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Microhistological analysis of sheep gastro-intestinal content to confirm poisonous plant ingestion

CRISTINA YAGUEDDU, M. SILVIA CID AND TOMAS LOPEZ

Yagueddú is graduate student, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (UNMdP), cc 1245 (7600), Mar del Plata, Argentina; Cid is professor, Facultad Ciencias Agrarias, UNMdP and research scientist, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); López is research scientist, Instituto Nacional de Tecnología Agropecuaria (INTA).

Abstract

The epidermal remains of 4 poisonous plant species that produce acute intoxication in ruminants were quantified by microhistological analysis in the gastro-intestinal content of sheep experimentally poisoned. These species were 'romerillo' or 'mío mío' (*Baccharis coridifolia* DC); 'duraznillo negro' (*Cestrum parqui* L'Hérit.); 'poison hemlock' (*Conium maculatum* L.), and 'sunchillo' (*Wedelia glauca* (Ort.) Hoff.). All of these species produce important economic losses of livestock in the Flooding Pampa, Buenos Aires, Argentina. The plants used for intoxication were at the vegetative stage of growth. Results indicate that the microhistological technique can be used to confirm the diagnosis of ruminant intoxication by duraznillo negro, romerillo, and sunchillo, but not by poison hemlock because digestion degrades its fragments beyond recognition. It would be convenient to sample the final sections of the digestive tract to confirm romerillo and sunchillo ingestion, because their fragments tend to concentrate there. The uniformity of duraznillo negro fragment distribution would allow identification of this species from any section of the digestive tract. However, the considerable variability in fragment distribution found among animals poisoned with the same plant species makes it necessary to sample more than 1 digestive region if only 1 animal is available for necropsy.

Key Words: toxic plants, *Baccharis coridifolia, Cestrum parqui, Conium maculatum, Wedelia glauca*

Resumen

Los restos epidérmicos de cuatro especies tóxicas que producen intoxicación aguda en rumiantes se cuantificaron por análisis microhistológico en el contenido gastrointestinal de ovinos intoxicados experimentalmente. Estas especies fueron 'romerillo'o 'mío mío" (*Baccharis coridifolia* DC); 'duraznillo negro" (*Cestrum parqui* L'Hérit.); 'cicuta' (*Conium maculatum* L.), y 'sunchillo'(*Wedelia glauca* (Ort.)Hoff.); todas ellas producen importantes pérdidas económicas de ganado en la Pampa Deprimida, Buenos Aires, Argentina. Las plantas usadas para las intoxicaciones se encontraban en estado vegetativo. Los resultados indicaron que la técnica microhistológica puede ser usada para confirmar el diagnóstico de ingestión de duraznillo negro, romerillo y sunchillo por rumiantes, pero no la de cicuta ya que la digestión degrada sus fragmentos hasta imposibilitar su reconocimiento. Se encontró suficiente muestrear las regiones finales del tracto digestivo para confirmar la ingestión tanto de romerillo como la de sunchillo, ya que los fragmentos de estas especies tienden a concentrarse allí. La uniformidad en la distribución de los fragmentos de duraznillo negro permitiría la identificación de esta especie en muestras de cualquier región del tracto digestivo. Sin embargo, la considerable variabilidad en la distribución de fragmentos encontrada entre los animales intoxicados con 1 misma especie hace conveniente muestrear más de una región si se dispone de sólo un animal para realizar necropsia.

Poisonous plants are a major cause of economic losses to livestock production around the world (Nielsen 1978, James et al. 1980, Everist 1981). For diagnostic purposes, it would be desirable to confirm the ingestion of poisonous plants by ruminants through the detection of their epidermal remains in the gastro-intestinal contents (Panter et al. 1987). The microhistological analysis quantifies the botanical composition of herbivore diets by the identification of the epidermal characters of the ingested species (Sparks and Malechek 1968, Holechek et al. 1982).

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'Romerillo' or 'mío mío' (*Baccharis coridifolia* DC), 'duraznillo negro' (*Cestrum parqui* L'Hérit.), 'poison hemlock' (*Conium maculatum* L.) and 'sunchillo' (*Wedelia glauca* (Ort.)Hoff.) are species poisonous to livestock (Ragonese and Milano 1984) and of great economic impact (T.López; unpublished data) in the Salado River Basin, Buenos Aires province, Argentina, where 20% of the country's livestock graze (Rearte 1996). Their lethal doses in sheep range from 1 to 10 gDM kg^{-1}LW. These species are primarily eaten during early vegetative stages, but some cases have been observed in which animals fed from them at later growth stages, (when other forages was scarce, or when the poisonous species were the only green plant material available). The purpose of this study was to assess whether microhistological analysis of the gastro-intestinal content of ruminants can confirm their ingestion.

Specific objectives of this study were to determine: (1) the dis-

(2) the number of points to sample in the rumen + reticulum; and (3) the effect of digestion upon the recognition of the epidermal fragments of the poisonous species.

Materials and Methods

Plants of the 4 above mentioned poisonous species were collected from different farms where cases of intoxication had previously been recorded. The collected material consisted of young shoots and fully developed leaves obtained from plants at the vegetative stage of growth. Dry matter percentage was estimated for each species in order to determine the amount of fresh plant material to be administered to the experimental animals. Three Corriedale ewes (average weight = 41.6 ± 4.7 kg) were randomly assigned to each poisonous species (Table 1). Sheep were provided by the Balcarce Experiment Station, Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina.

Sheep were housed in separate, roofed pens and fed with a half ration of oat-ryegrass hay during 72 hours prior to intoxication. Forage availability was restricted to simulate the conditions in which animals frequently ingest poisonous plants (Merril and Schuster 1978, James et al. 1980, Everist 1981). Water was offered ad libitum. The following doses were administered: duraznillo negro 10 g $kg.^{-1}$ LW (López et al. 1978); sunchillo 1.5 g $kg.^{-1}$ LW (Platonow and López 1978); and poison hemlock and romerillo 1.0 g $kg.^{-1}$ LW (T. López and G. Pinilla, unpublished data).

We used 2 procedures to administer the poisonous species to the sheep. Poison hemlock (as fresh plant material) was administered as a small bolus, which were introduced by hand in the sheep's mouths, and the sheep were allowed to chew it. The other 3 species were partially dried (60°C for 30 min), blended in a commercial blender (0.5 cm diameter), mixed in a warm water slurry and gavaged by stomach tube. Plant material mixed with the smallest amount of water required to provide the thickest slurry that flowed through the cannula.

Due to the large volume of plant material required for the duraznillo negro (10 g kg^{-1} lethal dose), a split dose was used. Sheep 1 was given the full lethal dose, but the full lethal dose would have exceeded the ruminal capacities of the other 2 sheep. Therefore, doses of duraznillo negro were 1/3 of the lethal doses for sheep 2 and 3. Twenty four hours later, sheep 1 dosed with duraznillo negro died, and sheep 2 and 3 showed the signs of poisoning usually produced by non-lethal doses of this species (lack of appetite, ataxia, lack of reaction to external stimuli, and recumbency). Both animals were sacrificed by electric shock. The animals dosed with the other species died within 24 hours after dosing.

The necropsies of the animals were performed, and digesta were taken from different sections of the digestive tract: rumen+reticulum, omasum + abomasum, duodenum, jejunum, ileum, cecum, colon, and rectum. Fecal samples were not collected because retention time of forage in sheep is greater than 24 hours (Faichney 1993). Except for the rumen + reticulum sections, the entire content of each of the other sections was collected as a single sample from each animal. The rumen + reticulum contents were sampled as follows. Excess water was removed by pressing the digesta over a 35 mesh sieve screen, the contents were weighed (Table 1), and 3 composite samples were obtained from each animal by sampling at random 15, 30, and 50 points. Previous analysis of rumen + reticulum contents of animals fatally poisoned under natural conditions had shown that samples of these sections taken at 10 or less points (20 g each) produced variable quantifications of the poisonous species fragments (C. Yagueddú, unpublished results).

All the samples were individually washed with tap water over a 200 mesh sieve screen to remove soluble substances which can coagulate and render a compact mass unsuitable for further processing. After drying for 24 hours in a forced air oven at 60° C, the samples were ground over a 1 mm (16 mesh) sieve screen. A representative amount of each sample was soaked in full strength household bleach to clear the material (30 to 60 sec). Each subsample was then washed over a 200 mesh sieve screen to remove the bleach and very small fragments (Sparks and Malechek 1968). A small portion of each sample was spread evenly and mounted on 5 microscope slides using gelatine: glycerine (1:7).

Table 1. Experimental feeding of poisonous species that produce acute intoxication in sheep.

Sheep No.	Live Weight	Rumen+reticulum Net content	Digestive tract Net content	Poisonous species fed		Expected poisonous fragment percentages assuming uniform distribution.
				Fresh	Dried	
	(kg)	(g)				(%)
Baccharis coridifolia (romerillo)						
Sheep 1	37.0	857	1224	91	37	3.0
Sheep 2	38.9	588	840	93	38	4.5
Sheep 3	42.0	777	1110	103	42	3.8
Cestrum parqui (duraznillo negro)						
Sheep 1	38.0	3650	5214	2077	380	7.3
Sheep 2 (*)	40.0	2750	3928	751	138	3.5
Sheep 3 (*)	50.0	4000	5714	909	166	2.9
Conium maculatum (poison hemlock)						
Sheep 1	39	3144	4491	153.5	39	0.9
Sheep 2	43	2940	4200	169.3	43	1.0
Sheep 3	49	3904	5577	192.9	49	0.9
Wedelia glauca (sunchillo)						
Sheep 1	33.5	2955	4221	288.8	50	1.2
Sheep 2	35.0	3791	5416	301.7	53	1.0
Sheep 3	33.0	3417	4881	284.5	50	1.0

(*)1/3 lethal dose administered.

Table 2 .Dry matter percentages (x±SD) of 4 poisonous species estimated by microhistological analysis, and the expected percentages assuming a uniform fragment distribution of fragments throughout the entire digestive tract.

R + R(15)	R + R(30)	R + R(50)	O + A	D	J	I	Ce	Co	Re	
				(Estimated%)						(Expected%)
Baccharis coridifolia (romerillo)										
2.6±0.7	1.3±1.0	3.6±2.9	3.5±4.1	2.9±2.0	5.8±4.4	16.2±9.9	6.9±5.3	15.1±15.9	0.0(1)	3.8±0.8
Cestrum parqui (duraznillo negro)										
Sheep1(lethal dose administered)										
8.6(2)	5.2	6.4	3.1	13.3	11.3	13.0	4.3	11.3	7.9	7.3
Sheep 2 and 3 (0.33 lethal dose administered)										
1.0±0.6	0.9±1.1	0.7±0.3	0.9±0.6	NC	1.4±1.7	0.8±0.7	1.7±1.8	3.2±4.2	0.3(2)	3.2±0.4
Wedelia glauca (sunchillo)										
1.7±1.9	0.6±0.4	0.8±1.2	5.8±6.9	0.9±1.0	7.0±8.4	2.2±2.2	8.7±7.5	9.9±9.8	8.3±5.7	1.1±0.1
Conium maculatum (poison hemlock)										
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9±0.1

R + R = rumen + reticulum (in parenthes is the number of points sampled for the composite), O + A = omasum + abomasum,
D = duodenum, J = jejunum, I = ileum, Ce = cecum, Co = colon, Re = rectum.
NC = No content.
When SD is not presented, it means that (1) for this section there was content only in 1 sheep, (2) the sample size was 1 sheep, (3) the toxic species percentage was the same in 3 sheep.

poisonous species and hay fragments were recorded. One of the basic assumptions of the microhistological technique outlined by Sparks and Malechek (1968) is that a 1 to 1 relationship exists between relative density and percent dry weight of identifiable fragments ground to a uniform size through a 1 mm screen. Accordingly, we recorded hay and poisonous species fragment density as an estimation of their relative dry weight (estimated percentages, EST %).

Samples of hay and various proportions of each poisonous plant were mixed by hand, processed and analyzed to evaluate the operator accuracy, as suggested by Holecheck and Gross (1982).

We calculated the expected percentage (EXP %) of poisonous species, based on the amount of poisonous plant administered and the estimated weight of the total gastrointestinal content, assuming a uniform distribution of the fragments throughout the digestive tract (Table 1). We registered the weight of R + R contents after removing water, but we did not register the net weight of the remaining sections of the digestive tract. The net contents of the entire digestive tracts were estimated considering that the rumino-reticular content is 70% of the total (Jarrige 1981).

For each poisonous species and sheep, we calculated the differences between the EST% for each digestive region and EXP%. This difference gives an indication of the species concentration in each section. If EST%–EXP% is greater than 0, the fragments of the poisonous species tend to concentrate in that section. Differences between EST% and EXP% were examined by the X^2 test for each animal, section of the digestive tract, and sample size in the rumen + reticulum.

To determine how ruminal digestion of plant fragments affected their subsequent identification, we estimated the percentages of cell wall by the neutral detergent fiber procedure (NDF) (Goering and Van Soest 1970) of the hay and of the poisonous species before and after a 24 hour in vitro digestion with rumen microbes. We stopped digestion at 24 hours because the poisonous species cause death within this time. Three samples of species and 3 samples of the oat-ryegrass hay were dried for 24 hours at 60° C, ground in a Willey mill with a 1 mm sieve screen, and divided in 2 subsamples for analysis of NDF and percentage of recognizable fragments before and after digestion. In vitro digestibilities were determined by a modified Tilley and Terry (1963) procedure, with a reduced incubation time with rumen microbes from 48 to 24 hours, and omitting the incubation with pepsin. After the 24 hours microbial digestion, the subsamples residues were recovered by filtration, dried, and weighed to calculate DM losses.

Variability among poisonous species and hay in dry matter digestibility, and percentages of the recognized fragments and cell wall before and after digestion, was estimated by ANOVA with a completely ramdomized design. Differences among means were determined by Tukey test. For each poisonous species and for the hay, the percentages of recognizable fragments and of cell wall before and after digestion were compared by ANOVA.

Results

Fragments of duraznillo negro, sunchillo, and romerillo were identified in several regions of the digestive tract of all the intoxicated sheep (Fig. 1, Table 2). However, poison hemlock fragments were not found in any section of the digestive tract of any of the 3 poisoned sheep.

Fragment distribution throughout the digestive tract and its variability among animals differed according to plant species. Romerillo fragments tended to concentrate in the gut regions. Duraznillo negro fragments had a rather uniform distribution in 2 sheep (sheep 1 and 2), and tended to have higher concentration in the last sections of the gut in the third one (Fig. 1). The distribution of sunchillo fragments had the highest variability among animals, showing peaks in omasum + abomasum and jejunum (sheep 1), cecum and colon (sheep 2), and colon (sheep 3) (Fig. 1). The increase in the number of points sampled in the rumen+reticulum compartment (from 15 to 50) did not produce a clear improvement in the fragment estimation for any of the 3 detectable species. Sampling from 15 points in the rumen + reticulum provides a representative sample.

The percentage of fragments recognized before in vitro digestion ranged from 20 to 50% for hay and duraznillo negro, respectively. Poison hemlock had the highest digestibility and was the species with the highest percentage of cell wall loss by digestion (Table 3). In this species, digestion impeded fragment recognition almost entirely, because the majority of the fragments remaining

after digestion were secondary cell walls of xylem cell vessels and cuticles. Digestion of the cell wall strongly reduced fragment recognition in duraznillo negro, but did not affect the recognition of the fragments of romerillo, sunchillo, and hay. In these 3 species cell wall loss by digestion was lower than 25% (Table 3).

Discussion

In cases of acute intoxications, affected animals are usually either found dead, or too late to initiate supportive treatment (López et al. 1991). Pathological, histological, and biochemical determinations do not always successfully diagnose plants responsible for deaths, since many poisonous species do not produce unequivocal pathological or histological signs. In other species the toxic compound is unknown. In addition, the chemical structure of the toxic compounds and tissue lesions can be altered if the poisoned animal has been dead for too long before the post mortem examination.

The microanalysis of feces has been used to quantify the poisonous plant ingestion by cattle and jackrabbits (Alipayou et al. 1993). However, this technique has seldom been used to confirm poisonous plant ingestion from digestive tracts of dead domestic herbivores. Panter et al. (1987) estimated percentages of 'death camas' (*Zygadenus paniculatus*) ranging from 8 to 17% in the rumen content of sheep intoxicated in the field, and other sheep

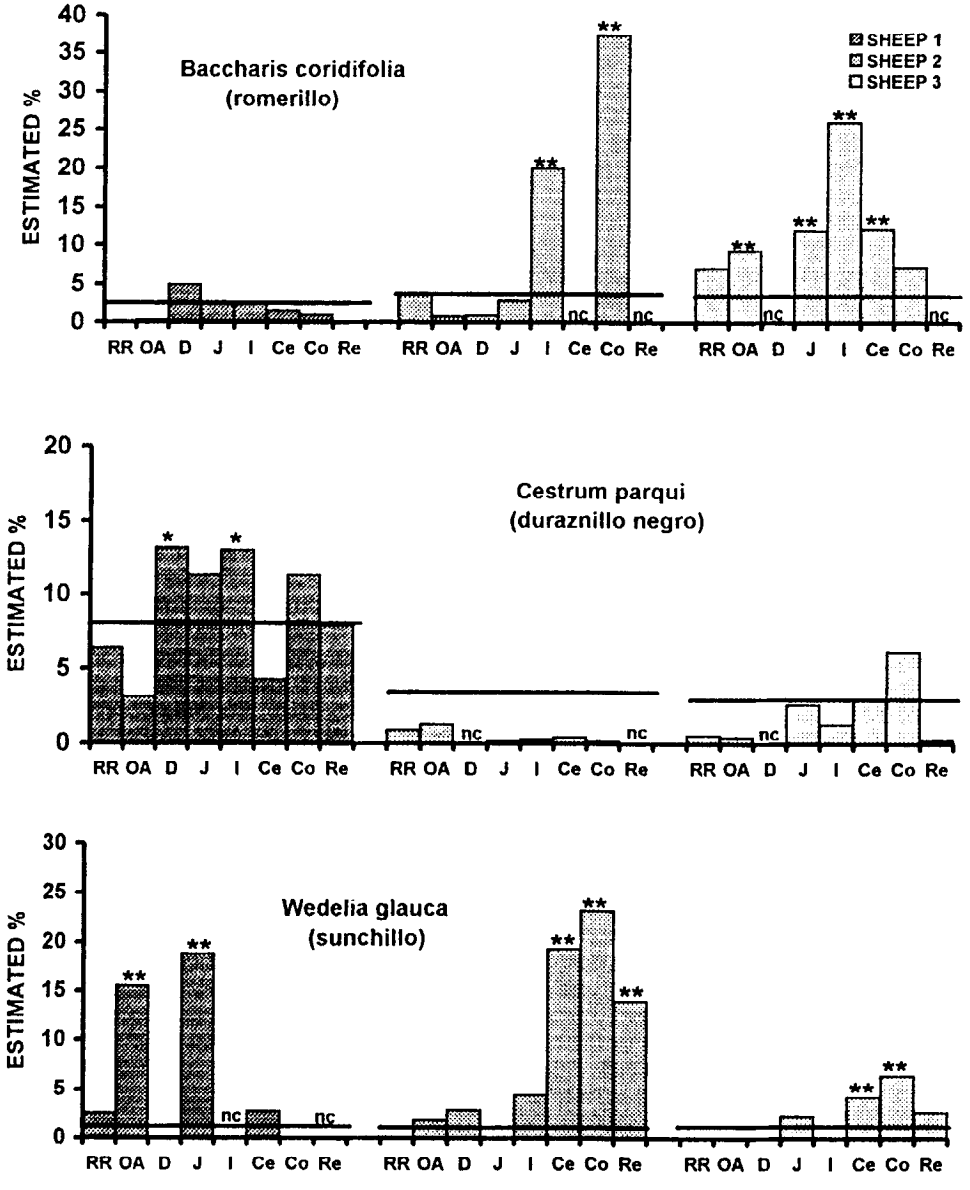

Fig. 1. Estimated percentages of species that produce acute intoxication in different sections of the digestive tract of experimentally poisoned sheep. RR = rumen + reticulum, OA = omasum + abomasum, D = duodenum, J = jejunum, I = ileum, Ce = cecum, Co = colon, Re = rectum, nc = no content. Percentages in R + R were obtained from 50 point samples. Horizontal lines indicate the expected percentages assuming a uniform distribution of the fragments throughout the digestive tract. Percentage of fragments estimated by microhistological analysis differs from the expected *($p < 0.05$), **($p < 0.01$).

Table 3. Dry matter digestibility and digestion effect on fragment recognition by microhistological analysis, and on cell wall percentages in 4 poisonous species that produce acute intoxication, and on oat-ryegrass hay (x ± SD) (n=3).

	Recognized Fragments		Cell Wall		Dry Matter Digestibility	Cell Wall Digested
	Before Digestion	After Digestion	Before Digestion	After Digestion		
	(%)		(% NDF)		(%)	
Baccharis coridifolia (romerillo)	28.5± 1.9b	23.2± 5.0ab	26.8±1.3b **	39.5±0.9b	45.8±0.4b	20.5±5.6b
Cestrum parqui (duraznillo negro)	49.3±11.6a **	10.1± 4.5bc	23.4±0.1c **	37.3±2.6b	52.5±0.8b	24.1±5.9b
Conium maculatum (poison hemlock)	40.3±15.9a *	1.5± 2.7c	15.5±0.7d **	30.4±1.1c	68.5±2.8a	38.2±6.0a
Wedelia glauca (sunchillo)	32.6± 9.0b	30.3±11.2a	26.9±0.1b **	39.8±0.9b	48.2±2.1b	23.3±3.2b
Oat+Ryegrass hay	19.8± 0.5c	16.1±3.3abc	66.9±1.8a *	75.5±3.1a	30.0±5.2c	21.5±4.7b

* ** Means of each species differ before and after digestion * ($p<0.05$), ** ($p<0.01$)
Means followed by different letters differ among species ($p<0.05$)

experimentally poisoned via stomach tube with doses ranging from 1.8 to 6.6 g DM kg^{-1}LW .

We did not oven-dry the net content of the rumen + reticulum sections before weighing them. The excess water was removed by pressing the content over a sieve screen. We may have underestimated the expected percentage of the poisonous species assuming a uniform distribution of their fragments throughout the digestive tract.

Degradation of epidermal fragments from digestion varies greatly among plant species. In general, grass epidermis resist digestion better than that of forbs and shrubs. Forbs and shrubs are usually highly digestible; as a result they can be underestimated by microhistological analysis (Leslie et al. 1983). Digestion reduced by almost 40% the amount of cell wall in poison hemlock. Epidermal cell walls before digestion were thin and they were almost completely degraded by digestion. After digestion only xylem secondary cell walls and cuticles remained, but these fragments do not allow species identification. Thus, the microanalysis of the digestive content can not confirm poison hemlock ingestion, at least when ingested at the vegetative stage of growth.

Digestion of cell wall in the other 3 species and in the hay was lower, ranging from 20 to 24%. Duraznillo negro fragments were recognized in different sections of the digestive tract, however the percentages of recognized fragments after digestion were low. Although the microanalysis of the digestive content would confirm duraznillo negro ingestion, the percentage of this species was probably underestimated. Cell wall from romerillo, sunchillo, and hay were more resistant to digestion than that of poison hemlock. The effect of digestion on romerillo, sunchillo, and hay fragment recognition was similar, however the percentage of sunchillo recognizable fragments before and after digestion was almost twice that of the hay, which suggests that this species may have been overestimated.

Species quantification by microanalysis is influenced by the effect of digestion on the percentage of recognizable fragments by species. Epidermis is covered by indigestible cutin that inhibits digestion, protecting cell walls of microbes attack. Cutinization increases with stage of growth (Wilson 1976) and aridity (Martin and Juniper 1970). Thus, it is possible that the percentage of recognizable fragments increases as plants develop. Variation in the resistance of epidermal fragments to digestion at different growth stages can be important in the detection of poison hemlock ingestion. The alkaloid concentration in the leaves of this species decreases in the reproductive stages of growth (de la Torre et al. 1997), which would increase the lethal dose. At those stages the epidermal resistance to digestion may be higher and in consequence, the possibility of this species fragment detection would increase. In the experimental conditions of our study the entire lethal dose of poison hemlock was administered in a short period. However, under field conditions it is possible that the animals ingest the plant material during longer periods, and they can be still consuming it when they die. This would result in fragments in the first sections of the digestive tract not altered by digestion.

Poisonous plants fragment distribution throughout the digestive tract showed different trends indicating that the regions of the digestive tract to be sampled in dead animals would depend on the poisonous species suspected to have produced the intoxication. In addition, for a given plant species there were individual variation among animals, which were more pronounced with romerillo and sunchillo. This implies that it would require sampling more than 1 poisoned animal in cases of suspected poisoning with these species. Although the fragments of the 3 species which were recognized tended to concentrate in duodenum and ileum, the actual volume or these sections is low, and might be void at the time of sampling under field conditions. If only 1 animal is necropsied and only 1 section is sampled, it would be possible that the poisonous plant fragments were not detected even though the species had been ingested. For this reason, we recommend that more than 1 region of the digestive tract be sampled if only 1 animal is available for necropsy. Quantification of sunchillo fragments in different regions of the digestive tract of a steer fatally poisoned in the field confirms the tendency of the fragments of this species to concentrate in the gut regions found in our experimental sheep for this plant species. In this case, the percentages of sunchillo estimated by microanalysis were <0.1, 1.5, 2.1 , and 2.8% in R + R, duodenum, jejunum and colon, respectively.

It is true that the R+R section is the easiest place to sample. However, although the duraznillo negro fragments were found in the R + R of the 3 sheep poisoned in our experiment, those of romerillo were found in the R + R of 2 sheep, and those of sunchil-

lo in the R + R of just 1 sheep. This indicates that if only R + R is going to be sampled, more than 1 animal should be sampled. In a field case of death by romerillo ingestion we analyzed the R + R of 2 steers, but we only found fragments of this species in 1 animal.

Our results indicate that the microhistological analysis of the gastro-intestinal content can be used to confirm the ingestion of some poisonous species that produce acute intoxication in ruminants, but not others in which digestion can make fragment identification impossible. In fact, the method has contributed to the diagnosis of 16 local plant poisoning cases since it was made available here (Balcarce Animal Health Group records; unpublished data).

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