Spartium junceum *L. poisoning in small ruminants*

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Keywords
Cytisine,
Sheep,
Spartium junceum L.,
Toxic plant.

Summary
An outbreak of neurological disorders in a flock of 20 sheep coming from a rural farm in Civitella Roveto, Italy, occurred in winter 2015. All the animals showed tonic-clonic convulsions followed by muscle paralysis associated with dilated pupils, tremor, tachycardia, tachypnea and diarrhea. The presence of bundles of dry broom of *Spartium junceum* L. in the feed, eaten by the animals supported the hypothesis of plant intoxication. Two animals died after worsening of clinical signs. The anatomopathological findings and the laboratory results ruled out viral or bacterial infections or accidental exposure to other toxics. Phytochemical study showed the presence of large amount of cytisine, a nicotinic acetylcholine receptor agonist, in all parts of the plant eaten by the animals. Clinical and pathological findings, the complete remission of clinical signs after the exclusion of dry broom from the diet, together with the results of phytochemical analyses results corroborated the hypothesis of *S. junceum* L. intoxication.

Avvelenamento da Spartium junceum *L. in piccoli ruminanti*

Parole chiave
Citisina,
Ginestra odorosa,
Pecore,
Piante tossiche.

Riassunto
Nell'inverno del 2015 è stato segnalato un sospetto di malattia infettiva con prevalente interessamento del sistema nervoso centrale in un allevamento ovi-caprino sito nel comune di Civitella Roveto (AQ). Tutti gli animali hanno mostrato convulsioni tonico-cloniche seguite da paralisi muscolare associata a pupille dilatate, tremore, tachicardia e tachipnea e in alcuni casi anche diarrea. La presenza di fascine secche di ginestra odorosa *Spartium junceum* L. con alte concentrazioni di citisina e metilcitisina e l'ingestione di tali piante da parte degli animali facevano ipotizzare un avvelenamento da tale pianta. L'esame anatomo-patologico, sierologico, batteriologico e virologico hanno escluso qualsiasi causa di malattia infettiva. L'esposizione degli animali a sostanze tossiche utilizzate in agricoltura o altre piante tossiche è stata esclusa dall'esame clinico, dai reperti anatomopatologici e dall'anamnesi ambientale. Dopo la rimozione delle fascine e il cambiamento della dieta è stata osservata una remissione completa dei segni clinici in tutto il gregge. Lo studio fitochimico ha mostrato che tra gli alcaloidi isolati era presente, in quantità maggiori in tutte le parti della pianta consumata dagli animali, la citosina, agonista del recettore dell'acetilcolina nicotinica. I risultati clinici e patologici associati alla remissione completa dei segni clinici dopo l'esclusione della fascina secca dalla dieta, nonché i risultati dell'analisi fitochimica hanno portato a fare una diagnosi finale di intossicazione da *S. junceum* L.

Spartium junceum L., commonly known as Spanish broom, is a species of flowering plant in the family *Leguminosae* (sub-family *Faboideae*, *Papilionoideae*) (Pignatti 1982). All parts of *S. junceum* L. contain cytisine, an alkaloid reported as a nicotinic acetylcholine receptor agonist (Greinwald *et al.* 1990). In humans, poisoning occurs after ingestion of seed, flower or other parts of the plant. Clinical signs appear within an hour of ingestion and include mild irritation of the mouth and throat, hypersalivation, diarrhea and vomit. Early signs of neurotoxicity include dilated pupils, headache, delirium, mental confusion; severe cases show tremor, tonic-clonic convulsions followed by muscle paralysis, and coma. Death may occur through respiratory failure because of central nervous system depression and muscle paralysis (Riccardi *et al.* 2006). To our knowledge no cases of poisoning from *S. junceum* L. have been reported in small and large ruminants.

An outbreak of neurological disorders in a flock of 20 sheep coming from a rural farm in Civitella Roveto (Aquila), Italy, occurred in winter 2015 (Table I). The main clinical signs observed were: dilated pupils, tachycardia, tachypnea and tremor. The animals began to stagger showing moderate to severe ataxia with unsteady posture, frequent falling pedalling and opisthotonos. Some of them presented also diarrhea. The clinical signs persisted for several days and the ram and lambs were the worst affected. After few days one lamb and the ram died. Because of clinical symptoms observed, the farm veterinarian suspected a possible involvement of infectious agents and notified the case to the Public Health Department. Field necropsies and laboratory analysis were carried out to ascertain the cause of death of animals and to exclude the presence of any infectious agents hazardous to both animals and humans.

Samples of brain, lung, kidneys and liver were collected, immersed in 10% buffered formalin (pH 7.4) and sent to diagnostic laboratory of University of Naples 'Federico II', Department of Veterinary Medicine and Animal Productions for histopathology evaluation. Other organs such as intestine and stomach were in an advanced stage of putrefaction and were not eligible for histopathology.

Coprological analysis was also performed collecting feces directly from the rectum of each sheep; samples were then processed by McMaster method. Blood samples from twelve animals were collected by jugular vein for haemato-biochemical investigations. Hematological parameters were performed using a semi-automatic blood cell counter (Genius S, SEAC Radom Group) and included total white blood cell (WBC), neutrophils (N), lymphocytes (L), monocyte (M), eosinophil (E), basophil (B), red blood cells (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin (MCH), corpuscular hemoglobin concentration (MCHC), platelet count (PLT) and mean platelet volume (MPV). Regarding biochemical examination, a semi-automatic chemical chemistry analyzer (OLOT, Spinreact) was used to analyze concentrations or activities of glucose, blood urea nitrogen (BUN), creatinine, triglycerides, total cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP), total bilirubin (T-Bil), gamma-glutamyl transferase (gamma-GT), amylase, serum calcium, uric acid, albumin and total serum proteins (TP). Serum samples were also tested for the presence of maedi-visna antibodies by agar gel immunodiffusion test (AGID).

At a later stage, the flock feeding behaviour was investigated and more information about clinical history and management of the flock was obtained. The owner reported that because of restricted economic conditions of the farm, in winter the diet of the animals was extremely poor and based almost exclusively on hay; moreover, the farmer reported that for about several weeks the animals used to eat parts of bundles of dry broom subsequently identified as *Spartium junceum* L., stored within the same paddock of the farm. Specimens of the suspect plant including stem, leaves and flowers were submitted to the laboratory at the University of Salerno, Department of Pharmacy, for evidence of toxic compounds; in the meantime, the suspected food was removed from the farm and animals were fed only with hay and generous quantity of water.

After collection, *S. junceum* materials (stem and bark) were analyzed. The samples, after a drying period in

Table I. *Clinical history of the flock after ingestion of* Spartium junceum *L.*

N° of animals	Species	Age	Sex	Neurological signs	Duration of the illness	Outcome
17	Sheep	> 2 years	Female	++	1 month	Complete remission of clinical signs after removal of bundles of brooms
1	Sheep	> 2 years	Male	++	1 month	**Death**
1	Sheep	3 weeks	Male	+++	1 month	**Death**
1	Sheep	12 weeks	Male	+++	1 month	Complete remission of clinical signs after removal of bundles of brooms

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the shade, were ground into a powder, kept at 10 °C and subjected to extraction within 12 h. According to literature (Barboni *et al.* 1994), 2 g dried, finely pulverized samples were added with 25 ml 1N H_2SO_4, stirred or shaked 15 min at room temperature and then filtered. This procedure was repeated twice. The combined solutions were made alkaline with NH_4OH 20% aqueous solution and extracted three times with 30 ml $CHCl_3$.

The $CHCl_3$ extracts was evaporated to dryness under vacuum at room temperature using rotary evaporator.

Alkaloid extracts were analyzed by gas chromatography-mass spectrometry (GC-MS) using a fused-silica capillary column (Me silicone, 20 cm x 0.2 mm, He carrier gas, column temperature 100 °C, 2 min isothermal, 10 °C min-1 to 300 °C, 5 min isothermal, split ratio 1:30; injector 250 °C) and a mass selective detector (detector temp. 280 °C, electron energy 70 eV). Alkaloids were identified by comparison with MS data in the literature (Ohmiya *et al.* 1974, Wink *et al.* 1983, Wink 1987, Greinwald *et al.* 1990).

All animals showed a light parasitic infestation from gastrointestinal strongyles (maximum level, 100 epg) and coccidian (maximum level, 50 opg) (Reeg *et al.* 2005, Chandrawathani *et al.* 2009). Haematological and biochemical parameters showed only an increase of AST (IU/l: 109.75 ± 11.01; normal range: 32-97 IU/l) and GGT (IU/l: 53.76 ± 3.75; normal range: 0-32 IU/l) levels, suggesting a mild hepatic damage (Aitken 2007). All sera tested by AGID test were negative for maedi-visna virus infection. Anatomo-pathological investigations performed on the ram and lamb showed abdominal distension, pale mucous membranes and poor nutritional status; there was diffuse moderate to severe bilateral congestion in pulmonary lobes with white foam fulfilling the lumen of the distal trachea, major bronchi, and intrapulmonary airways. Liver was slightly enlarged and congested; petechial subdural hemorrhages were also found. No macroscopically reliable lesions were detected in other organs, including kidneys. Microscopically, multifocal to coalescing areas of abundant eosinophilic cellular debris along with large numbers of macrophages and neutrophils, fibrin and edema (Figure 1A) and occasional hemorrhages were observed in lungs. Many alveolar septa were expanded from 2 to 4 times the normal width by similar inflammatory components. A similar exudate filled bronchial and bronchiolar lumina. The interlobular, perivascular, and subpleural interstitia were expanded up to 2 to 3 times normal by abundant fibrin deposition, occasional edema, and few neutrophils, macrophages, lymphocytes, plasma cells, and erythrocytes. There were multifocal areas of closely apposed alveoli (atelectasis) or expanded and coalescing alveolar spaces (emphysema). In the kidneys, the interstitium was mildly expanded by edema and congested blood vessels. In the brain, within the gray matter of cortex, there were multifocal areas of hemorrhages with many erytrophagocytic macrophages, severe congestion of blood vessels in cerebrum, cerebellum and mild gliosis (Figure 1B). In the liver, moderate congestion of the liver parenchyma and centrilobular (zone III) sinusoidal dilatation were evident. No other microscopically lesions were detected in other organs.

Phytochemical study identified five different alkaloids in dried aerial part of *S. junceum* L. at the following concentrations expressed as µg/g dry

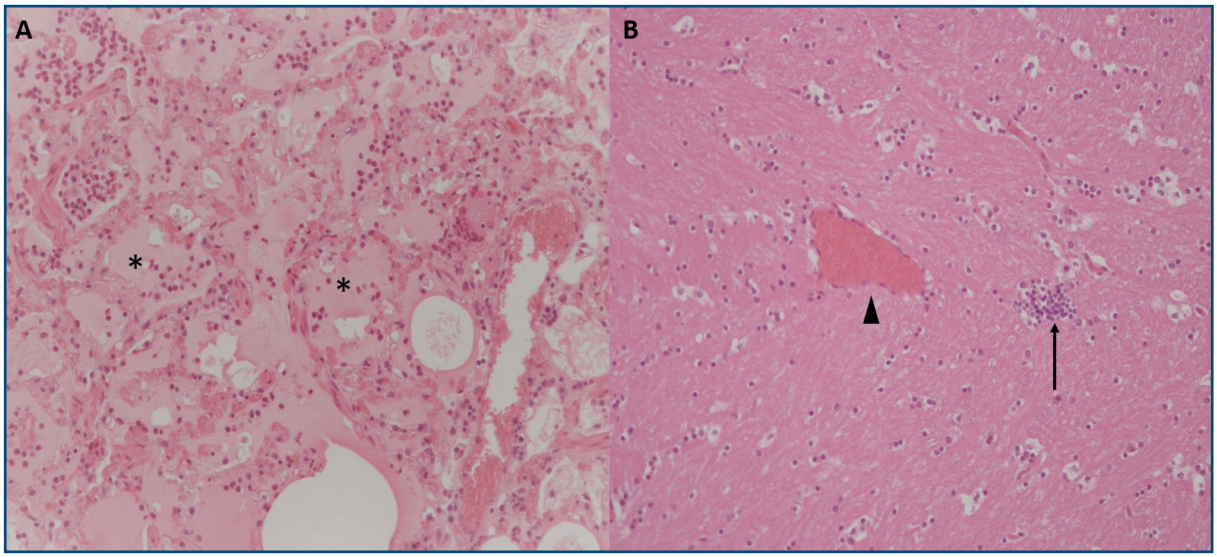

Figure 1. A. *Lamb. Lung. Eosinophilic amorphous material (*) fulfilling alveolar and bronchiolar spaces (severe diffuse pulmonary edema).* H&E stain; 200x magnification. **B.** *Lamb. Brain. Moderate congestion of a blood vessel (arrow head) and mild gliosis (arrow).* H&E stain; 400x magnification.

weight: cytisine (2,800.7 µg/g), N-methylcytisine (1,583.6 µg/g), lupanine (197.2 µg/g), anagyrine (380.7 µg/g), rhombifoline (73.5 µg/g).

Severe generalized weakness and clinical signs presented in animals, were consistent with well known, described nicotinic acetylcholine agonist toxicity, as exerted by some alkaloids. In small ruminants, the same clinical signs may be associated with some infectious diseases (i.e. maedi visna) or poisoning (Russo *et al.* 2018). Anyway, laboratory examinations did not point out any viral, bacterial or prionic disease. The animal's exposure to chemicals used in agriculture (i.e. pesticides) and other toxic substances was excluded on the basis of the environmental anamnesis.

Furthermore, the anatomopathological lesions described and the remission of clinical signs as a result of exclusion of *S. junceum* L. from diet, as well as the phytochemical analysis results, strongly corroborate the diagnosis of alkaloid poisoning. There is very little documentation of *S. junceum* L. intoxication in human and animals and no cases are reported in literature in small ruminants.

Ruminants have dietary preferences and usually exclude potentially poisonous plants. However, in some circumstances such as drought, droving or lack of forage, livestock are forced to eat, in a greater or lesser amount, toxic plants that they would otherwise avoid (Simmonds *et al.* 2000).

The toxicity seen in *S. junceum* L. is due to cytisine, an alkaloid with a bitter and unpleasant taste and a toxic component found within the genera of the *Fabaceae* and other plant families (Wink *et al.* 1983). Cytisine is a nicotinic acetylcholine receptor agonist and in humans has been documented to cause drowsiness, vomiting, vertigo, tachypnea, cold sweats, gastric pain, pupillary dilatation, agitation, and muscle contractions (Riccardi *et al.* 2006).

Literature data reported that the alkaloid pattern of *S. junceum* L. is strongly dependent on the vegetative cycle of plant; cytisine and N-methycitisine are the main alkaloids present in the aerial part of the plant whereas lupanin, anagyrine and rhombifoline are present in much lower concentrations (Greinwald *et al.* 1990, Barboni *et al.* 1994). Phytochemical study performed on dried aerial part of *S. junceum* L. revealed the presence of cytisine in relevant higher quantity compared to other alkaloids isolated in the same plant. Cytisine, lupanine, and rhombifoline concentrations proved to be higher than those found in the study of Barboni and colleagues (Barboni *et al.* 1994) but lower than those found by Greinwald and colleagues (Greinwald *et al.* 1990). The concentrations of N-methylcitisine and anagyrine found in this study were higher and lower, respectively, than those reported in other similar studies (Greinwald *et al.* 1990, Barboni *et al.* 1994).

The reported case demonstrates a strong causal association between clinical signs and ingestion of *S. junceum* L. In areas where *S. junceum* L. is abundant, the effect of cytisine poisoning in sheep should be considered to either prevent possible outbreaks or facilitate an early diagnosis.

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