Experimental Fascioliasis in Llamas

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ABSTRACT: Three llamas and 2 domestic sheep were inoculated orally with metacercariae of liver flukes, Fasciola hepatica. The prepatent period in llamas and sheep was 8–12 wk. Sizes of fluke eggs passed in feces were similar between llamas and sheep. At necropsy, the percentages of original inoculum recovered from the llamas and sheep were 24% and 22%, respectively. Sizes of flukes recovered from llamas were similar between llamas and sheep. The gross appearance of the livers from the llamas varied from slight discoloration with some bile duct thickening to marked fibrosis and scarring. Llama livers were similar histologically. Bile duct hyperplasia, portal fibrosis, and granulomas, often containing degenerated trematode eggs and necrotic debris, were hallmarks of infection. These changes resembled chronic fascioliasis in sheep. The data indicate that llamas, like domestic sheep, have low resistance to liver fluke infection.

KEY WORDS: Fasciola hepatica, liver flukes, experimental infection, llama, Lama glama, sheep, Ovis aries.

Fasciola hepatica is a prevalent and economically important trematode parasite of cattle and sheep in the United States. In endemic areas goats, rabbits, swine, horses, and man may also become infected (Leathers et al., 1982; Soulsby, 1982; Malone, 1986; Wescott and Foreyt, 1986). In the United States, natural infections of F. hepatica have been reported in 1 llama in Texas and 1 llama in Oregon (Cornick, 1988; Rickard and Bishop, 1991). The purpose of this study was to determine the prepatent period of F. hepatica in llamas, describe the lesions associated with mature infections, and compare them with those in domestic sheep.

Three healthy adult female llamas (Lama glama), 5–7 years old, were donated for research purposes because of reproductive or conformational problems. All were maintained on pasture and were supplemented with hay when needed. All pastures used were known fluke-free pastures and none of the animals had any history of liver fluke infections prior to experimental infection. All llamas were clinically normal. Two of the llamas (nos. 2 and 3) were also given larvae of meningeal worm, Parelaphostrongylus tenuis, on day 18 of this experiment. Because P. tenuis in llamas is confined to the neurologic system (Baumgartner et al., 1985; Krogdahl et al., 1987), it was considered that it would not directly affect the liver fluke infection. Two healthy domestic sheep (Ovis aries), 1.5-year-old wethers, were purchased as lambs from a known F. hepatica-free area, and were housed on pasture until the start of the experiment when they were moved indoors and housed on concrete.

On day 0, 250 (llama 1) or 500 (all other animals) metacercariae of F. hepatica (Baldwin Enterprises, Monmouth, Oregon) were administered to each animal orally either by stomach tube (llamas) or gelatin capsule (sheep). Rectal fecal samples were collected and animals were weighed at approximately 2-wk intervals throughout the trial. Animals were observed daily for signs of clinical parasitism.

Five grams of feces was examined at each sampling period for eggs of F. hepatica with a sedimentation technique. For llama 1, the samples were scored as negative or positive, and for the other animals, actual numbers of fluke eggs per gram of feces were determined. A minimum of 20 eggs from each positive sample were measured using a microscope equipped with an ocular micrometer.

On day 157 postinfection, llama 1 was euthanized for reasons unrelated to parasitism. On
of *F. hepatica* eggs were fixed in 10% neutral buffered formalin because of incoordination caused by *P. tenuis* infection (Rickard and Bishop, 1991). The following day sheep 2 was euthanized for comparative purposes. On day 83 postinfection, llama 3 died from causes related to *P. tenuis* infection (Forey et al., 1991). Sheep 1 was euthanized the next day for comparative purposes. At necropsy, the liver and duodenum were removed intact. Recovery and enumeration of flukes were as previously described (Rickard and Bishop, 1991) except live, intact flukes from all animals except llama 1 were measured to the nearest mm after relaxation in water. Representative pieces of liver were fixed in 10% neutral buffered formalin and were later processed by routine histologic techniques and stained in hematoxylin and eosin.

In the first llama, the prepatent period (PPP) of *F. hepatica* was 84 days, whereas in the other 2 llamas it was only 56 days (Table 1). For the sheep, the PPP was 63 days (Table 1), 1 wk longer than that for the llamas infected at the same time. The total number of liver flukes recovered from each animal, with percentage of original inoculum in parentheses was: llama 1, 82 (32.8%); llama 2, 68 (13.6%); llama 3, 154 (30.8%); sheep 1, 129 (25.8%); and sheep 2, 87 (17.4%). The mean lengths of flukes recovered from llamas 2 and 3 (13.0 ± 3.9 mm and 16.8 ± 3.0 mm, respectively) were slightly less than those from flukes of the same age recovered from sheep 2 and 1 (15.9 ± 3.7 mm and 17.7 ± 3.6 mm) (Table 2). However, the differences in size were minimal with almost complete overlap in range. Mean sizes of *F. hepatica* eggs were 123.2 × 68.6 mm in llamas (*N* = 240) and 125.4 × 67.8 mm in sheep (*N* = 200).

Weights of all llamas fluctuated during the experiment with some weight loss, but all were in good body condition at necropsy with adequate amounts of mesenteric and subcutaneous fat present. Both sheep were in excellent condition at the time of necropsy.

The gross appearance of the livers of llamas 1 and 2 was similar. Peripheral margins were slightly discolored being lighter than normal. Multifocal, hardened white nodules 1–2 mm in diameter were present throughout the livers. The ventral surfaces were slightly irregular with the main bile duct thickened. On cut surface, the bile ducts were irregularly thickened and contained flukes throughout the liver. The appearance of the liver of llama 3 was much different. Three cysts, about 1 cm in diameter, were present in the lateralmost section of the lateral lobe of the liver. White streaks on the capsular surface were evident, and the ventral surface was markedly irregular with numerous, raised firm nodules 2–10 mm in diameter. Similar, but less severe lesions, were present on the dorsal surface of the liver. On cut surface, the nodules and streaks were seen to be fibrotic bile ducts. Many ducts contained caseous, tan-green material as did the 3 cysts. The main bile duct was markedly and irregularly thickened and sacculated. The sacules contained bile and numerous flukes. Flukes were also found in bile ducts throughout much of the liver.

All 3 llama livers were similar on histologic examination; however, changes were more severe in llama 3. Regional differences in histologic changes were apparent. Bile duct hyperplasia (Fig. 1) and portal fibrosis were present in most areas. These changes were striking near the hilus where lobulation was accentuated by biliary hyperplasia that bridged adjacent portal areas. Bile ductules often contained basophilic granular material. Scattered granulomas containing necrotic debris and degenerated fluke eggs were present primarily in portal areas (Fig. 2). Eggs were also present in dilated bile ductules. Eosinophils, lymphocytes, plasma cells, and neutrophils were present.

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Days PI*</th>
<th>N</th>
<th>x ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Llama 2</td>
<td>69</td>
<td>33</td>
<td>13.0 ± 3.9</td>
<td>4–19</td>
</tr>
<tr>
<td>Sheep 2</td>
<td>70</td>
<td>51</td>
<td>15.9 ± 3.7</td>
<td>7–25</td>
</tr>
<tr>
<td>Llama 3</td>
<td>83</td>
<td>108</td>
<td>16.8 ± 3.0</td>
<td>8–27</td>
</tr>
<tr>
<td>Sheep 1</td>
<td>84</td>
<td>93</td>
<td>17.7 ± 3.6</td>
<td>7–25</td>
</tr>
</tbody>
</table>

* Days postinfection.

### Table 1. Numbers of *Fasciola hepatica* eggs per gram of feces from llamas and sheep.

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Days postinfection</th>
<th>N</th>
<th>x ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Llama 2</td>
<td>0–49</td>
<td>56</td>
<td>0.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Llama 3</td>
<td>0–49</td>
<td>63</td>
<td>6.6</td>
<td>27.8</td>
</tr>
<tr>
<td>Sheep 2</td>
<td>0–49</td>
<td>69</td>
<td>0.4</td>
<td>10.0</td>
</tr>
<tr>
<td>Sheep 1</td>
<td>0–49</td>
<td>77</td>
<td>0</td>
<td>2.4</td>
</tr>
</tbody>
</table>

NS = no sample as animal euthanized prior to the sampling date.
Figures 1, 2. 1. Biliary hyperplasia (arrows) in liver from llama 1. Scale bar = 100 μm. 2. Egg granuloma in liver from llama 1. Scale bar = 250 μm.
present surrounding the granulomas and throughout the portal areas. A calcified nodule was present in llama 1, corresponding to the white nodules seen on gross examination, and probably represented a fluke migration tract.

The appearance of the livers of both sheep was similar and did not differ substantially from previous descriptions (Dow et al., 1968; Rushton and Murray, 1977). Flukes were found in bile ducts throughout the livers of both animals. The majority of flukes were mature with eggs in their uteri.

The PPP of F. hepatica in sheep and cattle is variable, but is usually between 8 and 12 wk (Ross et al., 1966; Rushton and Murray, 1977; de Leon et al., 1981; Soulsby, 1982). Although the PPP in 2 llamas was 1 wk shorter than the sheep infected at the same time, the 8–12 wk PPP for all llamas and the 9 wk for the sheep are within the range in sheep and cattle.

Because the llamas were euthanized for reasons unrelated to the liver fluke infection, the patent period cannot be determined from these data. However, shedding of eggs was uninterrupted once it began and, in llama 1, continued for 9 wk.

Little difference existed in the numbers or sizes of flukes recovered from llamas and sheep at necropsy. The overall percentages of flukes recovered (llamas, 24%; sheep, 22%) were also similar. Yet, the severity of the gross pathologic changes present was somewhat dissimilar. Llamas 1 and 2 (82 and 68 flukes, respectively) had less severe lesions than sheep 2 (84 flukes). However, in llama 3 (154 flukes) gross lesions approximated those of the sheep. Histologic appearance of the llama livers was similar to that described for chronic fascioliasis in sheep and cattle (Ross et al., 1966; Dow et al., 1967, 1968; Rushton and Murray, 1977), including the presence of egg granulomas. A primary difference, however, between mature infections in cattle and sheep is the mineralization of bile ducts. This occurs in cattle beginning by week 16 of infection (Ross et al., 1966), but does not occur in sheep (Boray, 1969; Rushton and Murray, 1977). In the present study, no bile duct calcification was noted in llama 1 at 22 wk postinfection, indicating this may not be a feature of fascioliasis in llamas.

Various hosts differ in susceptibility to infection with F. hepatica and the degree of resistance has been cited as the underlying factor in the production of acute or chronic fascioliasis. Boray (1969) divided the more common hosts of F. hepatica into 3 groups based on an early, delayed, or low level of resistance. The early resistance hosts (group I; domestic pigs) possess tissues that are not suitable for the parasite resulting in a high degree of natural resistance. The infection is self-limiting without harming the host. The delayed resistance hosts (group II; cattle and horses) have a resistance which is acquired during the first weeks of a primary infection or during challenge infection. A delayed host reaction controls flukes during tissue migration, and chronic reactions including bile duct calcification lead to eventual elimination of infection. Mortality is not common. Group III hosts (sheep and goats) have low resistance resulting in severe tissue reactions that do not immobilize or eliminate the parasites. In the chronic condition, there is no calcification of the bile ducts and flukes often survive the life of the host. Mortality in both the acute and chronic phases is common. Neither acute nor chronic fascioliasis has been described in llamas in North America; however, both conditions are reported to occur in alpacas in South America (Hernandez and Condorena, 1967; Guerrero and Leguia, 1987). This, together with the histologic evidence, indicates that llamas may have low resistance to F. hepatica.

The cooperation of Dr. G. L. Zimmerman, M. K. Schuette, D. M. Mulrooney, J. K. Bishop, P. J. Reed, K. Hoffmann, J. E. Lagerquist, and many others is greatly appreciated.

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Research Note

Trichinella pseudospiralis Infections in Free-living Tasmanian Birds

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ABSTRACT: Muscle tissues from 91 birds comprising 13 species were examined for the presence of Trichinella pseudospiralis larvae. Trichinella infection was detected in 2 masked owls, Tyto novaehollandiae, and 1 marsh harrier, Circus aeruginosus. These findings confirm that carnivorous or carrion-feeding birds are naturally infected with this nematode. Intestinal infection was also achieved in a 6-day-old marsh harrier after oral dosing. The source of infections and the significance of avian hosts in the epizootiology of T. pseudospiralis are discussed.

KEY WORDS: Trichinella pseudospiralis, avian infections, Australia.

Following the detection of Trichinella pseudospiralis Garkavi, 1972, in Tasmania, investigations were commenced to determine which free-living vertebrate hosts are responsible for the transmission and maintenance of this parasitic infection (Obendorf et al., 1990). Studies to date have suggested that T. pseudospiralis in Tasmania is predominantly maintained by dasyurid marsupials, in particular Tasmanian devils, Sarcophilus harrisii, eastern quolls, Dasyurus viverrinus, and spotted-tailed quolls, D. maculatus.

In the northern hemisphere, there are several records of free-living carnivorous birds, particularly carrion feeders, being infected with Trichinella sp. presumed to be T. pseudospiralis (Boev et al., 1979). In the Tien Shan mountain region of U.S.S.R., T. pseudospiralis has also been recorded in 2 crows, Corvus frugilegus (Shaikenov, 1980), out of a total of 744 birds. It is also quite likely that T. pseudospiralis was recovered from a common buzzard, Buteo buteo, in Spain (Cale-ro et al., 1978). Records of Trichinella sp. in North American birds include the great horned owl, Bubo virginianus (Zimmermann and Hubbard, 1969), the pomarine jaeger, Stercorarius pomarinus (Rausch et al., 1956), and Cooper's hawk, Accipiter cooperi (Wheeldon et al., 1983).

In this study, 13 avian species with carnivorous habits were examined for the presence of Trichinella infection in muscles. Samples of muscle were obtained from birds killed as a result of road accidents, malicious shooting, or poisoning and trapping. Some forest ravens were obtained by authorized trapping. In addition, a 6-day-old raptor was experimentally infected with...