Prevalence, economic analysis and chemotherapeutic control of small ruminant fasciolosis in the Sargodha district of Punjab, Pakistan

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Economic losses, Fasciolosis, Levamisole, Oxyclozanide, Triclabendazole, Pakistan, Punjab, Sargodha.

Summary
This study describes the epidemiology, the economic significance of small ruminant fasciolosis in animals slaughtered in the abattoirs of the Sargodha district, Punjab, Pakistan between January and June 2012. *In vivo* fasciolicidal efficacy of commercially available compounds was examined using a randomised complete block design. Microscopically screened faecal samples revealed 40.51% positive animals for fasciolosis. The prevalent species included *Fasciola hepatica* (35.64%) and *Fasciola gigantica* (8.21%). Mixed infections were noted in 3.33% subjects. Prevalence rates were significantly higher in females (42.25%) than in males (39.52%; $p < 0.05$), and in adults (51.20%) compared to younger animals (33.98%; $p < 0.05$). The disease was recorded more often in emaciated animals (63.63%) followed in order by average (43.45%), thin (43.22%), and fat (32.12%) animals. Between January and June 2012, fasciolosis in Sargodha district, Punjab, Pakistan, was estimated to incur US$0.036 million and US$0.177 million direct (liver condemnation) and indirect (carcass depreciation) economic losses, respectively. *In vivo* fasciolicidal efficacy of oxyclozanide proved to be the most effective method of control, compared to triclabendazole, and levamisole. Results provide useful information on the frequency distribution of fasciolosis and its economic significance. Finally, data on *in vivo* fasciolicidal trials show that oxyclozanide is the most efficacious compound for the treatment of the disease in the district Sargodha, Punjab, Pakistan.

Prevalenza, analisi economica e controllo chemioterapico della fasciolosi in piccoli ruminanti nel distretto di Sargodha, Punjab, Pakistan

Parole chiave
Fasciolosi, Levamisolo, Ossiclozanide, Pakistan, Perdite economiche, Punjab, Sargodha, Triclabendazolo.

Riassunto
Lo studio prende in esame l'epidemiologia e l'impatto economico della fasciolosi nei piccoli ruminanti macellati tra gennaio e giugno 2012, nel distretto di Sargodha, Punjab, Pakistan. L'efficacia degli antielmintici in commercio è stata esaminata *in vivo* usando uno schema a randomizzazione completa. All'analisi microscopica dei campioni fecali, il 40,51% degli animali è risultato positivo alla fasciolosi. Le specie prevalenti sono state *Fasciola hepatica* (35,64%) e *Fasciola gigantica* (8,21%). Il 3,33% degli animali è risultato positivo per entrambe le infezioni. I tassi di prevalenza rilevati sono stati significativamente superiori nelle femmine (42,25%) rispetto ai maschi (39,52%) ($p < 0,05$) e negli esemplari adulti (51,20%) piuttosto che in quelli più giovani (33,98%, $p < 0,05$). La prevalenza registrata è stata più alta negli animali deperiti (63,63%), seguita nell'ordine da quella riscontata negli animali dalla corporatura media (43,45%), magra (43,22%) e grassa (32,12%). Nel periodo compreso tra gennaio e giugno 2012, i danni economici causati da fasciolosi nell'area oggetto di studio sono stati di US$ 0,036 milioni (perdite dirette: danneggiamento del fegato) e US$ 0,177 milioni (perdite indirette: minusvalenze a carico della carcassa). I risultati presentati forniscono informazioni utili sulla frequenza di distribuzione della

fasciolosi e sul conseguente impatto economico. Infine, i dati relativi allo studio *in vivo* dimostrano che l'ossiclozanide risulta essere il composto più efficace per il trattamento della malattia nel distretto di Sargodha, Punjab, Pakistan.

Introduction

Of the parasitic diseases, helminth infections are the most common and the most economically significant diseases among the production limiting factors affecting grazing ruminants worldwide (Perry and Randolph 1999). Among these, fasciolosis (hepatic distomatosis), caused by *Fasciola hepatica* and *Fasciola gigantica*, is one of the most significant and debilitating liver damaging diseases of ruminants worldwide. It has been estimated that about 6-7 hundred million ruminants are at risk of fasciolosis around the world (Mas-Coma *et al.* 2009). This disease can cause blood loss (between 0.2 ml and 0.5 ml of blood per fluke per day), reduced appetite, nitrogen retention, and impaired energy metabolism due to liver damage, reduction in weight gain and reduced milk production in animals. Damage of organs and other biochemical changes are due to the migratory and hematophagic activities of *Fasciola* (Dargie *et al.* 1979). Fasciolosis has been reported to cause a reduction in the protein and lipid contents of the infected livers (Anderson *et al.* 1977). Mortality is rare and usually only occurs in untreated animals. This disease is also emerging as a disease of public health significance (McManus and Dalton 2006). In Pakistan, variable prevalence of fasciolosis has been reported ranging from 0.66% to 40.31% in different districts of the Punjab Province (Maqbool *et al.* 2000, Ahmed *et al.* 2005, Tasawar *et al.* 2007, Asif *et al.* 2008, Ijaz *et al.* 2009, Khan *et al.* 2009). Furthermore, acute fasciolosis is more likely to occur than sub-acute and chronic forms in small ruminant population (Maqbool *et al.* 2000), this might be due to higher levels of pasture contamination, high density of small ruminants stocking in Pakistan, and lack of appropriate preventive measures.

Economic losses attributable to fasciolosis were estimated in Bangladesh between October 2007-September 2008 at approximately US$3 billion in terms of animal weight loss, declined fertility rate, and reduced milk yield (Hossain *et al.* 2011). In developing countries such as Pakistan, a higher proportion of the rural population (65.2%) receive a major share of their income from livestock[1], this further increases the importance of helminth infections. It is very difficult to control the disease and eradication is impossible as the parasite can survive in the host, snail, and environment for long periods. In addition to good farm management, it is imperative to exercise strategic anthelmintic therapy in order to maintain parasitic diseases at an acceptable level (Boray 2005). However, over-the-counter availability of drugs, low-quality brands, and an increased ratio of quackery lead to inappropriate drug administration (Ahmad M., personal observation), which can in turn reduce efficacy of available fasciolicidal compounds. Our study, therefore, was designed with the following goals:

- determine the prevalence of fasciolosis in the Sargodha district (Punjab Province, Pakistan);
- estimate economic losses attributable to small ruminant fasciolosis in the district;
- compare the *in vivo* chemotherapeutic efficacy of commercially available fasciolicides in 1 of the most heavily infected districts (Sargodha) of the Punjab Province in Pakistan for other animal species (Khan *et al.* 2009).

Materials and methods

Study area

The Sargodha district in Punjab Province, Pakistan is situated 32°08′00″N latitude and 72°67′00″E longitude. It lies between 100-200 m above sea level and is bound in the West and East by the Jhelum and Chenab Rivers, respectively. The soil of district Sargodha contains alluvial deposits which are more than 300 m thick and the mean Summer temperature is 41°C; whereas, in winter it decreases to as low as 6°C. The rainfall ranges from 3 mm to 84 mm, with an average of 26.75 mm. Its geographic location and climatic conditions make the area marshy favouring the propagation of snails, the intermediate host of this disease.

Sample size, animal data and coprological examination

Small ruminants (n = 390), including goats (n = 240) and sheep (n =150) brought in for slaughter in the

[1] Population Census Organization 2008. District Census Reports. Statistics division, Ministry of Economic Affairs and Statistics, Government of Pakistan, Islamabad, 612 pp.

local abattoirs of district Sargodha, were randomly selected and tagged for identification. Data, such as species, gender and age of animal, were recorded on a pre-designed form. Faecal samples of the animals were collected directly from the gastrointestinal tract in appropriately labelled plastic bottles containing 10% formalin solution (1:3). The samples collected were shipped to the Department of Parasitology of the University of Agriculture in Faisalabad, Pakistan for further processing. The sample size was estimated on the basis of 50% expected prevalence (Pexp) and at a 95% confidence interval in accordance with the formulae described by Thrusfield (Thrusfield 2007).

The standard sedimentation technique (indirect coprological examination) was used to detect the *Fasciola* eggs (Zajac and Conboy 2006). Quantitative assessment of fluke burden, *i.e.* number of eggs per gram (EPG), was performed using the modified McMaster technique (Whitlock 1948). The eggs of *Fasciola hepatica* and *Fasciola gigantica* are operculated and similar in shape; however, eggs of *F. hepatica* are smaller (150 µm by 90 µm) than those of *F. gigantica* (200 µm x 100µm) (Soulsby 1982).

Assessment of economic losses due to small ruminant fasciolosis

Direct economic losses due to liver condemnation caused by small ruminant fasciolosis were assessed by considering the overall prevalence of the disease in the abattoir and the retail market price of an average liver. Indirect economic losses were assessed by estimating the reduction in carcass yield of animals due to small ruminant fasciolosis. Direct and indirect economic losses due to fasciolosis were calculated over a period of 6 months (January to June 2012). Data on small ruminants slaughtered during this period were collected from the local municipal authorities. Direct and indirect economic losses were calculated using the procedure described by Ogunrinade and Ogurinade (Ogunrinade and Ogurinade 1980) explained below:

$$\text{Direct economic losses due to liver condemnation (LC)} = \text{CSR} \times \text{LC}_o \times p$$

Where:
LC = losses due to liver condemnation;
CSR = average number of animals slaughtered at abattoir during the study period;
LC_o = average cost of one liver in district Sargodha;
p = prevalence of the fasciolosis at the study abattoir.

Indirect economic losses were estimated as follows:

$$\text{CW} = \text{CSR} \times \text{CL} \times \text{MC} \times p \times (\underline{n})\ \text{kg}$$

Where:
CW = losses due to carcass weight reduction;
CSR = average number of animals slaughtered at abattoirs during the study period;
CL = carcass weight loss (10%) in individual animal due to fasciolosis;
MC = average price for 1 kg meat in district Sargodha, Punjab, Pakistan;
p = prevalence of fasciolosis in the study abattoir;
($\underline{n}$) kg = average carcass weight of animal.

In vivo fasciolicidal trials

The clinical trial to evaluate fasciolicides was conducted at the Livestock Experimental Station Khizraabad, Sargodha district, Pakistan in accordance with the recommendations of the World Association for Advancement of Veterinary Parasitology (WAAVP) and following the procedures published by Coles and colleagues (Coles *et al.* 2006) and Khan and colleagues (Khan *et al.* 2009). Confirmed positive female Kajli sheep of the same age (1.25 years) and similar mean EPG values were classified into 4 groups (A to D, n = 25 each) using randomised complete block design (RCBD). Animals belonging to group A were treated with normal saline as placebo and those in groups B, C, and D were treated with triclabendazole (10 mg/kg^{-1}), levamisole (10 mg/kg^{-1}), and oxyclozanide (16.6 mg/kg^{-1}), respectively. The post-treatment faecal egg count reduction test (FECRT) was used (Coles *et al.* 1992). Briefly, EPG was calculated on days 0, 7, 14, and 28 to compare the reduction in egg count against the initial reading and to measure the drug efficacy (E). The drug was considered effective if the result of the FECR exceeded 95% and the lower limit of the 95% confidence interval was greater than 90% as described in the WAAVP guidelines (Wood *et al.* 1995).

The following formula for FECR (%) was used:

$$\text{FECR (\%)} = \frac{\text{Pr-T EPG–Po-T EPG} \times 100}{\text{Pr–T EPG}}$$

Where:
FECR = faecal egg count reduction;
Pr-T EPG = pre-treatment EPG;
Po-T EPG = post-treatment EPG.

Statistical analyses

Microsoft Excel 2010 was used to calculate the descriptive statistics. Data collected on the age, gender, species, and body condition of each animal were transferred to Stata version 8.0 (Stata Corporation, Texas, USA). The analysis of variance (ANOVA) method was used to calculate *in vivo* efficacy of fasciolicides. The interpretation of results was performed using a 95% confidence interval.

Results

Of total 390 faecal samples, 158 (40.51%) revealed the presence of *Fasciola* eggs, which were detected

using conventional sedimentation techniques. The prevalence rate of *F. hepatica* (35.64%) was significantly higher ($p < 0.05$) than *F. gigantica* (8.21%); 3.33% animals showed mixed infections. The prevalence of fasciolosis was significant ($p < 0.05$) with respect to the age of the animals. Younger animals (70/206; 33.98%) were less prone to infection in comparison to adult animals (85/166; 51.20%). Fasciolosis was significantly higher ($p < 0.05$) in females (42.25%) than males (39.52%). The influence of breed on the occurrence of infection was found to be non-significant ($p < 0.05$). It was observed in the study area that sheep (42.2%) were more prone to fasciolosis than goats (39.58%), which might be attributable to many factors related to host or the environment. There was a significant relationship between animal body condition and disease prevalence ($p < 0.05$). Vulnerability to the disease was highest in emaciated animals (63.63%), followed in order by average (43.45%), thin (43.22%). and fat (32.12%) animals.

During the period under study, 85.700 small ruminants were slaughtered in the abattoirs in the 6 tehsils (administrative divisions) of Sargodha district. The prevalence of fasciolosis was 40.51% and the average value of liver (n = 1) and meat (per kg) were Rs 100 (US$1.06) and Rs 400 (US$4.25), respectively. The average carcass weight of slaughtered animals was approximately 12 kg. Direct and indirect economic losses over a period of 6 months were estimated at US$0.036 and US$0.177 million, respectively.

The FECRT for the candidate anthelmintics showed significant ($p < 0.05$) reduction in EPG of treated groups compared to controls (Table I). The reduction of EPG was significantly higher ($p < 0.05$) on days 14 and 28 post treatment than on day 7. The highest mean post-treatment reduction in EPG on day 28 post treatment ($p < 0.05$) was noted in the group treated with oxyclozanide, followed in order by the group treated with triclabendazole, and levamisole.

Discussion

Our results indicated a higher prevalence compared to other studies conducted in various parts of the world (Abunna *et al.* 2010, Alves *et al.* 2011, Hassan *et al.* 2011). Likewise, the prevalence rate was higher than the one previously reported in local surveys of small ruminant fasciolosis (range 0.66%-33.66 %) concerning other localities within the Punjab Province (Maqbool *et al.* 2000, Ahmed *et al.* 2005, Tasawar *et al.* 2007, Asif *et al.* 2008, Ijaz *et al.* 2009). These prevalence differences might be attributed to variations in agro-ecological conditions, such as altitude, rainfall, temperature, livestock management systems, and suitability of the environment for the survival of the parasite, as well as the availability of the intermediate host for the successful propagation of disease. Also relatively lower prevalence rates of this disease, compared to the prevalence rate described in this article, have been reported in different parts of the world (Ibarra *et al.* 1998, Kleiman *et al.* 2005, Molina *et al.* 2005, Mazid *et al.* 2006, Paz-Silva *et al.* 2007). Among age groups, our findings are concordant with other results (Hassan *et al.*, 2011, Abdulhakim and Addis 2012). The higher infection rate in older animals is likely due to the long life-cycle of this parasite and to the increased exposure of the animals. In contrast to our findings, Tasawar *et al.* (2007) reported a higher prevalence rate of fasciolosis in younger animals compared to adults that might be attributable to an acquired self-cure phenomenon (Ross 1967). It is recognised that the gender of the host influences the prevalence of small ruminant fasciolosis. Higher prevalence trends of fasciolosis in females have been well established (Ahmed *et al.* 2005, Mazid *et al.* 2006, Asif *et al.* 2008, Ijaz *et al.* 2009). However, these trends differ from the findings reported by Maqbool and colleagues (Maqbool *et al.* 2000) and Waruiru and colleagues (Waruiru *et al.* 2000), where gender was a non-significant determinant for the occurrence of disease. During our investigation, we observed that most of the older female goats were brought to the abattoir and this may have changed

Table I. In vivo *comparative efficacy (%) of the commercially available fasciolicides in sheep (*Ovis aries*) naturally infected with* Fasciola hepatica *in the Sargodha district, Punjab, Pakistan between January and June 2012.*

Group	Mean EPG ± SE of *Fasciola hepatica* (% efficacy of drugs)				Mean ± SE
	0	7	14	28	
Control	320±28.3^{a} (00)	308±27.0^{a} (00)	296±19.6^{a} (00)	280±20.8^{a} (00)	301.0±12.0^{A}
Triclabendazole	312±26.0^{a} (2.5)	140±16.3^{bc} (54.5)	48±11.7^{cde} (83.78)	56±14.2^{cde} (80.0)	139.0±13.8^{BC}
Levamisole	332±25.6^{a} (3.7)	160±23.8^{b} (46.25)	76±15.6^{b-e} (74.32)	96±15.8^{b-e} (65.67)	166.0±14.4^{B}
Oxyclozanide	308±20.8^{a} (3.7)	112±19.4^{bcd} (63.63)	36±09.8^{de} (87.84)	12±6.63^{e} (95.71)	117.0±13.9^{C}
Mean ± SE	318±12.5^{A}	180±13.3^{B}	114.0±12.9^{C}	111±12.7^{C}	

Means sharing similar superscript letters in a row or in a column are statistically non-significant (P > 0.05). Small superscript letters represent comparison among interaction means and capital superscript letters are used for overall mean.

the physiological state that creates stress in animals (Selim *et al.* 1997).

Host species is an important factor for disease occurrence. Some species are more likely to become infected in comparison to other species due to their eating habits. Normally, helminth infection is greater in sheep than in goats due to different grazing habits, *i.e.* sheep are low grazers, whereas, goats browse rather than graze (Soulsby 1982). A comparatively higher prevalence rate of small ruminant fasciolosis in sheep than goats has been well-documented (Asif *et al.* 2008, Ijaz *et al.* 2009, Kifle and Hiko 2011, Abdulhakim and Addis 2012). However, a higher prevalence rate of fasciolosis has also been reported in goats compared to sheep (Ahmed *et al.* 2005, Mungube *et al.* 2006, Yadav *et al.* 2008, Ahmadi and Meshkehkar 2010). Similar to what we report here, other studies have described higher prevalence rates of fasciolosis in animals with poor body condition, in comparison to animals in average and good body condition (Abdulhakim and Addis 2012, Hassan *et al.* 2011).

To our knowledge, no investigation has been previously conducted concerning the economic losses due to fasciolosis in Pakistan. Our estimated economic losses are supported by the analysis provided by Hossain and colleagues (Hossain *et al.* 2011), while Kifle and Hiko (Kifle and Hiko 2011) documented even higher economic losses due to fasciolosis; Selim and colleagues (Selim *et al.* 1997) estimated losses of 7.54% corresponding to US$ 28.86 per thousand livers of slaughtered goats.

The prevalence of liver condemnations due to fasciolosis decreased from 7.37%, 1.80%, and 4.41% in 1999-2000 to 4.64%, 1.12%, and 2.80% in 2007-2008 for cattle, sheep, and goats, respectively (Ahmadi and Meshkehkar 2010). Direct and indirect economic losses, up to US$0.036 and US$0.177 million, respectively, should not be under-estimated in the context of a developing country like Pakistan.

Oxyclozanide gave promising results for the treatment of this infection confirming other studies (Khan *et al.* 2009, Olaechea *et al.* 2011). The relatively lower efficacy of triclabendazole and levamisole could be due to the development of resistance, as these two anthelmintics have been used frequently in the study area (Ijaz *et al.* 2009). The development of anthelmintic resistance in field animals is due to the use of drugs as hit and trial method against parasitic infection, inadequate dose level, low protein diet, environmental toxicity, and poor efficacy of anti-parasitic agent (Smith and Sherman 2009). The probable contributing factors to reduced effectiveness of anthelmintics in general, and fasciolicidal compounds in particular, might include the following: (i) the over-the-counter availability of the drugs, (ii) low-quality brands, and (iii) increased quackery, which can lead to inappropriate drug administration.

Fascioliasis was found to be a highly prevalent and economically important disease of small ruminants in the districts of Sargodha, Punjab, Pakistan. The observed level of infection suggests the existence of suitable climatic conditions for the development and survival of the parasite in the area of the study animals. In the present circumstances, using less efficacious compounds will further increase the economic burden on smallholder farmers. Consequently, periodic monitoring of the compounds is highly recommended and should be accompanied to the implementation of all the relevant preventive strategies to minimise the damages.

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