FASCIOLIASIS IN SHEEP IN THE HUMAN HIGH ENDEMIC REGION OF THE NORTHERN BOLIVIAN ALTIPLANO

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ABSTRACT: Fasciola hepatica infection was studied by means of coprological surveys carried out in sheep from the Northern Bolivian Altiplano. This area, located at an altitude of 3800-4100 m, between Lake Titicaca and the valley of the city of La Paz, has proved to be the fascioliasis endemic region in which the highest prevalences and intensities in human subjects have been found. A total of 1600 ovines from 12 different zones belonging to the provinces of Omulouyos, Los Andes and Ingavi were surveyed in the April-October period. The prevalences found were relatively high and very homogeneous, with a total prevalence of 61.6% and a range of 49.1-87.0%. The high prevalences detected may be emphasized, above all taking into account that only coprological techniques were applied. The high fascioliasis prevalences and the relatively reduced range found in sheep from localities where the parasite is present disagree with the usually low prevalences and large heterogeneity detected in cattle from the same Altiplanic area. No significant difference was found according to host sex. Among the different breeds studied, there appears to be a higher susceptibility for fascioliasis infection in the Corriedale breed (84.0% prevalence) when compared to Merino, Criollo and Mestizo sheep. Sheep may be considered the main animal reservoir host of fascioliasis in the Northern Bolivian Altiplano, where they develop a very important role in the contamination of the external milieu and participate decisively in the transmission of the disease. However, they cannot alone explain the high transmission rates to human subjects known to occur in the Northern Bolivian Altiplano, as prevalences and egg output in Altiplanic sheep are similar to those found in areas of other regions and countries where human subjects become infected only sporadically and isolated.

KEY WORDS: Liver fluke, Fasciola hepatica, fascioliasis, sheep, coprological surveys, Bolivian Northern Altiplano.

INTRODUCTION

In recent years the Northern Bolivian Altiplano, between Lake Titicaca and the valley of the city of La Paz, has proved to be not only a human endemic region of infection caused by Fasciola hepatica (Linnæus, 1758) (Trematoda: Fasciolidae), but also the area known to present the highest human prevalences and intensities of fascioliasis: prevalences up to 70% by coprological methods and up to 100% by serological techniques (HILL-YUR et al., 1992; MAS-COMA et al., 1995; BJÖRLAND et al., 1995; ESTEBAN et al., 1997a, b; ANGÜES et al., 1997; STRAUSS et al., 1997); intensities including an egg output up to more than 5000 eggs per gram of faeces (ESTEBAN et al., 1997a, b).

Several multidisciplinary studies were undertaken to understand how the liver fluke is able to reach such high transmission rates to human subjects, owing to the very important health problem it represents, 2.5 million people being at risk.

As already pointed out by OVIDEO, BARGUES & MAS-COMA (1995), the very high altitude (3800-4100 m) of this endemic region represents an additional question, as the liver fluke does not inhabit such extreme altitudes in other parts of the world. Moreover, research studies carried out recently have demonstrated that the lymnaeids that transmit F. hepatica in the Bolivian Altiplano are not Lymnaea viatrix d’Orbigny, 1835 and Lymnaea cumensis Pfeiffer, 1839 as noted by UEFNO et al. (1975), but that they belong to only one species, the European Lymnaea truncatula (Müller, 1774) (BARGUES & MAS-COMA, 1997; BARGUES et al., 1997; JABBOUR-ZAHAB et al., 1997). This European lymnaeaid is also a species which does not reach high altitudes (see the review by OVIDEO, BARGUES & MAS-COMA, 1995) and how it has colonized such extreme conditions of the Northern Bolivian Altiplano (see FUENTES et al., 1998) remains to be disentangled.

Investigations on animal reservoir host species performed have included all the potential mammal definitive host species inhabiting the Altiplano endemic region. Cattle have proved to be a main reservoir host, although prevalences and egg output in bovines could not by themselves explain the high transmission rates detected...
in the Altiplano (UENO et al., 1975; MAS-COMA et al., 1995; BUCHON & MAS-COMA, 1995; HILLYER et al., 1996; BUCHON et al., 1997). Prevalences and egg output in pigs and donkeys showed that these two domestic animal species must be considered as secondary reservoir hosts in the Bolivian Altiplano, and that goats, horses, llamas and alpacas need not be considered in general control programmes owing to their very reduced populations (MAS-COMA et al., 1997). Studies on wild mammal species such as lagomorphs and rodents have proved that, contrary to what is known in other regions, they do not participate in the transmission of the disease in the Altiplano (FUENTES et al., 1997).

Concerning Altiplanic sheep, there are several studies on prevalences both by coprological (UENO et al., 1975; MAS-COMA et al., 1995) as well as by serological methods (HILLYER et al., 1996), but they refer to a few given localities. At any rate, UENO et al. (1975) already emphasized the important reservoir role of ovines in the Altiplano. The main participation of sheep in the transmission of fascioliasis in the Northern Bolivian Altiplano was recently confirmed by egg output results obtained by MAS-COMA et al. (1997). However, there is a lack of a general survey on sheep covering the whole endemic region. This is the purpose of the present paper.

MATERIAL AND METHODS

Animal samples: A total of 1600 faecal samples from sheep were collected from different zones of the Northern Bolivian Altiplano belonging to three provinces of the Department of La Paz: A) Omasuyos province: Achacachi-Chua-Huatajata-Huarina zone (310 samples); B) Los Andes province: Batallas zone (170 samples), Pucarani-Laja-Tambillo zone (230), Catavi Bajo zone (100), Lacaya zone (100) and Chojashihu zone (100); C) Ingavi province: Huacullani zone (100 samples), Cala Cala zone (100), Chiripa zone (100), Taraco zone (100), Santa Rosa zone (100) and Tiwanaku-Guaqui zone (90). Ovines of the Corriedale, Merino, Criollo and Mestizo breeds (Mestizo = crossbreeds Criollo x Corriedale or Criollo x Merino) aged 1 month to 6 years were studied. The geographical distribution of the zones surveyed is shown in Fig. 1.
Stool sample collection: Only coprological methods were carried out. Faecal samples were directly collected from the rectum of ovines in the April-October period. The number of samples from each community was a minimum of 10% of the sheep population. The surveys were made at random and carried out so that the sample size in each zone was always largely assured to be representative. Qualitative analyses giving rise to prevalence results (Table 1) as well as investigations on the relationship of prevalences with host sex and breeds (Table 2) were made in all zones surveyed. Studies on the relationship of prevalences with age groups (Table 3) were only conducted in 800 sheep from the zones of Catavi Bajo, Lackay, Chojasihu, Huacullani, Cala Cala, Chiripa, Taraco and Santa Rosa. Similarly, analyses on the relationship of prevalences with the type of owners (Table 4) could only be performed in 800 sheep from the zones of Achacachi, Chua, Huatajata, Huatajata, Battallas, Pucarani, Laja-Tambillo, Tiwanaku and Guaqui; three types of owners were distinguished according to the number of sheep in their herds: small owners (between 1 and 20 sheep per owner), medium owners (between 21 and 50 sheep per owner) and big owners (more than 50 sheep per owner).

Stool sample preparation and study: In the laboratory, before examination under light microscope for *F. hepatica* eggs, from each stool sample a quantity of 5 g were prepared according to the Watanabe sedimentation method modified by Uno (1970). This technique is similar to that described by Dennis, Stone & Swanson (1954) in its first steps and is generally known in Bolivia simply as the National Institute of Animal Health (I.A.H.) sedimentation technique. Faecal samples were collected directly from the rectum of animals, placed in numbered plastic bags for transport to the laboratory, and maintained in a freezer until examination. Of each sample, 5 g of faeces were weighed and placed in a large container, to which 200-250 ml of tap water and 2-3 drops of liquid detergent solution were added. The final mixture was homogenised with a palette. The mixture was then strained through a coarse mesh sieve, adding 250-300 ml tap water in a flooding, swirling motion through the faecal material for a maximum recovery in a conical flask. The obtained solution was allowed to stand for 8-10 min. and the supernatant was siphoned off down to about 50 ml remainder. The remaining material in the conical flask was rewarshed with tap water in a similar way and sufficient to refill up to 50 ml as done in the previous step. The solution was again allowed to stand for 8-10 min. and afterwards the supernatant was removed down to 15-20 ml remainder. This sediment was well shaken and transferred to a Petri dish, shaken again once in the Petri dish to a total homogeneity of the sediment and allowed to stand for 1 min. The dish was then carefully inclined so that the sediment flows and the water originated a division of the dish into 2 equal portions. At the time of the inclination, pipetting was carried out slowly with a small 0.3-0.5 ml Pasteur pipette along the white line of the sediment; if needed, this latter process was repeated. The liquid pipetted was then placed on a microscopic slide, two drops of 5% methyl green were added and a final homogeneity obtained by mixing with a small palette or a cover-slip. Finally, a cover-slip was placed and the sample studied under the microscope at 100-400x. Brownish eggs of *F. hepatica* were easily distinguishable among the green-coloured debris. If no eggs were found after studying 3 slides, the sample was considered negative.

**RESULTS**

*F. hepatica* infection prevalences in sheep according to the zones studied are shown in Table 1. The total prevalence in the Northern Bolivian Altiplano is 61.6%, that is, 987 ovines detected shedding eggs among the 1600 surveyed. The prevalences found are very homogeneous, with a maximum range of 87.0% prevalence in the Catavi Bajo zone and a minimum range of 49.1% prevalence in the Pucarani-Laja-Tambillo zone. Results obtained in the studies on the relationship of prevalences with sex and breeds are shown in Table 2. No significant difference was found between the prevalences detected in male and female sheep. Concerning the different breeds studied, there appears to be a higher susceptibility for fascioliasis infection in the Corriedale breed (84.0%) compared to the others (57.0-63.4%). Results obtained in the studies on the relationship of prevalences with age groups are shown in Table 3. Ovines aged less than 4 months appear to be the most infected (88.0%), whereas the prevalence decreases relatively with age, up to the 2-6 year-old group (57.2%).

The relationship of prevalences with the type of owner is shown in Table 4. No significant difference is found among the different types of owners.
between fascioliasis prevalences in sheep according to small, medium and big owners.

**DISCUSSION**

The fascioliasis infection prevalences found in sheep from the Northern Bolivian Altiplano (Table 1) are relatively high (range 49.1-87.0%; mean 61.6%). These findings are consistent with the results obtained by other investigators in *F. hepatica* infection surveys carried out in Altiplano sheep with coprological methods in localities where the liver fluke was detected: Achocalla (78.4% positive of 125 ovines analysed), Belen (66.7% of 90), Chirapaca (78.3% of 46), and Viacha (88.3% of 179) (UENO et al., 1975); Chirapaca (100% of 7), Belen (62.5% of 8), Viacha (68.6% of 265), Pajchani (100% of 5), Huarina - Cota Cota Alta (100% of 13), Guauqui (0% of 6), San Antonio (0% of 6), Patorani (0% of 5), Achuta (0% of 5), Tiwanaku and Guaqui, in the Bolivian Northern Altiplano, according to the type of owners.

The high prevalences found in the present study could be related to the year period (April-October) in which stool samples were collected. According to UENO et al. (1975), outbreaks of acute fascioliasis in the Northern Bolivian Altiplano are recorded over a period from the end of April to the beginning of August, especially from May to July, that is, in the dry season.

The high fascioliasis prevalences and the relatively reduced range found in sheep from localities where the parasite is present disagree with the usually low prevalences and large heterogeneity detected in cattle from the same Altiplano area (BUCHON et al., 1997). These differences between *F. hepatica* infection in sheep and cattle may be understood by taking into account that:

- the life-span of the parasite in sheep can be as long as 11 years (DAWES & HUGHES, 1964; SMITHERS, 1982), whereas in cattle the parasites generally survive not more than 1 year (SMITHERS, 1982);

- observations of numerous investigators suggest that sheep are relatively susceptible to reinfection and flukes may accumulate in their livers, while adult cattle are more resistant to reinfection (HUILLER & HUILLER, 1986) and may self-cure; significant drops in egg excretion levels becoming egg-negative after 1 year of infection (DE LEON, QUIÑONES & HILLYER, 1981) are presumably due to acquired resistance and worm elimination; thus in an area endemic for fascioliasis one would expect a relatively high prevalence rate in sheep and the higher infection rates in sheep over cattle are not surprising (HILLYER et al., 1996);

- the contamination of the milieu in the Bolivian Altiplano, and consequently also the incidence rates, may be very high, as deduced from the high prevalence rates detected in sheep treated with fasciocicides once or twice a year (prevalences of 66.7% in Belén and 88.3% in Viacha), and from the high number of infective metacercariae found attached to aquatic plants (UENO et al., 1975);

- in the Bolivian Altiplano sheep are usually moved by their owners throughout larger pasture fields, so that they are daily able to visit more places and, hence, have a higher probability of visiting transmission foci (water collections inhabited by miracidia) where they become infected.

### Table 3. *F. hepatica* infection prevalences found in 800 sheep from the zones of Catavi Bajo, LaCaya, Chojasisihi, Huacullan, Cala Cala, Chiripa, Taraco and Santa Rosa, in the Bolivian Northern Altiplano, according to age groups.

<table>
<thead>
<tr>
<th>Age</th>
<th>No. sheep studied</th>
<th>No. positive</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4 months</td>
<td>50</td>
<td>44</td>
<td>88.0</td>
</tr>
<tr>
<td>4 months - 1 year</td>
<td>170</td>
<td>123</td>
<td>72.3</td>
</tr>
<tr>
<td>1-2 years</td>
<td>250</td>
<td>192</td>
<td>76.8</td>
</tr>
<tr>
<td>2-4 years</td>
<td>330</td>
<td>189</td>
<td>57.2</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>800</strong></td>
<td><strong>548</strong></td>
<td><strong>68.5</strong></td>
</tr>
</tbody>
</table>

### Table 4. *F. hepatica* infection prevalences found in 800 sheep from the zones of Achacachi, Chua, Huatajata, Huarina, Batallas, Pucarani, Laja, Tambillo, Tiwanaku and Guaqui, in the Bolivian Northern Altiplano, according to the type of owners.

<table>
<thead>
<tr>
<th>Type of owner</th>
<th>No. ovines per owner</th>
<th>No. sheep studied</th>
<th>No. positive</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>1-20</td>
<td>198</td>
<td>116</td>
<td>58.5</td>
</tr>
<tr>
<td>Medium</td>
<td>21-50</td>
<td>144</td>
<td>79</td>
<td>54.8</td>
</tr>
<tr>
<td>Big</td>
<td>&gt;50</td>
<td>458</td>
<td>244</td>
<td>53.2</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>800</strong></td>
<td><strong>439</strong></td>
<td><strong>54.8</strong></td>
<td></td>
</tr>
</tbody>
</table>
Concerning the relationship of *F. hepatica* infection and the different sheep breeds analysed (Table 2), the marked difference between the prevalence rates found between the two pure strains, Corriedale (84.0%) and Merino (57.0%), may be *a priori* surprising. High fascioliasis prevalences in coprological surveys as well as a high intensity of liver infection in necropsies were already found in Corriedale ovines from Belén and Viana in previous studies carried out by Morales Lavandénz (in MAS-COMA *et al.*, 1995). At any rate, the different geographical origins of these two breeds may account for this difference of susceptibility to *F. hepatica* infection: Corriedale sheep are originally raised in New Zealand while Merino sheep were originally raised in Spain. As already noted by Ueno, Bargues & Mas-Coma (1995), it is known, first, that the parasite *F. hepatica* is of European origin and has spread all over the world with the exportation of European livestock and, second, that the Spanish colonization of South America, begun five centuries ago, included the importation of European domestic animals (Posnansky, 1982). In the Northern Bolivian Altiplano, present-day livestock includes several European races of sheep, cattle, goats, pigs, horses, donkeys and rabbits, besides autohomous species such as llamas and alpacas (MAS-COMA *et al.*, 1995). Hence, the Merino breed may be more resistant to the liver fluke, even more than strains later developed in Latin America such as Criollo (57.3% prevalence) or than crossbreeds such as Mestizo (63.4%).

The prevalence decrease according to age groups (Table 3) may presumably be related to an ensemble of circumstances, including the death of the most infected young ovines, the appearance of some level of acquired resistance, and a lower egg production by old liver fluke adults. Concerning the first above-mentioned circumstance, Ueno *et al.* (1975) already noted in their studies about sheep fascioliasis in the Northern Bolivian Altiplano that: about 38% of faecal samples collected from Altiplanic sheep revealed egg counts of more than 1000 eggs per gram (in sheep it is known that the presence of 300-600 eggs per gram of faeces indicate an infection likely to be pathogenic); the majority of these sheep were in a very poor bodily condition; big outbreaks of acute fascioliasis have been observed among sheep every year inflicting a heavy loss on animal production; in the case of sheep kept on farms heavily contaminated with the liver fluke the mortality rate was estimated to be not less than 15 to 25% annually.

In the same sense, it has already been stated that domestic animals from the Northern Bolivian Altiplano are very highly affected owing to the injuries caused, even including an almost total extermination of sheep (MAS-COMA *et al.*, 1995). The need for appropriate treatments was already emphasized by Ueno & Morales (1973). Several years ago rural families could have up to 200 and 250 sheep, whereas today only a few families with no more than 15-20 individuals can be seen. The low production of meat and milk caused by severe infection levels ranging between 20 and 100% in numerous communities makes livestock exploitation very difficult. This becomes extremely important in the Altiplano, because sheep and cattle are the main sources of income in subsistence agriculture (MAS-COMA *et al.*, 1995).

Concerning the studies conducted to analyse whether there is a relationship between fascioliasis prevalences and the type of owners (Table 4), the results obtained suggest that the size of sheep herds has no influence in the Altiplano endemic area. This is presumably due to the liver fluke life cycle pattern and the type of transmission: foci linked to fresh water collections necessarily inhabited by lymnaeid snails, but also to the sheep herd management customs of the Aymaras living in the endemic region.

As it is known that the egg output of the adult flukes in sheep is relatively high. Daily egg output per fluke is dependent on the number of flukes in the liver and varies from 4000 to 50000 and the average numbers range from 8800 to 25100 during 13 to 19 weeks after infection (Bo- ray, 1969). Daily egg output per adult fluke is generally inversely proportional to the intensity of the fluke burden. In moderate infections the daily egg output is usually constant, while in heavy infections the egg output varies considerably (Chen & Moit, 1990).

In the Northern Bolivian Altiplano, studies on egg output in sheep have been carried out by different authors. Ueno *et al.* (1975) noted egg counts of more than 1000 eggs per gram, Morales Lavandénz (in MAS-COMA *et al.*, 1995) found averages of 1203.13.2 and 4.5 eggs/5 g of faeces in sheep with high (n = 25), moderate (n = 4) and low (n = 9) infection, respectively. MAS-COMA *et al.* (1997) detected a number of eggs per gram of faeces ranging between 3 and 145, with a mean of 44.7, which gives an estimation of 3000-435000 *F. hepatica* eggs shed with faeces by a sheep per day. This latter amount is similar to that estimated for Altiplanic cattle (MAS-COMA *et al.*, 1997; Buchon *et al.*, 1997).

Together with cattle, sheep are the most numerous domestic animals in all zones of the Altiplanic endemic area and they usually constitute part of the livestock of Aymara families (MAS-COMA *et al.*, 1995, 1997). According to the prevalence and egg output results found, it can be concluded that ovines may be considered the main animal reservoir host of fascioliasis in the Northern Bolivian Altiplano. Sheep develop a very important role in the contamination of the external milieu and participate decisively in the transmission of the disease in this area. However, in spite of the high prevalences and egg output numbers detected in sheep, they cannot alone explain the high transmission rates to human subjects known to occur in the Northern Bolivian Altiplano, as prevalences and egg output in Altiplanic sheep are similar to those found in areas of other regions and countries where human subjects become infected only sporadically and isolately.
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