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REGULAR ARTICLES

Cross-sectional prevalence of *Fasciola gigantica* infections in beef cattle in Botswana

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Abstract
A cross-sectional study was carried out to determine the prevalence and distribution of *Fasciola gigantica* infections in communally grazed and ranch-grazed beef cattle through coprological examination. A total of 8646 cattle (4618 adults, 2843 weaners and 1185 calves) faecal samples were collected during the 24 months of study. Results from this study indicated that only 64 (0.74%; 95% CI 0.57, 0.94%) of the samples were positive for *F. gigantica* eggs. The positive samples were detected in one (Central) of the six study districts and was restricted to the Tuli Block (commercial) farms in Machaneng village in eastern Botswana. The prevalence of fluke eggs was significantly higher in adult cattle (12.85%; 95% CI 9.72, 16.54%) than weaners (6.49%; 95% CI 3.40, 11.06%) and calves (0.79%; 95% CI 0.02, 4.31%), ($\chi^2(2) = 19.01$, $p < 0.001$). Thus, adults (OR = 18.57; 95% CI 2.54, 135.81%) were approximately 20 times more likely to be infected than calves. By taking liver inspection as a gold standard for diagnosis of fasciolosis, the sensitivity of the sedimentation technique was found to be 72.41% and the specificity 100%, with moderate relationship ($\kappa = 0.53$; 95% CI 0.31–0.75) between the two methods. This study has demonstrated that infection of cattle from Botswana, with *F. gigantica*, was low and distribution of the fluke appeared to be linked to suitable environmental conditions for the intermediate host snail. However, detailed surveillance studies, involving more cattle and areas, are required to verify the true prevalence. Such information would assist in the design of appropriate, strategic and effective fluke control programmes.

Keywords Beef cattle · Botswana · Coprology · *Fasciola gigantica* · Prevalence

Introduction

Fasciolosis has been recognised as one of the most important parasitic diseases in tropical countries limiting the productivity of ruminants, in particular cattle (Keyyu et al. 2005b). The prevalence of the disease in cattle has been found to vary depending on a number of environmental and management factors. The disease occurs in areas where environmental conditions, typically low swampy or marshland areas, exist for the survival and proliferation of the snail intermediate host (Pfukenyi et al. 2006; McGavin and Zachary 2007). Such suitable conditions exist in the north-western region of Botswana, where the disease is believed to be endemic.

Infection with the tropical fluke, *F. gigantica*, is regarded as one of the most important helminth infections of ruminants in Asia and Africa (Harrison et al. 1996; Roberts and Suhardono 1996; Wamae et al. 1998) due to its wide distribution and spectrum of definitive hosts (Rondelaud and Abrous 2001). It is considered a major source of production losses in domestic ruminants (Mage et al. 2002), since even subclinical infections can result in reduced feed efficiency, poor weight gains, decline in milk production, poor reproductive performance, poor carcass quality, reduced work output in draught animals and condemnation of livers at slaughter (Pfukenyi et al. 2006). Infection with *Fasciola* can also be a predisposing factor to other livestock infections, such as infectious necrotic hepatitis and salmonellosis (Ogunrinade and Ogunrinade 1980).

The epidemiology and economic losses of fasciolosis are well known in most countries, with the prevalence of *F. gigantica* infection well documented in many tropical

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countries in Africa (Mzembe and Chaudhry 1981; Tembely et al. 1988; Kithuka et al. 2002; Keyyu et al. 2005b; Phiri et al. 2005a; Phiri et al. 2005b; Pfukenyi et al. 2006). However, there are no well-authenticated reports on the prevalence and possible impact of fasciolosis in cattle in Botswana. The existing data on the disease are based on a few laboratory reports in some areas of the country and cover only a few months of the year. The objective of the present study was to determine the prevalence and distribution of *F. gigantica* in cattle in Botswana.

Materials and methods

Study location

The study was undertaken in six of the nine districts of Botswana (Southern, Southeast, Kweneng, Kgatleng, Central and Northeast districts—Fig. 1). These districts are located between two villages at the extreme ends of the country, Ramatlabama in the south, bordering South Africa, and Ramokgwebana in the north-east, bordering Zimbabwe. The study area was located between latitude 20° 36′ 38″ and 25° 39′ 55″ S and longitude 25° 34′ 23″ and 27° 36′ 50″ E. The districts were included in the study owing to their topographical features and the prevailing weather conditions. These areas are characterised by hills, valleys, rivers, streams, drainage depressions, dams (natural and man-made), lakes and swampy or marshland areas. These rivers, streams, dams, lakes and marshlands serve as watering places for livestock.

Dip tanks and crushes, used as handling facilities for livestock, and boreholes, used as water sources, were chosen as study sites. Cattle are regularly taken to the crushes for husbandry practices such as dipping, deworming, branding and loading/off-loading for transportation as well as for individual or national livestock census, mass (national) vaccinations throughout the year, and to the boreholes for drinking water, especially during the dry season. A few crushes/dip tanks and boreholes were randomly selected from each of the six study districts.

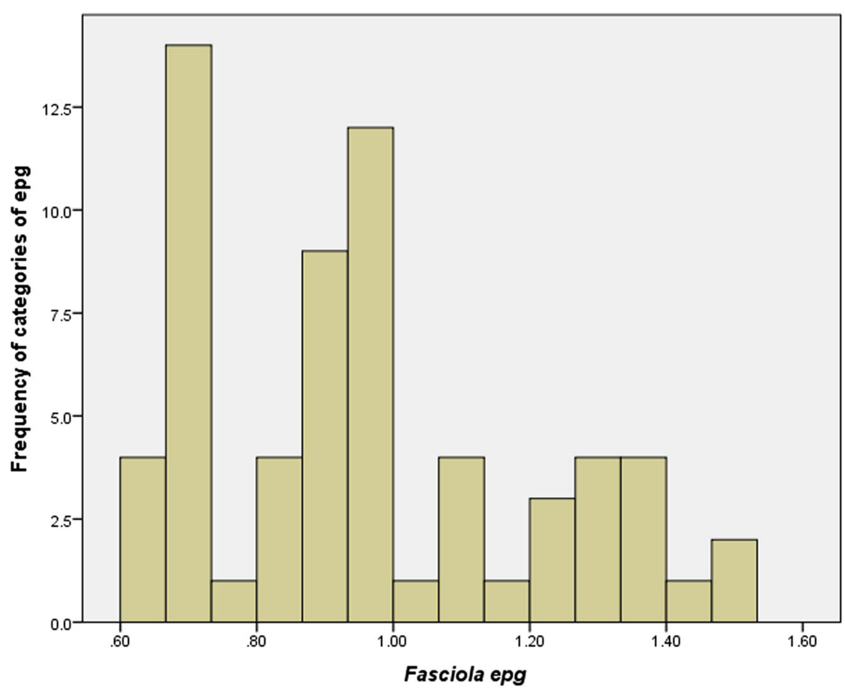
Study animals

The breed of the sampled animals was recorded, and included Brahman, Simmental, Charolais, Limousin, Hereford, Tuli, the native Tswana, Nguni and their crosses. Cattle were categorised into three age groups such as calves (≤ 12 months old), weaners (> 12 to ≤ 24 months old) and adults (> 24 months old) and were further subdivided into male and female animals.

Cattle management systems

Cattle were managed under the traditional or communal and commercial or ranch beef cattle systems. In the traditional, commonly referred to as the '*cattle post*' system in Botswana, farmers kept small herds of between five and 50 animals per herd. Animals were grazed on unfenced tribally administered communal rangeland and watered at central watering points such as hand dug wells, dams, boreholes and rivers. Cattle-grazed natural pastures during both the wet and dry seasons, and supplementation was rarely undertaken.

Fig. 1 Infection intensity (epg of faeces) of *F. gigantica* at Tuli Block farms in Machaneng



Crop residues, available after harvesting, were grazed in situ rather than harvested as feed for livestock during the dry season, when natural pastures deteriorated both in quantity and quality. Livestock were allowed to wander freely over the grazing land during the day but were kraaled close to the households at night. Some farmers practised crop production in small fields in addition to livestock production. The traditional cattle herds were given little or no effective disease control, apart from the annual national vaccinations conducted by the government. The common breeds kept were the Brahman, Simmental and Tswana, and their crosses.

The commercial farmers raised their cattle on leasehold or freehold fenced large grazing lands or ranches, and had far more cattle (hundreds to thousands) than the traditional farmers. The sector adopted an improved management system, with supplementary feeding of livestock whenever necessary. Although cattle still used the extensive rangelands, most farmers are wealthy and can afford to buy either locally or imported feed resources, either roughage or concentrates. Animals were let out onto pasture to graze and could roam for several days before returning to drink. Cattle in the commercial sector were also kraaled regularly, but not handled frequently as in the traditional sector, unless some husbandry practices had to be carried out. In some farms, intensive livestock production systems, such as feedlotting, were practised. Disease control was much better in the commercial sector than in the traditional sector. The majority of cattle kept on commercial farms were exotic beef cattle reeds, Tswana and Nguni, and their crosses.

Selection of farms and sampling of animals

The study was an observational cross-sectional study conducted in six districts across the country. A stratified random sampling method (proportional to size) was used to select animals from districts, villages, farms, management systems, age, gender and breed groups. Farms in each district or village were categorised as either traditional or commercial. Animals on each farm were categorised as calves (≤ 12 months), weaners (> 12 to ≤24 months) or adults (> 24 months). The survey covered the period from June 2011 to May 2013. The total number of animals sampled was 8646.

Faeces were collected per rectum from cattle in a crush, using a gloved hand, and then placed into 40-g universal bottles, labelled, put in a cooler box and transported to the Veterinary Parasitology Laboratory at the Botswana University of Agriculture and Natural Resources in Gaborone. The presence of *F. gigantica* eggs was determined quantitatively by the standard sedimentation technique (Foreyt 2001; Zajac and Conboy 2006). The eggs of *F. gigantica* were distinguished from those of *Paramphistomum* species on the basis of their colour. *Fasciola* eggs are yellow-brown and those of *Paramphistomum* are colourless (Taira et al. 2003; Zajac and Conboy 2006).

Sensitivity and specificity of the faecal examination method

The sensitivity and specificity of the method was computed by taking liver inspection at postmortem as the gold standard for the diagnosis of fasciolosis. Cohen's kappa (κ) statistic was used to determine the degree of agreement between the two methods of liver fluke diagnosis. The kappa value was interpreted as described by Dohoo et al. (2009).

Statistical analysis

The prevalence of *F. gigantica* was calculated along with their 95% confidence intervals for each sampling site and group of animals (Thrusfield 2003; Dohoo et al. 2009). Data obtained for the prevalence of bovine fasciolosis were entered and validated in a Microsoft Excel 2007 (for Windows) spreadsheet and later transferred into the IBM Statistics Programme for Social Sciences (IBM SPSS) version 21.0 for Windows (IBM Corporation, Somers, New York, USA) for analysis.

The proportion of animals with *Fasciola* eggs in the faeces was compared between geographic location (district of origin), age, gender and breed using Pearson's Chi-square (χ^2) test for independence and odds ratios (OR) and their 95% CI were calculated to identify risk factors for infection. The effect of age, gender and breed on transformed faecal egg counts [Log_{10} (egg count + 1)] were analysed using analysis of variance (ANOVA). The intensity of infection was based on the number of eggs per gram (epg), and classified into three levels, namely *low intensity*: egg count ≤ 10 epg, *moderate intensity*: egg count between 10 and 25 epg and *high intensity*: egg count ≥ 25 epg. In all analyses, the statistical level was considered significant if $p < 0.05$.

Results

The overall prevalence of *F. gigantica* infection was 0.74% (95% CI 0.57 to 0.94%) with only 64 animals positive for *Fasciola* eggs from a total of 8646 cattle (4618 adults, 2843 weaners and 1185 calves) faecal samples examined during the 24 months of study. Of the 8646 cattle sampled, 7052 originated from communal farms and 1594 from commercial farms. The prevalence according to district of origin was as follows: Central, 2.34% (95% CI 1.81, 2.98%); Kgatleng, 0% (0.00, 0.29%); Kweneng, 0% (0.00, 0.33%); North-east, 0% (0.00, 0.77%); South-east, 0% (0.00, 0.25%) and Southern, 0% (0.00, 0.23%). The disease was found in only one (Central district) of the six districts studied, and was restricted to only one (Machaneng) of the 25 villages sampled, with all the positive animals originating from a cluster of commercial farms in the Tuli Block area, east of the village. A total of 709 cattle faecal samples were obtained from these farms, giving a prevalence of 9.03% (95% CI 7.02, 11.38%) in this study area.

The prevalence, as indicated in Table 1, was significantly higher in adults (12.85%; 95% CI 9.72, 16.54%) than in weaners (6.49%; 95% CI 3.40, 11.06%) and calves (0.79%; 95% CI 0.02 to 4.31%), (χ^2 (2) = 19.01, $p < 0.001$). By calculating odds ratios, it was clearly demonstrated that infection with *F. gigantica* was more common in adult cattle (OR = 18.57; 95% CI 2.54, 135.81%) and weaners (OR = 8.74; 95% CI 1.12, 68.09) than calves.

Female cattle (7.76%; 95% CI 5.90, 9.98%) had a significantly higher prevalence than males (1.27%; 95% CI 0.58, 2.40%), (χ^2 (1) = 9.73, $p = 0.002 < 0.05$). Females were three times more likely to be infected than males (OR = 3.01; 95% CI 1.46, 6.21). When gender were separated into individual age categories, only adult females showed a significantly higher prevalence than males (χ^2 (1) = 4.28, $p = 0.04 < 0.05$) while weaners and calves did not show significant gender differences, (χ^2 (1) = 1.27, $p = 0.26 > 0.05$) and (χ^2 (1) = 0.77, $p = 0.38 > 0.05$), respectively.

A comparison of the prevalence among different cattle breeds (Table 2) revealed that the pure Brahman (8.33%; 95% CI 5.56, 11.89%) and Brahman crosses (12.80%; 95% CI 9.18, 17.21%) were positive for *F. gigantica* eggs. In contrast, the Nguni breed was not infected (0%; 95% CI 0.00, 3.77%), and this was significantly lower than that of the Brahman and Brahman crosses (χ^2 (2) = 14.73, $p = 0.001 < 0.05$).

The intensity of infection based on egg per gram (epg) of faeces, regardless of age, gender or breed, showed that almost all cattle examined (97.18%; 95% CI 95.68, 98.27%) had either no or low infection level. Only a few (2.40%; 95% CI 1.40, 3.81%) had a moderate infection and a negligible number of animals (0.40%; 95% CI 0.09, 1.23%) had severe infections, with a mean egg count of 0.98 ± 0.14 epg. Of the 64 *Fasciola* spp. egg-positive animals, the majority (44–68.75%) had a low count, ≤ 10 epg, 17 (26.56%) had a moderate infection (> 10≤ 25 epg) and only three (4.69%) had evidence of high infections (> 25 epg) (Table 3 and Fig. 1).

Discussion

The 0.74% prevalence determined in the present study has indicated that although liver fluke infection occurs in beef cattle in Botswana, it was only present in a few animals amongst the sampled herds and not as widespread throughout the country as anticipated. This is the first systematic report of infection with *Fasciola* in the areas covered in this study in Botswana. Prior to this study, it was believed that infection was widely distributed in the country, albeit based on a few unsubstantiated records (Department of Veterinary Services 2008). These results, therefore, indicate that the geographical distribution of *F. gigantica* infection is narrower than previously envisaged, being prevalent in only one of the six study districts, and localised within the Tuli Block area in Machaneng village, in the eastern margin of the country. This is due to the fact that the Central district has larger permanent water bodies than most districts in Botswana where it is relatively drier. The prevalence of 0.74% (2.34% in the Central district and 9.03% in the Tuli Block) was significantly lower than that reported from neighbouring countries including Malawi, Zambia and Zimbabwe, which ranged in prevalence from 15 to 37% (Mzembe and Chaudhry 1981; Phiri et al. 2005b; Pfukenyi et al. 2006).

The occurrence of fasciolosis in cattle in different areas around the world is influenced by a variety of factors (Maqbool et al. 2002) including hosts, parasites, environmental and climatic conditions, snail population and the choice of diagnostic techniques used to detect infection or in some situations the livestock management practices and prevalence may differ between continents, countries or even within a country (Yilma 2000; Keyyu et al. 2006; Yildrim et al. 2007; Copeman and Copland 2008; Abunna et al. 2010). During the 24 months of study, the mean annual rainfall recorded was less than 100 mm in all the areas studied, which was far below the normal annual average of 500 to 650 mm (Department of

Table 1 Age and gender-specific prevalence in Tuli Block farms in Machaneng village

Age class	Gender	No. examined		No. positive		Prevalence (%) (95% CI)	
Adult	Female	397	305	51	45	12.85 (9.72, 16.54)[a]	14.75
	Male		92		6		6.52[a]
Weaner	Female	185	127	12	10	6.49 (3.40, 11.06)[b]	7.87
	Male		58		2		3.44[b]
Calves	Female	127	55	1	0	0.79(0.02, 4.31)[c]	0
	Male		72		1		1.38[c]
Total	Female	709	487	64	55	9.03 (7.02, 11.38)	11.29
	Male		222		9		4.05

[a,b,c] Values with different superscript in a column are significantly different ($p < 0.001$)

Table 2 Breed-specific prevalence in Tuli Block farms in Machaneng village

Breed	Fasciola faecal result No. examined	No. positive	Prevalence (%) (95% CI)
Brahman	324	27	8.33 (5.56, 11.89)[a]
Brahman cross	289	37	12.80 (9.18, 17.21)[a]
Nguni	96	0	0 (0.00, 3.77)[b]
Total	709	64	9.03 (7.02, 11.38)

[a,b] Values with different superscript in a column are significantly different ($p = 0.001$)

Meteorological Services 2003). As a result, this low precipitation could have negatively affected the prevalence of infection with the fluke. The mean annual temperature, though, ranged from 12 to 26 °C, which was suitable for the proliferation of the intermediate host snail as well as development of the intra-molluscan stages of *F. gigantica* (Soulsby 1982; Taylor et al. 2007). However, temperature alone is not sufficient to support the development of *Fasciola* infection, with adequate moisture as an additional requirement for growth.

The Tuli Block, the only place where *Fasciola* infection was detected, is located on the fringes of the Limpopo River, which holds a large amount of water during the year and thus serves as a good source of drinking water for cattle in the area. Therefore, this study area presents prominent epidemiological significance due to its geographical location. The prevalence of fasciolosis observed in this study ecological zone of eastern Botswana is consistent with reports from other African countries with similar environmental conditions. In Kenya, Malawi, Tanzania, Zambia and Zimbabwe, the pattern of distribution of fasciolosis usually follows zones of high rainfall, high livestock density and wetland areas infested with the intermediate host snail (Bitakaramire and Bwangamoi 1969; Mzembe and Chaudhry 1981; Kithuka et al. 2002; Keyyu et al. 2005b; Phiri et al. 2005a; Phiri et al. 2005b; Pfukenyi et al. 2006). The study in Zimbabwe found that fasciolosis was more prevalent in high rainfall areas of the highveld than in drier ones in the lowveld (Pfukenyi et al. 2006) since high rainfall areas favour the development and survival of both the intermediate host snail and the intra-molluscan stages of the parasite (Malone et al. 1984; Malone et al. 1998; Yilma and Malone 1998; Yadav et al. 2007) and thus increase the likelihood of infection in livestock. This is further supported by recent reports in Britain where the emergence of fasciolosis in cattle was linked with high rainfall, which favoured the development of *Lymnaea truncatula* intermediate host and the free-living stages of *F. hepatica* (Pritchard et al. 2005).

Table 3 Intensity of infection with *F. gigantica* in different categories of cattle from the Tuli Block farms in Machaneng village

Animal category	Number infected Low (≤ 10 epg)	Moderate (> 10 ≤ 25 epg	High (> 25 epg)
Adult	35	14	2
Weaner	8	3	1
Calves	1	0	0
Total	44	17	3

It is a fact that large water bodies provide good sources of water for drinking by animals, but also increase the risk of infection (Ogunrinade and Ogunrinade 1980), and the high population of livestock in such areas helps to maintain the disease (Kithuka et al. 2002). In accordance, a high prevalence of fasciolosis was reported in wetter areas of Kenya where flood plains exist, allowing accumulations of large water masses which favour the survival of the intermediate host snail, *Lymnaea natalensis* (Bitakaramire and Bwangamoi 1969). Similar findings have been reported in Tanzania where the grazing of cattle in marshland areas exposed them to contaminated pastures (Keyyu et al. 2005b). These reports are in agreement with the findings of a similar study in Zambia, which showed that the occurrence of wetlands and high livestock density in grazing areas increase the risk of infection with *F. gigantica* (Phiri et al. 2005a). The present study, however, did not include the North-west district of Botswana, where the wetlands of the country, the Okavango delta, exist, due to the limitation of resources. The area is believed to be endemic for fasciolosis, and presumably high prevalence, owing to the marshy pastures, which serve grazing and drinking sources for livestock. These pastures are potential habitats for the snail intermediate host, and as a consequence are likely to increase the risk of infection with *F. gigantica*.

The prevalence of fasciolosis in West Africa varies widely according to the availability and distribution of the intermediate host snail (Schillhorn Van Veen et al. 1980) with higher prevalence recorded where favourable climatic conditions prevail (Mekroud et al. 2004). A study in Mali found a higher prevalence in cattle grazing the wet inland delta of the Niger river (Tembely et al. 1988) and similarly in Nigeria, a higher prevalence was attributed to the time of increased cattle water contact (Schillhorn Van Veen et al. 1980). In East Africa (Ethiopia,) the prevalence of bovine fasciolosis tends to differ with locality, depending on climatic and ecological conditions

(Yilma 2000; Abunna et al. 2010). Other parts of the world reported higher prevalence in wetter countries with mild temperatures (Dorchies 2006) and that the disease has become endemic in lowland areas with extensively seasonally flooded grazing pastures (Rangel-Ruiz et al. 1999; Faria et al. 2005).

The prevalence of infection with *F. gigantica* has been reported to increase with age (Gonzalez-Lanza et al. 1989; Spithill et al. 1999). Similarly in the current study, albeit the overall low prevalence, older cattle had a significantly higher prevalence than younger ones, which is in concord with results reported from other countries (Baldock and Arthur 1985; Gonzalez-Lanza et al. 1989; Holland et al. 2000; Waruiru et al. 2000; Keyyu et al. 2005b; Phiri et al. 2005a; Pfukenyi et al. 2006). The higher infection in older cattle is believed to be associated with increased opportunity for infection from pastures as cattle become more exposed when they get older (Schillhorn Van Veen et al. 1980; Baldock and Arthur 1985; Waruiru et al. 2000; Keyyu et al. 2005b; Pfukenyi et al. 2006). The present study observed gender differences, with a statistically higher prevalence in female than male cattle. These findings are consistent with those reported in other parts of Africa, including Sierra-Leone (Asanji and Williams 1984), Zambia (Phiri et al. 2005a) and Egypt (Kuchai et al. 2011). Similar results have also been found from other parts of the world including Turkey (Yildrim et al. 2007) and Switzerland (Ducommun and Pfister 1991). The higher prevalence in females could be explained by the fact that female to male ratio is usually high since most, if not all, female cattle are attributed to the practice of replacement for breeding and in some cases milk production purposes, as reported by Phiri et al. (2005a). Also, stress associated with pregnancy and parturition may increase the risk of infection in females (Spithill et al. 1999).

This study also noted breed differences, with a significantly higher prevalence in pure Brahman (8.33%) and Brahman crosses (12.20%) than the indigenous Nguni cattle (0%). These findings are in agreement with a similar study in South Africa where a lower *Fasciola* egg count was reported in Nguni than Bonsmara and Angus breeds (Ndlovu et al. 2009). In the present study, Nguni cattle regularly drank water directly from the river and, thus, were expected to have a higher prevalence than other breeds which were watered through water pipes. However, it was the other breeds which showed infection whereas the Nguni did not. In contrast, studies in other countries have indicated a higher prevalence in traditional than *Bos taurus* breeds (Tembely et al. 1988; Kato et al. 2005; Keyyu et al. 2006) while others found no breed differences (Sánchez-Andrade et al. 2002; Dorchies 2006; Yildrim et al. 2007; Yeneneh et al. 2012). The absence of infection in Nguni cattle could be due to innate resistance, as opposed to acquired immunity that occurs over time, since both breeds were exposed to the same conditions on the farm. Genetics may, in fact, play a major role in determining differences in resistance and resilience to *F. gigantica* infection (Molina 2005). Generally, acquired resistance is known to be only partially protective, with cattle remaining susceptible to re-infection every year (Spithill et al. 1999).

The present study observed *Fasciola* infections in cattle reared under modern system of farming. This could be explained by the fact that the Limpopo River, which provided drinking water for the cattle in the ranches, was not accessible to communally grazed cattle and therefore was probably the source of infection. These findings were in contrast to studies from other tropical countries which have indicated that higher prevalence is generally associated with traditional or communal grazing of cattle, than with modern farming (FAO 1994; Maqbool et al. 2002; Keyyu et al. 2005a; Yildrim et al. 2007; Khan et al. 2009; Abunna et al. 2010; Tsegaye et al. 2012). A study in Japan found a higher prevalence of fasciolosis in cattle grazed on potentially contaminated pastures than those that remained kraaled, thereby reducing the risk of infection (Kato et al. 2005). The high prevalence in communal grazing areas has been ascribed to allowing cattle to drink water or graze contaminated pastures adjacent to irrigation canals, river banks or dams (Spithill et al. 1999; Suon et al. 2006; Munguía-Xóchihua et al. 2007) and to insufficient adoption of anthelmintic treatment and control measures for flukes (Phiri et al. 2005a; Yildrim et al. 2007); whereas, modern systems provide clean water and grazing pastures, as well as regular treatment of their livestock against helminth parasites.

In general, most studies have observed seasonal variation with regard to the prevalence of fasciolosis, with prevalence tending to be higher during wet than dry seasons. However, the present study did not establish any seasonal pattern of *F. gigantica* infection probably as a result of limitations associated with logistical problems and therefore failure to carry out monthly visits to the farms. Observations from most African countries have indicated higher prevalence during the rainy than dry periods (Phiri et al. 2005b; Keyyu et al. 2006; Adedokun et al. 2008; Kuchai et al. 2011). In contrast, reports from elsewhere by Khan et al. (2009) and Faria et al. (2005) found a higher prevalence during the dry season. Other studies, however, have reported *Fasciola* infections throughout the year (Amato et al. 1986; Morel and Mahato 1987; Roy and Tandon 1992; Rangel-Ruiz et al. 1999; Holland et al. 2000; Yilma 2000; Maqbool et al. 2002; Keyyu et al. 2005b; Phiri et al. 2005b; Mungube et al. 2006; Pfukenyi et al. 2006; Adedokun et al. 2008; Kuchai et al. 2011).

The mean intensity of infection was extremely low in this study, which could be associated with acquired resistance due to frequent contact of the trematode with the animals, thereby enhancing the development of immunity which provides some protection (Bouvry and Rau 1986; Munguía-Xóchihua et al. 2007). Acquired resistance has been reported in cattle, goats and sheep (Spithill et al. 1999) but with considerable variation (Hurtrez-Boussès et al. 2001). The present findings are suggestive of a capability by ruminants to mount immune responses that could kill *Fasciola* (Spithill et al. 1999) with cattle probably showing that ability to develop acquired immunological resistance (Hillyer et al. 1996; Hurtrez-Boussès et al. 2001). The findings reported in this study are amongst the lowest reported in Africa. Therefore, bovine fasciolosis could be regarded as a parasitic disease of low prevalence especially in the widespread semi-arid regions of the country. However, this low prevalence might be attributed to the sedimentation technique used, which is well known for its low sensitivity and thus characteristically poor detection of fluke eggs by this method. In contrast, reports from other parts of the world showed much higher prevalence and infection rates (Hillyer et al. 1996; Esteban et al. 2003; Awad et al. 2009). Therefore, the use of serological tests, which are more sensitive for the diagnosis of *Fasciola* infection, would provide more accurate estimates of the prevalence in developing countries like Botswana, where molecular diagnosis of fasciolosis is still in its infancy (Hillyer 1999; Spithill et al. 1999; Awad et al. 2009).

The results from the current study have indicated a low prevalence of *F. gigantica* infection in cattle, with the disease being localised only in the Tuli Block ranches in the Central district. However, the disease could still have a higher prevalence in other districts of Botswana, in particular the Northwest, which were not included in the study. Therefore, detailed epidemiological surveillance studies, involving more cattle and areas, are required to confirm the accurate prevalence and distribution of the disease. Additionally, monthly visits would be necessary to determine if any seasonal pattern exists. Such data would assist to design appropriate control programmes for the farmers, since in order to accomplish an effective flukicide treatment, a properly timed strategic programme has to be implemented. Furthermore, the design of future approaches for the control of bovine fasciolosis in Botswana would require a good understanding of the epidemiology of this trematode disease.

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Compliance with ethical standards

The study was approved by Murdoch University Research Animal Ethics Committee.

Conflict of interest The authors declare that they have no conflict of interest.

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