before death.

CASE HISTORY

Environmental anamnesis and early history

The farm comprises about 650 ha and is located at an altitude of about 55 m above sea level. Of the total area of the farm, about 400 ha is pasture; the remaining 250 ha is covered by tropical dry forest. Formerly, some pastures were used for rice culture. The average temperature in the area is 25°C and the annual rainfall is 800 mm. The year is divided into a wet season (May-November) and a dry season (December-April), with almost all rainfall falling during the wet season.

Farm management is principally dedicated to the breeding of beef cattle, with an average cattle population of 400. There are small populations of goats and sheep. The horses and mules are used for work purposes but some are used for breeding with the intent to sell the offspring. The original equine population was about 110 animals.

Outbreaks of VEE in 1970 (3,6) had led to annual vaccination campaigns in the whole of Costa Rica. These campaigns were discontinued in the early 1980s. Thereafter the horses on the farm were not vaccinated and the only preventive measure taken consisted of regular bimonthly deworming with ivermectin.

The death rate among the horse population had been high for years, but rose in recent years with the result that in a 4-year period about 75% of the original horse population died, leaving not more than 25 horses alive. Two main clinical manifestations were observed. The acute form had a sudden onset in seemingly healthy animals and was characterized by violent neurological signs such as head pressing, grasping of barbed wire fences and breaking through them, and sometimes aggressive behaviour towards other animals. Death occurred within a few hours. In the chronic form the horses were dull and listless. The animals lost their appetite, rapidly became emaciated, and seemed to wander aimlessly. These animals wasted away in a few weeks or months. Treatment, consisting of the administration of broad-spectrum antibiotics, was given to several ani-

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mals by the owner but without positive results. Only one animal, which had suffered the chronic form of the disease, was reported to have survived. This animal had been treated in the same way as the others but had been transported to another location. Animals under 1 year of age were not affected.

Horses

After various attempts to lower the death rate had failed, the owner contacted the Veterinary School. Owing to the remoteness and the difficult accessibility of the farm, it was decided that two sick animals should be sent to the Veterinary School for observation (horses A and B).

Both animals were examined clinically and subjected to various diagnostic procedures. Horse A died within a month and was unfortunately not available for necropsy, but horse B was euthanized after 3 months and was examined pathologically.

Six months later a third horse, which had been transported from the farm to a stable in the Central Valley 7 months earlier, died after showing acute neurological signs. This horse was presented for necropsy (horse C).

Because horses continued to die, the farm was visited to study local conditions and to take blood samples for serological examinations from the entire remaining horse population, which had decreased to 14 horses and 2 mules at that time (horses D-Q and mules X,Y). One sick horse was encountered and was transported to the Veterinary School for further diagnostic procedures. This horse was killed and necropsy was performed (horse D). Horses A-D were all mares belonging to the local criollo breed and ranged in age from 31/2 to 8 years. The average body weight was about 400 kg.

Clinical observations

All sick horses examined showed the chronic form of the syndrome. The acute form was not observed. The animals were dull and listless, showing little interest in their environment. They had little appetite and rapidly became emaciated. All animals showed an ataxic gait to some extent. Further signs were involuntary movements, decreased postural reflexes, and hypersensitivity and facial paralysis in some animals.

Laboratory findings

Samples from horses A, B, and D were collected for haematological tests, analysis of cerebrospinal fluid, and parasitological investigations. In horses B and D the haematocrit was elevated (46% and 43% respectively), and the white blood cell count was relatively high in horses A and D (13.9 G/l and 17.9 G/l respectively). In both cases the percentage of neutrophils was high (78% and 85%). Other haematological variables were normal. In horse D blood chemistry revealed high values for aspartate aminotransferase (AST) (174 IU/l, normal values for this laboratory: 68-74), for alanine aminotransferase (ALT) (9.5 IU/l, normal: 1.6-7.0) and for total bilirubin (7.9 mg/dl, normal: 0.2-2.0). In the same horse blood urea nitrogen was lower than normal (5.9 mg/dl, normal: 10-30). In the cerebrospinal fluid total protein, glucose, neutrophils and erythrocytes were determined and were within the normal ranges in all cases. Bacteriological culture of the cerebrospinal fluid in horses A and B yielded negative. Faeces did not contain internal parasites.

Serology

Serum from horses A and B was screened for Equine infectious anaemia (EIA) and Leptospirosis, and serum from all horses was examined for the presence of antibodies against VEE (except for horse C, which was only examined postmortem). Both horses A and B were negative for EIA. Horse A was positive for *L. hardjo* (1:400) and *L. ballum* (1:100). Horse B was positive for *L. hardjo* (1:200), *L. ballum* (1:100) and *L. pyrogenes* (1:100). Table 1 presents the results of the serological tests for VEE.

Postmortem findings

Necropsy of horse B was performed with special attention being paid to the brain. Meningeal oedema and congestion were evident, but no specific brain damage could be found. Gross liver damage was noted in this horse, but the liver was not investigated histologically. In horses C and D macroscopic liver damage was evident and in these cases a thorough histological examination was performed of all macroscopically affected organs.

Table 1. Serological results of tests for Venezuelan equine encephalitis (VEE). Titres expressed as plaque reduction neutralizing titres, 50% indicates the serum dilution capable of neutralizing 50% of plaques and 80% indicates serum dilution capable of neutralizing 80% of plaques of VEE (Trinidad strain).

Horse	Age (yrs)/sex	History (anecdotal information obtained from farm personnel)	VEE titre 50%	VEE titre 80%
A	8/F	Depressive symptoms, died at vet school	640	160
B	3.5/F	Depressive symptoms, died at vet school	<10	-
C	7/F	Acute encephalitis, postmortally examined at vet school	ND	ND
D	3.5/F	Depressive symptoms, died	2560	640
E	4/F	Acute encephalitis, died in 4 hrs	<10	<10
F	5/M	Depression, lived	<10	<10
G	5/F	Depressive symptoms, died	<10	<10
H	2/F	No symptoms	320	160
I	7/M	No symptoms	2560	640
J	6/F	Depressive symptoms, died	320	80
K	4/M	Depressive symptoms, died	<10	-
L	6/F	Depressive symptoms, died	<10	-
M	5/F	Acute encephalitis, excitation, died	<10	-
N	8/M	No symptoms	1280	160
O	1/M	No symptoms	80	20
P	3/F	No symptoms	640	160
Q	5/F	Acute encephalitis, excitation, died	<10	-
X	8/Mu	No symptoms	160	40
Y	8/Mu	No symptoms	10	<10

F = female; M = male; Mu = mule; ND = Not Determined.

Both horses C and D were cachectic at necropsy and had skin lesions caused by *Dermatophilus congolensis* infection. They showed icteric mucosae and an icteric intima of the aorta. Both the thoracic and the abdominal cavity contained moderate volumes of an amber-coloured fluid. The liver was markedly reduced in size with a pale zonal pattern and a firm consistency. The kidneys showed adherence of the capsule and yellowish spots in the glomerular zone. The lungs had multiple nodules with purulent exudate. As in horse B, in these horses the brain was characterized by meningeal oedema and congestion.

At the microscopic level the liver appeared to be severely damaged with interlobular fibrosis, hepatocellular anisomorphia characterized by megalocytosis, retention of bile pigments, vacuolar and fatty degeneration, proliferation of the bile ducts, and focal parenchymal haemorrhage. In the kidney epithelial degeneration, megalocytosis (although to a lesser extent), and retention of bile pigment in the tubular cytoplasm could be observed. The lungs were characterized by multiple areas of purulent to granulomatous pneumonia. In the spleen retention of bile pigment was seen. The cerebral cortex showed satelitosis, neuronophagia, and gliosis.

Taxonomic and toxicological investigations

As the clinical and pathological symptoms pointed more and more towards hepatoencephalopathy caused by the ingestion of toxic plants, the farm was visited a second time to investigate the presence of potentially hepatotoxic plants in the pastures the horses normally grazed.

The pasture appeared to be rather wet (the area was formerly used for the growing rice). Natural grasses (*Brisantha, Transvala, Angleton, Ciperaceas, Jaragua*) and many other plants grew there. The following plants that showed signs of having been eaten were categorized as potentially hepatotoxic and sampled for laboratory investigations: *Acacia spp, Amaranthus espinosus, Crotalaria spp, Crotton spp, Esterculaceas, Heliotropium spp, Milleria quinqueflora, Pipertuberculatum spp, Mimosaceas, Sida acuta, Sida rombifolia.* Toxicological investigations showed that of these plants *Heliotropium indicum* contained considerable quantities of pyrrolizidine alkaloids.

DISCUSSION

Hepatic encephalopathy caused by the ingestion of pyrrolizidine alkaloids is a well-documented cause of neurological disorders in horses. In Europe and Northern America the poisoning is mainly caused by tansy ragwort (*Senecio Jacobaea*) (5). Syndromes closely resembling the one caused by tansy ragwort have been reported in Canada as Alsike clover poisoning and in France, also presumably caused by a clover species (12, 14). Under the tropical conditions of Central America, *Crotalaria spp* and *Heliotropium spp* are the most widely dispersed plants that could cause the syndrome. Although *Heliotropium spp* are commonly indicated as possible causes of hepatic encephalopathy in horses (7, 15), no reports could be found of massive intoxication of horses by this plant. In the present study *Heliotropium indicum* was the most probable cause of the syndrome because, of all potentially toxic plants that were examined, only this species contained considerable quantities of pyrrolizidine alkaloids.

It is important to point out that the presence of toxic plants does not necessarily lead to clinical disease. Most toxic plants are relatively unpalatable and will not be eaten when sufficient grass of good quality is available (7). Therefore, it is necessary to check not only for the presence of poisonous plants, but also to verify that they have been consumed. In this case, the lack of sufficient grass, together with the abundance of a number of toxic plant species in the pasture in which the horses were kept, forced the horses to consume the poisonous plants. In fact, they were seen eating them by the authors. Apparently, chronic ingestion of low volumes of toxic plants can provoke clinical hepatic encephalopathy. Craig *et al.* demonstrated that in the case of tansy ragwort (*Senecio jacobaea*) toxicosis, a relatively low intake of 5% dry matter for 60 days was sufficient to provoke fatal hepatoencephalopathy in ponies (2). In the Costa Rican farm, the horses remained in the contaminated pasture for years, and all year round, although the intake of toxic plants would not have been consistently high because of seasonal variations in environmental and climatological conditions.

The clinical signs observed coincided well with those described in the literature. Although there are no clinical features that distinguish hepatic encephalopathy from other causes of cerebral dysfunction (11), all the clinical symptoms encountered have been described as possible manifestations of the syndrome. Depression and slight-to-severe ataxia were the most consistent clinical symptoms in the three horses (A,B,D) examined. Craig *et al.* (2) described both the acute and the chronic forms of the disease. They proposed the term 'chronic-delayed' for the symptomatology described in this paper as acute. The seemingly 'acute' form is in fact the sudden expression in an apparently healthy animal of long-existing chronic liver failure. The differential response or resistance of individual animals to the pyrrolizidine alkaloid toxin may reflect each animal's rate of bacterial detoxification in the gastrointestinal tract, rate of conversion of the non-toxic pyrrolizidine alkaloid to the toxic active pyrrole by the liver, antioxidant status, and exact amount ingested (2). Craig *et al.* reported that horses with the chronic-delayed form died as long as 346 days after exposure to the toxic plants had ended. This long interval between exposure to the toxic alkaloids and the onset of clinical signs explains why horse C, which was removed from the original farm and transported to a different site free from toxic plants, died of the acute (or 'chronic-delayed') form 7 months after it had left the farm.

Haematological variables for horses A,B and D did not show important changes. The haematocrits of horses B and D were relatively high, but this may have been due to stress and dehydration during the long trip in an open trailer. The white blood cell counts in horse A, but more so in horse D, were somewhat elevated, indicating the presence of an inflammatory process. In horse D this may have been due to the relatively extensive *Dermatophilus congolensis* infection the horse was suffering from.

The blood chemistry of horse D showed relatively high values for aspartate aminotransferase (AST) and, to a lesser extent, for alanine aminotransferase (ALT). Serum levels of these enzymes may be elevated in hepatic encephalopathy, but they are not liver specific (11). Unfortunately, the local laboratory facilities did not permit the determination of more specific enzymes such as gamma glutamyl transferase (2, 5). Total bilirubin was significantly raised above the normal level of 0.2-2 mg/dl, reflecting possible liver damage. Blood urea nitrogen (BUN) was low in horse D. As urea production occurs almost exclusively in the liver, a decrease in BUN is

frequently seen in liver failure (15).

Cerebrospinal fluid variables for the horses A, B, and D were within the normal range. These findings are consistent with the lesions found in the cerebrum, which were degenerative in nature and not inflammatory, which argues strongly against the original presumptive diagnosis of encephalitis.

The serological investigations for Equine infectious anaemia yielded negative results for horses A and B. The presence of low titres of antibodies against some *Leptospira* species was considered to be of no importance as no abortions or other manifestations of clinical leptospirosis had been observed in the horse population over the past 8 years by the owner, who is a retired veterinarian. There were high titres of antibodies to VEE in some horses of this unvaccinated population. This suggests that the virus was present in the region. As the pathological findings of the examined horses were consistent with damage caused by a hepatotoxic agent and not with the clinical manifestation of VEE, the disease was discarded as a possible main cause of the high mortality among these horses with signs of nervous system involvement. However, the clinical signs shown by horses suffering from the chronic form were also compatible with VEE enzootic and/or epizootic activity. Some interference of this disease in the overall clinical picture of the herd can therefore not be ruled out.

The postmortem findings for both horses C and D were very suggestive of pyrrolizidine alkaloid intoxication. A strongly fibrotic liver with microscopically detected megalocytosis, retention of bile pigment, interlobular fibrosis, and proliferation of the bile ducts is characteristic for this disorder (7). Secondary lesions in the brain characterized by satelitosis, neuronophagia, and gliosis also have been reported.

That animals younger than about 1 year were not affected may be explained by the fact that alkaloids are not known to be transferred by milk and by the long interval between the onset of liver damage and the occurrence of clinical signs. No clinical signs were seen in the cattle, sheep, or goat populations on the farm. Sheep and goats are known to be about 30 times less sensitive to pyrrolizidine alkaloid poisoning than horses. Cattle are only slightly less susceptible than horses (7), but the average length of stay of the beef cattle on

this farm was about $2^{1}/2$ years whereas the horses were kept on the farm for their whole working life.

Furthermore, the better pastures with virtually no harmful weeds were designated for the cattle while the horses were kept permanently in one large pasture with only some natural grasses and a dense vegetation of all kinds of weeds and toxic plants.

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