RESUMO - Toxicidade das larvas de Perreyia flavipes.
Larvas frescas ou descongeladas de Perreyia flavipes foram moídas e misturadas com água e administradas de forma oral a ovinos. Com 5mg/kg, não foram observados achados clínicos ou enzimáticos. Doses únicas de 7,5 e 10mg/kg induziram a sinais clínicos característicos de intoxicação pelas larvas de Perreyia sp., os níveis de GGT e AST estavam aumentados e as curvas glicêmicas estavam diminuídas. Contudo, doses de 5, 10 e 15mg/kg repetidas em intervalos de 30 ou 15 dias não causaram doença ou causaram doença discreta seguida de morte, respectivamente. Estes achados indicam que estes animais provavelmente desenvolveram algum grau de tolerância para as toxinas presentes nas larvas de Perreyia flavipes. O exame ultraestrutural do fígado revelou proliferação do retículo endoplasmático liso de hepatócitos, o que pode ser associado a um aumento na capacidade de metabolizar toxinas e consequentemente levar à tolerância observada no presente estudo. Outras investigações poderão esclarecer se os efeitos de tal tolerância poderiam ser aplicados como medida de controle da intoxicação por P. flavipes ou outras doenças hepatotóxicas. Além disso, os resultados clínico-patológicos foram discutidos.

INDEX TERMS: Perreyia flavipes larvas, intoxication, tolerance, sheep.

INTRODUCTION
The ingestion of larval insects from the suborder Symphyta (sawfly) (order Hymenoptera) has caused worldwide outbreaks of acute hepatic necrosis in livestock (Dadswell et al. 1985, Thamsborg et al. 1987, Smith 1990, Dutra et al. 1997). Larvae have been linked to spontaneous poisoning and deaths in different species and countries including the following: larvae of Lophyrotoma interrupta (Pergidae) poisoning cattle and sheep in Australia (Da-
dswell et al. 1985, McKenzie et al. 1985), larvae of *Arge pullata* (Argidae) poisoning sheep and goats in Denmark (Thamsborg et al. 1987), larvae of *Perreyia lepida* (Pergidae) suspected of poisoning pigs in Brazil (Camargo, 1955), and larvae of *Perreyia flavipes* (Pergidae) poisoning cattle and sheep in Uruguay (Dutra 1997, Dutra et al. 1997) as well as cattle (Raymundo et al. 2009, Soares et al. 2008), sheep (Raymundo et al. 2008) and pigs (Jonck et al. 2010) in Brazil. Experimental intoxication by *Perreyia flavipes* larvae has been conducted in pigs (Soares et al. 2001), cattle and sheep (Dutra 1997). Sawfly larval poisoning has been attributed to D-amino acid containing peptides (Kannan et al. 1988, Oelrichs et al. 1999). The palatability of the insect in association with nutritional deficiencies has been indicated as the main risk factor involved in the intoxications (McKenzie et al. 1985, Dutra 1997). This communication reports toxicity aspects of experimentally induced *P. flavipes* larvae poisoning in sheep and discusses clinicopathological findings.

**MATERIALS AND METHODS**

Compact masses of sawfly larvae subsequently identified as *Perreyia flavipes* (Smith 1990) were collected in farms in Rio Grande do Sul and Santa Catarina, where outbreaks of *P. flavipes* poisoning in cattle and sheep had occurred. Seven 2-year-old mixed breed sheep were forced fed with fresh or thawed larvae (ground and mixed with water) through a syringe (with the top cut) as shown in Table 1. Hepatic biopsies were collected from sheep 5 and 6 before the first and after each dose and then submitted for ultrastructural study. Animals were clinically examined twice daily. Blood samples were collected before and after (twice daily) dosing sheep with larvae to determine glycemic curves and serum activities of gamma glutamyl transferase (GGT) and aspartate aminotransferase (AST). Necropsies were performed immediately after death and a number of tissue samples were collected, fixed in buffered 10% formalin, processed for histology, and stained by hematoxylin and eosin (Allen 1992).

**RESULTS**

A summary of the clinical evolution is presented in Table 1. Clinical signs included anorexia, apathy, weakness, reluctance to move, difficulties in standing, palpebral ptosis, muscular tremors (especially head and neck), dried feces, increased salivation, depression, sternal or lateral recumbence, nystagmus, opisthotonos, paddling, vocalization, coma and death. Figure 1 includes the GGT and AST analysis as well as the glycemic curves. Main macroscopic lesions were ascites, hepatomegaly, yellowish liver, enhanced lobular pattern, diffuse hepatic subcapsular congestion and hemorrhage, gallbladder distended and walls edematous. There were also varying degrees of hemorrhage and edema scattered in several organs. Prominent microscopic lesions

**Table 1. Experimental intoxication of sheep with *Perreyia flavipes* larvae. Data concerning animals, doses, larvae, and clinical evolution**

<table>
<thead>
<tr>
<th>Sheep</th>
<th>Dose 1 (g/kg)</th>
<th>Time (h) to clinical signs</th>
<th>Dose 2 (g/kg)</th>
<th>Time (h) to clinical signs</th>
<th>Dose 3 (g/kg)</th>
<th>Time (h) to clinical signs</th>
<th>Time (h) to death</th>
<th>Larvae</th>
<th>Total dose (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>10</td>
<td>47</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Fresh</td>
<td>395</td>
</tr>
<tr>
<td>2*</td>
<td>7.5</td>
<td>45</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Fresh</td>
<td>244</td>
</tr>
<tr>
<td>3*</td>
<td>5</td>
<td>No signs</td>
<td>-</td>
<td>-</td>
<td>No signs</td>
<td>Not died</td>
<td>Thawed</td>
<td>Control</td>
<td>1221</td>
</tr>
<tr>
<td>4*</td>
<td>10</td>
<td>42</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>49</td>
<td>Thawed</td>
<td>Fresh</td>
<td>285</td>
</tr>
<tr>
<td>5†</td>
<td>5</td>
<td>No signs</td>
<td>10</td>
<td>No signs</td>
<td>15</td>
<td>No signs</td>
<td>Thawed</td>
<td>Not died</td>
<td>1158</td>
</tr>
<tr>
<td>6†</td>
<td>5</td>
<td>No signs</td>
<td>10</td>
<td>48, mild signs</td>
<td>15</td>
<td>56</td>
<td>Thawed</td>
<td>Not died</td>
<td>1221</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Not died</td>
<td>Control</td>
<td>Control</td>
</tr>
</tbody>
</table>

*Sheep receiving single doses. † Sheep dosed at 30 days intervals. ‡ Sheep dosed at 15 days intervals.
included centrilobular coagulative or massive necrosis associated with centrilobular congestion and hemorrhage. Necrotic hepatocytes had vacuolated cytoplasms and nuclei, which were also pyknotic with condensed chromatin, karyorrhexis or karyolysis. Vacuolated renal tubular cells and lymphoid depletion associated with diffuse necrosis of the germinal centers were also observed in lymphoid tissues. Ultrastructural evaluations of biopsy sections showed moderate (7 days after the 5g/kg dose) and severe (7 days after the 10g/kg dose) proliferation of the smooth endoplasmic reticulum (Fig. 2). Sinusoidal dilatation and a few necrotic hepatocytes could be observed 7 days after the 10g/kg dose.

**DISCUSSION**

Clinicopathological findings were comparable to those observed in spontaneous *P. flavipes* poisoning in sheep (Raymundo et al. 2008, Dutra et al. 1997). The smallest toxic dose (STD) of *Perreyia flavipes* larvae to sheep was 7.5 g/kg. Similar results were reported for toxicity of *Lophyrotoma interrupta* and *Arge pullata* larvae to ruminants (Mckenzie 1985, Thamsborg et al. 1987). As previously suggested (Raymundo et al., 2008), sheep were more susceptible to *P. flavipes* larvae toxicity than were cattle, in which the STD was reported to be 40g/kg (Dutra et al., 1997).

Neuromuscular disorders are common to many diseases and may be linked to hypoglycemia in association with hepatic detoxicant agents (Radostitis et al. 2002). Hepatic necrosis may cause hypoglycemia because the remaining hepatic tissue may be insufficient to maintain adequate glycemic control (Colodel et al. 2000). Biochemical analysis of the blood was consistent with hepatic lesions, i.e., GGT and AST values were considerably elevated in the affected animals (Cornelius 1989). Similar AST serum profiles have been described for *P. flavipes* larvae toxicity in pigs (Soares et al. 2001). Electron microscopy revealed hepatic and vascular lesions. Although little is known regarding the mechanism of action of the toxins lophyrotomin and pergidin (Oelrichs et al. 1999) present in *P. flavipes* larvae, the typical cavitary edema observed in this cases suggests increased vascular permeability. Fibrin extravasation is an additional feature of those cavitary fluids because clots formed when the fluids were exposed to air. Similar findings have been described in intoxications by *Xanthium cavanillesii* (Loretti et al. 1999) and *Microcystis aeruginosa* (Jackson et al. 1985). Hemorrhages are expected consequences of acute hepatic intoxication because the liver maintains important hemostatic mechanisms (Badyak 1988). Sinusoidal dilatation has been observed in *Xanthium cavanillesii* (Loretti et al. 1999) and *Microcystis aeruginosa* (Jackson et al. 1985) poisonings and indicates vascular lesions. Proliferation of the smooth endoplasmic reticulum has been associated with an increased production of detoxifying enzymes (Cheville 1994) and could explain an increased ability to metabolize toxins, consequently leading to the development of apparent tolerance observed here. Sheep 6 developed mild disease after receiving 10g/kg but had only a few compromised hepatocytes, and the sheep was able to recuperate. The clinical evolution observed in sheep 5 and 6 suggested that these animals may have developed some degree of tolerance to the toxins in *P. flavipes* larvae. Although it is uncertain whether the mechanism is through hepatic detoxification or ruminal flora adaptation, sheep have been reported as being able to acquire tolerance to pyrrolizidine alkaloids (Cheeke 1998, Kellerman et al. 2005), especially after initial and light ingestions of plants containing these substances. This characteristic has been studied with the goal of developing control measures to poisoning by pyrrolizidine alkaloid-containing plants (Holloway 1968, Riet-Correa et al. 2007). The aforementioned discussion may suggest similar alternative control measures for *Perreyia sp.* larvae toxicity.

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**REFERENCES**


