Dynamics of *Fasciola hepatica* transmission in the Andean Patagonian valleys, Argentina

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Abstract

We described the transmission dynamics of *Fasciola hepatica* at its southern distribution range. Studies of prevalence and egg output in cattle and population dynamics and infection in snails were performed in a farm in the Andean Patagonian valleys, Argentina, between December 1998 and February 2002. Snail surveys were conducted from spring to autumn. Infection was diagnosed coprologically in the whole herd at the beginning and end of the study, and in a cohort of heifers at the beginning and end of 2001. A twice-a-year anthelmintic treatment was implemented in 1999. The relationship of the variables mentioned above with temperature and rainfall was determined. *Lymnaea viatrix* showed a life-span of about 15 months and an annual pattern of population dynamics. Specimens were frequently found in temporary environments and lagoons, and rarely in streams. Snail abundance and soil-water availability were directly related in temporary environments and inversely related in lagoons. Overall prevalence in *L. viatrix* was 0.67% (range: 0.9–14%) and infection was detected in summer and autumn. At the beginning of the study, calves were the least infected age group (15%). Prevalences and median egg counts in grazing animals were similar at the beginning (heifers: 81%, 3.3 epg; cows: 60%, 1.3 epg) and end of the study (heifers and cows: around 51%, 1 epg). Likewise, the prevalence in the cohort of heifers remained similar (around 40%) between surveys. Transmission to cattle was highly effective despite the short activity period and the low infection rate of snails, and the regular anthelmintic treatment. There would be two seasonal transmission peaks, one in summer–autumn, when infected snails were present, and the other in early spring due to overwintering metacercariae. Some recommendations based on the climatic conditions of the region are provided to minimize snail infection and ultimately to reduce the incidence of fasciolosis in cattle.

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1. Introduction

Fasciolosis, caused by *Fasciola hepatica* (Trematoda: Digenea), is a major disease of livestock that produces important economic losses due to mortality, liver condemnation, reduced production of meat, milk, and wool, and expenditures for anthelmintics (Dargie, 1987; Mas-Coma et al., 1995; Hillyer and Apt, 1997). The disease has a cosmopolitan distribution with cases reported from Scandinavia to New Zealand and Southern Argentina (Torgerson and Claxton, 1999). The life cycle of *F. hepatica* includes snails of the family Lymnaeidae (Gasteropoda: Pulmonata) acting as intermediate hosts (Malek, 1985) and free-living stages (eggs, miracidia, cercariae, and metacercariae). Temperature and moisture

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are key factors for the development and survival of both snails and extramammalian stages, which may take place between 10 and 30 °C in presence of wet soil or freshwater environments (Torgerson and Claxton, 1999). When these factors become limiting, snails adopt survival strategies such as hibernation and aestivation (Boray, 1969; Ollerenshaw, 1971a; Malek, 1985). On the other hand, *F. hepatica* eggs and metacercariae may remain alive for long periods under low temperatures, but they are susceptible to heat and drought (Boray, 1969; Luzón-Peña et al., 1994; Andrews, 1999).

In endemic regions, chronic fasciolosis is the most common form of liver fluke infection in cattle (Boray, 1999) as a consequence of prolonged intake of metacercariae from contaminated pastures. Clinical disease is only likely in young cattle because bovines may develop some immunity to the parasite with age (Gonzalez-Lanza et al., 1989; Buchon et al., 1997), but older-resistant-animals develop liver fibrosis associated with decreased production (Boray, 1999). In both cases, however, infected individuals contribute to pasture contamination with fluke eggs (Buchon et al., 1997).

Eradication of fasciolosis is rarely a practical option and control needs to be aimed at the reduction of the disease (Torgerson and Claxton, 1999). In this context, the epidemiology of the parasite provides the basis to design strategic control programmes according to the climatic conditions of each region (Stromberg and Averbeck, 1999). Over the last decades, several epidemiological studies dealing with the main factors involved in the transmission dynamics have been conducted worldwide (Ollerenshaw, 1971a; Malone et al., 1984; Amato et al., 1986; Alcaínó et al., 1993; Zukowski et al., 1993; Buchon et al., 1997; Claxton et al., 1997; Yilma and Malone, 1998; Fuentes et al., 2001; Cruz-Mendoza et al., 2005).

In Argentina, no longitudinal epidemiological research has been performed at present. In regard to the intermediate hosts of *F. hepatica*, *Lymnaea diaphana*, and *L. plicata* were described in the fifties (Hubendick, 1951; Hylton Scott, 1953) and seldom cited thereafter (Santa Cruz, 1979), *L. columella* appears to be restricted to the northeast (Castellanos and Landoni, 1981; Paraeense, 1982; Prepelitchi et al., 2003), while *L. viatrix* is more widely distributed (Paraeense, 1976; Lombardero et al., 1979; Castellanos and Landoni, 1981; Paraeense, 1982; Rossanigo et al., 1983; Venturini and Fonrouge, 1985; Durand et al., 2002; Rubel et al., 2005; Cucher et al., 2006), with its southernmost limit in Santa Cruz Province (49°59’S latitude, 68°55’W longitude) (Paraeense, 2005). So far, *L. columella* and *L. viatrix* are the only species that have been found naturally infected with *F. hepatica* (Prepelitchi et al., 2003; Rubel et al., 2005; Cucher et al., 2006). On the other hand, official reports of liver condemnation rates for cattle at abattoirs during the three years prior to this study ranged between 0 and 80% for different provinces of the country (SENASA), with highest prevalences in the Patagonian provinces. The aim of the present study was to describe and analyse the transmission dynamics of fasciolosis in the Andean Patagonian valleys, in order to provide information for effective control strategies.

2. Materials and methods

2.1. Study area

The study was conducted between December 1998 and February 2002, in a cattle farm with a history of chronic fasciolosis, covering an area of around 2500 ha, at the northwest of Chubut Province, Patagonia, Argentina (42°32’S latitude, 71°34’W longitude, Fig. 1). The farm is located within the Andean Patagonian valleys, a cattle-rearing area in the Andean

Fig. 1. Map of Argentina showing the location of Patagonian provinces. Star indicates the studied area within the Andean Patagonian valleys.
Precordillera at 500–600 m asl. The climate in the region is temperate cold and humid. Precipitation concentrates between May and September and the snowy period extends mainly between July and August. The valley area has a glacial origin, and is characterised by deep, clayish–sandy–loam soils, creating favorable conditions for water retention (Salazar et al., 1990). The region comprises permanent and temporary freshwater environments. The former are represented by lagoons, rivers and streams, and the latter by ponds, ditches and water-saturated flat areas of varying surface (locally called “mallín”), which are the most predominating environments in the region (Brinson and Malvárez, 2002). Most of these environments become temporarily interconnected in early spring, when the valley is flooded due to melting snow, and their sizes decrease rapidly with increasing temperature. Freshwater environments, all of which were potential habitats for snails, were identified and georeferenced during two field surveys made at the beginning of the study (Fig. 2).

2.2. Abiotic variables

Data of daily mean and maximum temperatures were obtained from the meteorological station at the Esquel Airport (NCDC, Argentine Meteorological Service). This station was selected because it was the nearest (50 km) to the cattle farm and had the most complete data records. Rainfall values were recorded in situ using a pluviometer. A water budget analysis was made following Thornthwaite and Mather (1955), based on a field capacity of 200 mm.

The inactivity period of the intermediate host (hibernation) was determined by using maximum daily temperatures below 10 °C (Aziz and Raut, 1996; Claxton et al., 1999). Thus, hibernation was assumed to take place between May and September. Mean daily temperatures below 10 °C were also considered for pre and posthibernation periods of presumed slow snail development.

2.3. Snail study

A total of nine surveys involving all the freshwater environments were conducted between November 1999 and February 2002 to collect lymnaeid snails by hand or with a net. These surveys were grouped into the following periods: period 1, spring 1999–autumn 2000 (November 1999, January and March 2000); period 2, spring 2000–autumn 2001 (November 2000, January and March 2001); period 3, spring 2001–summer 2002 (October and November 2001, February 2002) (Fig. 3).
Samples were taken by the same operator on every occasion. To determine the snail population structure and dynamics (size distribution and proportion of mature and immature individuals) and infection prevalence with *F. hepatica*, lymnaeid snails were collected for at least 2 h in each environment, in an area representing approximately 20% of the surface suitable for snail development. In order to assess whether the type of environment (temporary or permanent) has any effect on the dynamics and abundance of snails, these variables were compared between mallín C and lagoon D (Fig. 2). The mallín C was selected at random, while the lagoon D showed more favorable conditions for snails than other permanent environments. Snail abundance was estimated using the timed-collection method, that is, total number of snails found during 30–45 min (adjusted to 10 min). Collected snails were placed alive between wet cotton pads in properly labelled plastic containers. Once in the laboratory, they were measured from the apex to the anterior margin of the shell using a light microscope equipped with a calibrated ocular scale. Specimens longer than 4 mm were considered as sexually mature (Venturini and Fonrouge, 1985). Within this group, snails were classified as middle-sized (4–8 mm) and large (>8 mm).

*F. hepatica* infection in snails was determined on the basis of cercarial morphology. To stimulate cercarial shedding, collected specimens were placed individually in 10-ml plastic vials containing dechlorinated tap water, and then exposed to artificial light. The free-swimming cercariae were examined under light microscopy. Snails not shedding cercariae were dissected to reveal concealed infections. To confirm the taxonomic identity of the parasite (Jones, 2005), metacercariae were orally administered to Wistar rats, and adult flukes were recovered from the bile ducts (Kleiman et al., 2004a). About 25 nonshedding snails collected during the first and second study periods were preserved in Raillet–Henry’s solution (Paraense, 1984), and submitted to Dr. W. Lobato Paraense at the Department of Malacology of Oswaldo Cruz Institute, Rio de Janeiro, Brazil, for species identification by features of the shell and internal organs (Paraense, 1976). The remaining snails were assigned to the same species...
of the already identified individuals, on the basis of common morphological features.

2.4. Herd study

The study was conducted between December 1998 and December 2001. The herd was exclusively Hereford, born and raised on the farm. During the study, mean livestock density was around 0.3 head per hectare. Cattle management, common to the rest of the farms in the region, consisted in keeping the herd at a short distance up from the base of the slopes when valleys were covered by snow or flooded; cattle fed on the 2 m tall bamboo *Chusquea culeou* at these overwintering sites. Then, when weather conditions got milder, the herd was brought back down to the plain. At the beginning of the study animals were free to move at will, thus having access to any freshwater environment of the valley. A deferred-rotation grazing system was implemented from May 2000 onwards, after the construction of paddocks (Fig. 2). Information on this management activity was recorded and related to the infection outcome in snails. In regard to anthelmintic treatments, animals had been sporadically treated with Triclabendazole until 1998. Thereafter, the farm managers implemented a regular treatment with Closantel in the entire herd during autumn and by the end of spring, but this scheme varied slightly depending upon their convenience. In general terms, calves born in spring received the first treatment the following autumn, after weaning.

To evaluate the parasitological status of the herd and of the different age classes before and after the implementation of regular treatment, the faecal prevalence of *F. hepatica* and the number of eggs per gram of faeces (epg) were estimated at the beginning and end of the study. Cattle were classified into the following age classes of epidemiological importance: calves (up to six months old), heifers (after weaning, animals older than six months–first parturition), and cows (after first parturition–up to nine years old). The sampling of calves was delayed two months after the first survey to obtain a convenient size for handling. Calves could not be sampled in the second survey due to operational constraints.

In order to assess fluctuations in prevalence of fasciolosis in young animals subject to regular treatment with anthelmintics, faecal samples were taken in January and December 2001 from a cohort of 45-tagged animals aged 15 months.

In all cases, individual faecal samples of about 20 g were directly obtained from the rectum of heifers and cows with disposable gloves. In calves, samples were collected from the anus during spontaneous defecation. Faecal samples were placed in plastic flasks stored in coolers with ice packs and once in the laboratory they were kept at $-20^\circ$ C until being used. Three grams from each sample were processed using a coprological sieving method (Kleiman et al., 2005). In each positive sample, *F. hepatica* eggs were identified according to distinctive morphological features (Ash et al., 1994), and counted and expressed as epg.

2.5. Statistical analyses

To analyse the structure of the snail population, the length of all snails was compared among seasons using the Kruskal–Wallis test followed by the Dunn test, if required (Zar, 1999), and the proportions of sexually immature and mature snails were compared among surveys using the homogeneity test with a posteriori comparisons (Fleiss, 1981). In both cases, the analysis only included data from the surveys of November, January, and March, which were considered as representative of spring, summer, and autumn, respectively. The collections from October 2001 and February 2002 were excluded to prevent bias due to environmental heterogeneity.

The overall prevalence of *F. hepatica* in cattle was compared between samplings using the homogeneity test. Theoretical sample sizes were estimated on a basis of 900–1000 bovines, yielding 150 animals per survey (Zar, 1999). In the practice, 129 and 230 individuals were sampled in the field at the beginning and end of the study, respectively. In both surveys, the number of animals sampled from each age class was proportional to the number of individuals in each age class for the entire herd. The homogeneity test was also applied to compare the proportion of infected animals in each age class within and between samplings, with a posteriori comparisons. The epg for each age class was compared within and between samplings using the Kruskal–Wallis test followed by the Dunn test, if required. The prevalences in heifers were compared at the beginning and end of 2001 by the Mc Nemar test (Zar, 1999). For all comparisons, a value of $p < 0.05$ was considered significant.

3. Results

3.1. Snail study

All snails collected were taxonomically identified as *Lymnaea viatrix* Orbigny, 1835. This species, which
was present throughout the study, was found frequently in temporary freshwater environments and in the lagoons, only sporadically in one stream, and never in the river. A total of 2594 individuals were collected, ranging between 1.7 and 13.7 mm in length. Among these, 1633 snails were examined for infection with _F. hepatica._

Fig. 3 shows the population dynamics of _L. viatrix_ along the study periods in relation to temperature, precipitation, and water budget.

**Period 1:** In November 1999 (n = 92), 67% of the snails were large (>8 mm), and the remainder were middle-sized adults (4–8 mm), indicating that the population had reached sexual maturity after hibernation. In January 2000 (n = 653), the appearance of 23% immature individuals (<4 mm) showed the recruitment of a new generation between November 1999 and January 2000. Snails greater than 8 mm decreased to 34%, and 43% of the population was composed of middle-sized adults. In March 2000 (n = 490), prior to hibernation, snails less or equal to 4 mm dropped to 11%, middle-sized adults increased to 88%, and large snails were absent.

**Period 2:** In November 2000 (n = 130), all snails were adults, similarly to November 1999. Forty-five percent of the population was composed of large snails and the rest were middle-sized adults. In January 2001 (n = 396), the recruitment of a new generation was again indicated by the presence of immature snails (29%). Large snails decreased to 13% and middle-sized adults represented 58% of the total population. In March 2001 (n = 304), snails less or equal to 4 mm decreased to 15%, middle-sized adults increased to 75% and only 9.5% of the individuals were greater than 8 mm.

**Period 3:** During October 2001 (n = 78), 10% of the individuals were sexually immature. These snails were not considered as recently born because they were about 4 mm long. Middle-sized and large adults represented 66 and 24% of the total population, respectively. In November 2001 (n = 173), there was a small proportion of immature snails (4%). Among adults, 54% were middle-sized and the remainder were large snails. Finally, in February 2002 (n = 278), 83% of the individuals were middle-sized adults and 14% were large snails. In contrast to that observed in the previous summer periods, there was a negligible proportion of snails less or equal to 4 mm. The absence of recently born snails took place after the following situations: (1) precipitation levels higher in winter 2001 than in winter 1999 and 2000 (566 mm versus 297 and 460 mm, respectively), (2) maximum temperatures above 10 °C about one month earlier than in the other periods.

The observations described above were confirmed by statistical analyses. Snail sizes showed significant differences among seasons (p < 0.05). The median length was maximum in spring (n = 395, Me = 7.9, Q1–Q3: 6.8–9.4) and minimum in autumn (n = 794, Me = 4.9, Q1–Q3: 4.4–5.6), while it showed intermediate values in summer (n = 1049, Me = 6.2, Q1–Q3: 4–8) (Fig. 4). The proportion of sexually mature and immature snails differed significantly among surveys (p < 0.05). A posteriori comparisons revealed the following three homogeneous groups: (1) November 1999, 2000, and 2001 (spring) (p > 0.05), (2) March 2000 and 2001 (autumn) (p > 0.05), and (3) January 2000 and 2001 (summer) (p > 0.05).

In spring, when almost the whole population was composed of sexually mature individuals, mean seasonal temperatures (MST) ranged between 9.7 and 11.8 °C and the soil was saturated. During summer, when the recruitment of new individuals was indicated by the appearance of a high proportion of recently born snails (except for period 3), MST fluctuated between 12.8 and 15 °C and soil-moisture conditions varied from field capacity to water deficit. In early autumn, when middle-sized individuals predominated and large snails were absent, MST ranged between 5.3 and 6.2 °C and soil-moisture conditions were similar to those registered during summer. Finally, during hibernation, MST ranged between 2.7 and 3.8 °C and precipitation reached a maximum.

Field observations indicated that both permanent and temporary environments showed the highest water levels in spring and then gradually shrunk with increasing temperature. By autumn, a wide muddy shore surrounded the lagoon D, while the mallín C was
almost dried out. Both environments were subject to temporal variations in snail abundance (Fig. 5). In each study period, maximum snail abundance was higher in the lagoon than in the mallín, and highest values in one environment matched with lowest values in the other. In the lagoon snails were concentrated on the shore and in the mallín they were spread over the surface. In both environments, snail dynamics resembled that of the whole population, except for recruitment, which was simultaneously detected only during summer of the first period. In the second period immature snails were found in the mallín in summer and in the lagoon in autumn. This lag in the reproductive event of snails between environments coincided with increased rainfall before the survey of summer.

Overall prevalence of *F. hepatica* in *L. viatrix* was 0.67% (11/1633). Infected snails were only found in three of the environments. Snail infection rates were about 6% (7/119) for the lagoon D in summer 2000, 2% (1/53) for mallín C, 14% (1/7) for a stream in autumn 2001, and 0.9% (2/222) for the lagoon D in summer 2002 (Fig. 2). No infected snails were detected during spring. All parasitised snails were adults measuring between 4.5 and 9.4 mm, with a median of 7.55 mm.

### 3.2. Herd study

Heifers and cows had received the last anthelmintic treatment seven months before the surveys, and calves were never treated. At the beginning of the study (December 1998), overall prevalence of *F. hepatica* in cattle was 52% (95% confidence interval: 43 to 61%). Prevalence differed significantly among age classes (*p* < 0.05); heifers and cows were more infected than calves (*p* < 0.05), but there was no significant difference between them (*p* > 0.05) (calves: 15%, 5/33; heifers: 81%, 17/21; cows: 60%, 45/75). In regard to egg numbers, heifers showed the highest median epg value (median epg = 3.3, *Q*1−*Q*3: 0.85−7.7, range: 0.3−30) followed by cows (median epg = 1.3, *Q*1−*Q*3: 0.7−2.3, range: 0.3−10), and calves (median epg = 0.7, *Q*1−*Q*3: 0.3−1.45, range: 0.3−1.7). Statistically significant differences in epg were only found between heifers and calves (*p* < 0.05). At the end of the study (December 2001), overall prevalence of *F. hepatica* in cattle was 50.9% (95% confidence interval: 47 to 54%). Grazing animals showed almost identical infection levels (50.8%, 60/118 for heifers versus 50.9%, 57/112 for cows; *p* > 0.05) and a median of about 1 epg (*Q*1−*Q*3: 0.3−3.5, range: 0.3−13.3 for heifers versus *Q*1−*Q*3: 0.7−2.5, range: 0.3−16 for cows; *p* > 0.05). Overall prevalences in cattle were similar at the beginning and the end of the study (*p* > 0.05). Although infection levels decreased 30% and less than 10% of the initial values for heifers and cows, respectively, none of these age groups showed significant differences between surveys (*p* > 0.05). Likewise, neither heifers nor cows showed significant differences when epg medians were compared between surveys (*p* > 0.05).

The cohort of heifers was first treated in May (autumn) 2000 after weaning; the treatment corresponding to spring 2000 was delayed until January (summer) 2001, and a third treatment was conducted in May 2001. There was no significant difference in prevalence between January 2001 (pre-treatment) and December 2001 (40%, 18/45 versus 46.7%, 21/45; *p* > 0.05). Most of the heifers that were positive for *F. hepatica* in January became negative by December (78%, 14/18), but as much as 63% (17/27) of the initially negative animals became infected.

### 3.3. Cattle management and snail infection

The implemented deferred-rotation grazing system limited the access of cattle to certain freshwater environments. The location of these environments with respect to paddocks is shown in Fig. 2. Based on the information provided by the farmers, Fig. 6 shows the periods when the cattle had access to the different environments, the subsequent snail samplings conducted in these environments and the mean monthly temperatures. To analyse the relation between the presence of the cattle and the infection outcome in snails, only those environments harboring more than 30 snails in each survey were included. In mallín A, cattle were present from late winter 2000 and no infected snails were found in the surveys of spring 2000 and summer 2001; mean temperatures were above 10 °C for only two weeks until the first snail survey, and ranged...
between 10 and 14 °C for one month until the second one. Cattle had access to mallín B and mallín C from about midspring 2000 and snails were negative in summer and positive in autumn 2001, respectively. Temperature ranged between 10 and 14 °C for only one month before the snail survey in mallín B, and for three months before the snail survey in mallín C. In the lagoon D, cattle were present from late spring 2001 to late summer 2002 with a gap of one month, and snails were infected in the survey of summer. Temperatures rose above 10 °C three months before the snail survey, and rapidly reached 16 °C.

4. Discussion

In the Andean Patagonian valleys L. viatrix appears as the intermediate host for F. hepatica due to its regular presence in most of the freshwater environments and the finding of infected individuals in the three studied periods. Although the study area is within the distribution range of this species, this is the first study revealing its epidemiological importance in the Patagonian region. During the three-year study period, L. viatrix showed an annual pattern of population dynamics (Fig. 3). The new generation born during early summer reached intermediate sizes before hibernation. Thus, snails resuming activity in spring were sexually mature individuals that oviposited at the end of this season, which led to an overlapping of generations in summer. Based in our results, the estimated life-span of L. viatrix in the Andean Patagonian valleys is about 15 months. Similar results were found by Kendall and Ollerenshaw (1963) for L. truncatula from Scotland, where humidity is not a restrictive factor and temperatures are comparable to those of the study area. The impact of critical environmental conditions on snail population structure is likely to depend upon the lymnaeid species. For example, large individuals of L. tomentosa suffered the highest mortality when exposed to desiccation, whereas specimens of L. truncatula were not negatively affected by the long, cold winter (Boray, 1969). In the present study, sizes of L. viatrix after hibernation were similar to or higher than those before hibernation, suggesting that cold stress did not cause differential survival among snails of different sizes. In addition, there was a high percentage of sexually mature individuals after the inactivity period. This result, which was also observed for post-aestivating L. bulimoides from USA (Malone et al., 1984) may be interpreted as a reproductive strategy that enables snails to reproduce soon after winter, thereby providing enough time for the new generation to develop before the onset of unfavorable environmental conditions. On the other hand, experimental studies performed at constant temperatures indicate that snail development does not occur below 10 °C (Claxton et al., 1999; Aziz and Raut, 1996). In the field, however, the uncontrolled and fluctuating environmental conditions make it difficult to establish the most meaningful temperature value triggering snail activity, as reflected by the use of mean monthly temperature above 10 °C by some authors (Ollerenshaw, 1971a; Boray, 1999; Malone, 1994) and minimum temperatures above 10 °C by others (Malone et al., 1984). Based on the analysis of our data, maximum temperatures were selected because the use of mean monthly temperatures may have led to misleading conclusions. The latter were not consistent with field observations indicating that snails were active in spring of the second and third periods, when mean temperatures fluctuated slightly around 10 °C (Fig. 3). It is well known that the development of the intermediate host is dependent on the simultaneous occurrence of appropriate conditions of temperature and humidity (Boray, 1969; Ollerenshaw, 1971b; Malone et al., 1984; Roberts and Suhardono, 1996). In the study area, precipitation concentrates in winter, but due to high soil-moisture storage capacity, suitable habitats for the intermediate host are available between spring and autumn, when maximum temperatures are above 10 °C. Thus, the limiting factor for the development of L. viatrix in the Andean Patagonian valleys would be temperature rather than humidity.

The lack of recently born snails during the third period would be partially explained by the fact that the last summer sampling was conducted in February.
instead of January (Fig. 3). However, a more likely explanation is that maximum temperatures above 10 °C occurring almost one month earlier than in the previous periods (see arrows in Fig. 3) would have led to a shorter period of inactivity, an earlier reproductive event and an increased growth rate.

Snail infection was not detected in spring, suggesting that infected individuals would not survive adverse winter conditions. In epidemiological surveys conducted in Great Britain, Ollerenshaw (1971a) attributed the low overwintering infection rate of snails to the increased mortality of diseased individuals. These results may indicate that only those parasites infecting snails during spring or summer will develop to the metacercaria stage. On the other hand, it is also possible that we would have missed snail infection in spring taking into account the lower number of snails sampled with respect to summer–autumn, and the low prevalences recorded along the study. In the present study, all infected snails were middle-sized and large specimens. The importance of snail size for parasite transmission was pointed out by Ollerenshaw (1971b), who found that 85% of infected L. truncatula were large individuals, and that large snails harbored at least ten times more reidiae than small snails.

Snails were frequently found in all environments except for the river and a stream, where they were never or occasionally observed, respectively. Although fast-flowing water bodies are not suitable for snail development (Boray, 1969), this stream, which crosses different temporary environments as it runs through the valley, may act as a dispersal corridor for the snail (Fig. 2). The role of springs and streams in the migration of lymnaeids has already been mentioned (Boray, 1969; Rondelaud et al., 2005). Furthermore, the fact that a snail collected from this stream was infected suggests that it would also be involved in the transmission and spread of the parasite.

Both lagoons and temporary environments are likely to act as transmission foci because they offer suitable conditions for the complete development of snails, and may harbor infected snails. Their variation in the water level from spring to autumn inversely influenced snail abundance. In the mallín, snail abundance was negatively affected by decreased soil humidity, while in the lagoon it was positively affected by the formation of a muddy shore. The importance of muddy habitats for snails would be evidenced by the absence of individuals and a delay in the recruitment after the heavy precipitation recorded in January 2001, which increased the level of the water body. Knowledge of the periods of maximum snail abundance is useful when designing management strategies, in order to minimize snail infection and ultimately to reduce the incidence of fasciolosis in cattle. Consequently, in the Andean Patagonian valleys the encounter between cattle and snails should be minimized during spring in temporary environments, and during autumn in lagoons.

In the study area, the high prevalences of F. hepatica in cattle may indicate an effective transmission of the liver fluke, despite of the relatively short activity period and the low infection rate of snails. Records of high prevalence in livestock along with low prevalence in snails have also been documented in other regions (Malone et al., 1984; Rognlie et al., 1996; Parr and Gray, 2000; Rubel et al., 2005). When compared to grazing animals, milking calves showed lower prevalence, probably due to reduced exposure to infection (Gonzalez-Lanza et al., 1989; Torgerson and Claxton, 1999), and lower epg. In turn, there were no significant differences in the prevalence of F. hepatica in heifers and cows, in agreement with previous studies reporting resistance to re-infection in bovines older than 1–2 years (Buchon et al., 1997; Gonzalez-Lanza et al., 1989). In our study, overall prevalences (about 50%) were higher and epg values (range: 0.3–30) lower than those reported for other endemic areas of fasciolosis (Gonzalez-Lanza et al., 1989; Cringoli et al., 2002). Although fluke egg counts are not reliable as a measure of the parasite burden, they can be of value for monitoring the egg excretion pattern (Duménigo et al., 2000; Love and Hutchinson, 2003).

Temperature is known to affect the developmental rate from egg to cercaria stages. Egg embryonation begins at temperatures between 8 and 12 °C, miracidial development can be completed below 10 °C (Valenzuela et al., 1979), but hatching is only triggered above this temperature (Alcaíno et al., 1993). Egg development time to hatching lasted 70 and 35 days at constant temperatures of 10 and 15 °C, respectively (Claxton et al., 1999), and in the field, eggs took 60 days to hatch at mean monthly temperatures between 10 and 16 °C (Alcaíno et al., 1993). On the basis of results obtained by Ollerenshaw (1971a,b), the development period from egg to cercaria would last for about four months at a constant temperature of 15 °C. Our analysis of the relationship between the presence of cattle and subsequent infection in snails may indicate that F. hepatica requires approximately three months to develop from egg to cercaria at temperatures ranging between 10 and 16 °C (Fig. 6). According to background information provided by other authors and assuming the death of infected snails during winter, the absence of snail immature infections in the surveys of
spring suggests that fluke eggs did not hatch immediately after temperature had risen above 10 °C. On the other hand, the more favorable environmental conditions during the third period as compared to the second one (temperatures rising above 10 °C sooner and reaching higher values faster) would have increased the developmental rate of parasite eggs and larvae; although no data are available, it is reasonable to presume that snail infection may have started earlier than when it was detected.

The fact that overall cattle prevalence and egg output were similar at the beginning and at the end of the study suggests that the regular anthelmintic treatment did not reduce parasite transmission to cattle. The study of the cohort of heifers made during 2001 may provide some information on this matter. Taking into account that they received two treatments with Closantel, one in summer and the other before winter, and that they were kept on the slope (unsuitable for fasciolosis transmission) during winter, it was surprising that prevalence remained at high levels by the end of the year. This result could be explained by: (1) sanitary measures failing to reduce fluke burden; the main factors influencing treatment outcome are dates of drug administration, class of drug and dose, and alternation of drug classes (Gaasenbeek et al., 2001; Sangster, 2001); (2) overwintering metacercariae infecting heifers in early spring. This assumption would also be supported by the occurrence of infected calves in mid-summer, since they were born during the previous spring and had no time to develop mature infections derived from metacercariae produced in summer. The survival of metacercariae exposed to freezing winter conditions has already been documented by Ollershen-shaw (1971a) in the United Kingdom; or (3) a combination of both factors.

In the Andean Patagonian valleys, cattle may not represent the only definitive host of *F. hepatica*, since sheep and wild hares (*Lepus europaeus*) and coypus (*Myocastor coypus*) occur in the region. In contrast to the large-scale production of bovines, exploitation of sheep varies according to the economic situation, and they were seldom reared during the study period. On the other hand, naturally infected hares were found in very low prevalences (0.08%), but they appear to play a secondary role in the transmission of *F. hepatica* because of their high abundance (Kleiman et al., 2004b). The potential reservoir role of coypus and other wild species presumably undetected during the study still remains unknown. In fact, *M. coypus* and other wild rodents were reported to be hosts of the parasite elsewhere (Ménard et al., 2001).

The relevance of local studies is ascertained by the fact that variation in *F. hepatica* prevalence among sites may be masked when data are averaged over an extensive region. For example, official records for the period 1998–2001 reported prevalences of about 9% for the whole Chubut Province (SENASA), while we obtained a value of 50% for the Andean Patagonian valleys. The present work is the first longitudinal study on fasciolosis in Argentina based on an epidemiological approach. Previous research performed in Argentina has only focused either on cattle prevalence (for example, Dwinger et al., 1982) or on snail population dynamics and infection rate (Venturini and Fonrouge, 1985; Prepelitchi et al., 2003; Cucher et al., 2006), except for a focus study from a human case (Rubel et al., 2005). In addition, different regions show different epidemiological scenarios, but local anthelmintic schemes emulate those of the major traditional cattle-rearing area located in the central part of the country, or drenching is carried out at the convenience of the producer. Even worst, ecological management is often ignored notwithstanding the general consensus on the benefits of integrated programs (Roberts and Suhardono, 1996).

Our results may indicate the presence of suitable environments for the development of lymnaeid snails all over the valley, making it difficult to find “safe” grazing sites for cattle. Thus, a preventive measure would be to minimize faecal egg output before animals are allowed to graze in a paddock with high snail abundance. The repeated use of this scheme may lead to a decrease in pasture contamination and, consequently, to a reduced risk of cattle infection. The design of programs for the control of fasciolosis requires the knowledge of when and where the risk of infection is highest. Particularly in the Andean Patagonian valleys, there would be two seasonal transmission peaks, a major one between summer and autumn and a minor one in early spring. A first treatment in summer would prevent clinical fasciolosis, and a second one before the stressing winter period would improve the sanitary condition of animals. In addition, both lagoons and temporary freshwater environments appear as transmission foci, with the former showing higher snail abundances in autumn and the latter in spring, while streams may act as dispersal corridors for infected snails. Hence, a parasitological evaluation in early spring, when the herd returns to the valley, would be useful to determine the need for a third treatment before allowing cattle to graze in temporary environments, the most representative environments in the region. When this is not feasible, it is recommended to minimize the number of bovines or time spent in a paddock with a mallín.
Besides the treatment schedule, the class of drug and dosing must also be considered to obtain encouraging results. In the Andean Patagonian valleys, drugs should be selected in relation to the time when animals are expected to harbor adult parasites only (in early spring, unless the parasitological examination is negative) or both adults and juveniles (in summer and autumn). In regard to dosing, observations made in the field indicate that farmers first estimate the mean herd weight roughly by sight and then calculate a single dose based on such value. Thus, animals exceeding the mean weight receive a subtherapeutic dose.

This study provides information that can serve as a basis for the planning of fasciolosis control in the Andean Patagonian valleys and contributes to the knowledge of its epidemiology at the southern distribution range of _F. hepatica_.

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