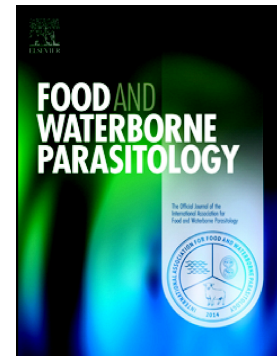


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Infection rates of *Fasciola* spp. in cattle slaughtered at 13 abattoirs in six of nine provinces of South Africa

Sophy Nukeri ^{a,b,*}, Mokgadi Pulane Malatji ^a, Msawenkosi I. Sithole ^a, Philile I. Ngcamphalala ^a, Ignore Nyagura ^a, Danisile Tembe ^a, Innocent Siyanda Ndlovu ^a, Mamohale Chaisi ^{a,b,c}, Samson Mukaratirwa ^{a,d}

^a*School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville Campus, Durban 4001, South Africa*

^b*Foundational Biodiversity Science, South African National Biodiversity Institute, Pretoria 0001, South Africa*

^c*Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort 0110, South Africa*

^d*One Health Center for Zoonoses and Tropical Veterinary Medicine, Ross University School of Veterinary Medicine, Basseterre, St Kitts and Nevis*

ABSTRACT

Fasciolosis or liver fluke infection is a snail-borne disease that affects the productivity of domestic ruminants including liver condemnation especially in cattle. However, there is paucity of information on the burden of infection due to lack of accurate data on liver condemnation related to correct geographical origin of infected animals. This distorts perception of the geographical occurrence of fasciolosis, particularly in South Africa among other countries. We aimed to determine the infection rates of *Fasciola* spp. in cattle slaughtered at selected abattoirs in South Africa. A total of 13 abattoirs consisting of 10 high throughput and three low throughput abattoirs across six provinces, were visited to screen for liver fluke infection in slaughtered cattle including tracing of the animals' geographical origin. A total of 57 livers from 673 slaughtered cattle (8.5%) were found infected with *Fasciola* spp. at 10 abattoirs. The highest infection rate from the study was 37% and the lowest 4.5%. Tracing of infected cattle showed that some abattoirs slaughtered cattle originating from other provinces. Forty-nine percent (29/57) of infected cattle had moderate body condition score (BCS) and recorded the highest intensity of fluke infection (>100 flukes per liver). Furthermore, young animals had high infection rates (51%; 29/57) compared to adults (49%; 28/57) and females (54%; 31/57) compared to males (46%; 26/57). The overall results highlighted the presence of *Fasciola* spp. in five provinces of South Africa, represented by more than four agro-ecological zones. *Fasciola hepatica* occurred in all provinces while both *Fasciola* spp. co-occurred in one province. Furthermore, *F. hepatica* constituted the highest percentage (74%; 710/960) of *Fasciola* spp. collected. *Fasciola gigantica* were collected from abattoirs in one province and constituted 26% (250/960) of the total collected flukes. Results from the study provide information on the burden of fasciolosis in cattle. This is based on abattoir

surveys in South Africa considering the traced geographical origin of animals slaughtered. Using data from surveys of this nature might support efforts to map the geographical distribution of fasciolosis in South Africa. Thus, contribute towards the development of effective control and treatment programs of fasciolosis to mitigate the burden of the disease in cattle.

Keywords:

Fasciolosis; Cattle; Infection rate; Abattoirs; South Africa; Cattle tracing

1. Introduction

Fasciolosis significantly affects the health and productivity of ruminant animals (Mathewos et al., 2023) including human health (Tanabe et al., 2024). This disease is globally distributed (Hurtrez-Boussès et al., 2001; Mas-Coma, 2005) and causes economic loss in livestock production (Khan et al., 2013). It is commonly caused by *Fasciola hepatica* and *F. gigantica*. The former has a cosmopolitan distribution, occurring in five continents except Antarctica (Admassu et al., 2015), whilst the latter is restricted to the tropical and subtropical regions of Asia and Africa (Robinson and Dalton, 2009). Previous reports estimated a global economic loss of US\$3.2 billion annually due to fasciolosis in livestock (Mas-Coma et al., 2005). These losses were reported to be caused by reduced productivity, liver condemnations, reduction in the value of carcasses, and mortality (Bekele and Getachew, 2010). Although the zoonotic nature of this disease has raised public health concerns, human fasciolosis is considered as an emerging disease (Rosas-Hostos Infantes et al., 2023). Previous research indicated that about 17 million people are infected with fasciolosis, with 180 million at risk of infection globally (Ashrafi et al., 2015).

Fasciola spp. have a heteroxenous life cycle requiring both freshwater snails as intermediate hosts (IHs) and mammalian spp. including humans as definitive hosts. Over 20 Lymnaeidae snail species have been linked with the epidemiology and transmission of both *F. hepatica* and *F. gigantica* globally (Malatji et al., 2019). They act as a host for the larval stage of *Fasciola* species, which then exit into the environment as cercaria. This then become the metacercariae, infective stage for definitive hosts after encysting on vegetation. Hence, making them important in establishing the geographical distribution of fasciolosis in animals and humans (Bargues et al., 2001).

Various domesticated mammals such as cattle, sheep, goats, pigs, and donkeys, as well as wild ruminants and human have been documented to be infected by *Fasciola* spp. (Valero et al., 2001; Malatji and Mukaratirwa, 2020). According to Mas-Coma et al. (1999), infection rates in livestock in some endemic areas can reach up to 90%. Infected ruminants tend to have pathological damages of

the liver leading to condemnation during slaughter. Keyyu et al. (2006) reported 100% rate of liver condemnation from cattle slaughtered in selected abattoirs in Tanzania. These condemnations, either partially or wholly, contribute towards the high economic loss in livestock production (Jaja et al., 2017). The current study aimed to estimate the geographical/provincial infection rates of *Fasciola* spp. in cattle slaughtered at 16 abattoirs located in six provinces of SA.

2. Methods

2.1 Sampling areas

Liver samples from slaughtered cattle were examined from ten high throughput abattoirs (HTA), and three low through-put abattoirs (LTA) of the Limpopo, Gauteng, Mpumalanga, Free State, KwaZulu-Natal and Eastern Cape provinces (Fig. 1). Limpopo province is the warmest and northernmost province in SA characterised by arid, semi-arid and sub-humid climate with greater rainfall (Mpandeli et al., 2015). Gauteng province is the smallest province in SA, located on the highveld with a high-altitude grassland. Its northern part is more subtropical and mostly a dry savanna habitat (Pfab et al., 2017). Mpumalanga province is located 1500–2000 m above sea level and is divided into the lowveld and highveld regions (Climate-Data, 2017). Free State, the third-largest province in the country is characterised by both a semi-arid and a humid subtropical climate (Moeletsi et al., 2011). KwaZulu-Natal province is situated in the subtropics with balanced precipitation and evaporation as a result of the humid air over the warm Mozambique Channel (Tyson and Preston-Whyte, 2000). The Eastern Cape, situated 586–2371 m above sea level is characterised by cold semi-arid climate to temperate oceanic climate and occasionally covered in snow (Markus et al., 2006).

2.2 Liver fluke survey

To determine the relative occurrence of fasciolosis, 13 cattle abattoirs were visited between June 2021 to December 2022. Livers from slaughtered cattle were assessed for liver fluke infection. The expected sample size ($n = 384$) was determined following a formula from Thrusfield (2005), based on the previous prevalence (39.6%) of fasciolosis in the Eastern Cape province (Jaja et al., 2017), 95% confidence interval and 5 % desired absolute precision:

$$N = 1.962 (P_{exp} (1 - P_{exp}) / d^2,$$

where N = required sample size

P_{exp} = expected prevalence

d = desired absolute precision.

However, we examined 673 slaughtered cattle for liver fluke infection (621 from 10 HTAs and 52 from three LTAs) (Table 1). From the 673 examined, 239 cattle were from Limpopo, 198 from KwaZulu-Natal, 116 from Mpumalanga, 73 from Gauteng, 25 from Free State, and 22 from Eastern Cape province (Table 1). Information on the origin of the infected cattle was obtained from the abattoir managers and/or meat inspectors.

2.3 *Antemortem analysis*

2.3.1 *Age and gender*

The sex of each animal was confirmed and recorded before slaughter. The information on age were obtained from abattoir personnel prior to sample collection and categorised as 0-4 years (young) and >4 years (adult).

2.3.2 *Body condition assessment*

Body condition scoring (BCS) of each animal was based on a five-point scale as described by Nicholson and Butterworth (1986). Each animal was scored on a scale from 1-5, where 1 represented very poor or emaciated, 2 represented poor, 3 represented good, 4 represented fat, and 5 represented excessively fat (Nicholson and Butterworth, 1986). Thereafter, the scores were re-arranged to fit into a three-point scale (1-2) poor, (3-4) moderate, and (five) good (Jaja et al., 2017). The scoring was performed by an author, and a trained meat inspector or animal health technician from each abattoir.

2.4 *Postmortem analysis*

2.4.1 *Liver fluke recovery and species identification*

Whole liver from each animal was cut into big chunks and hand compressed to release flukes from the bile ducts. All sampled livers were further sliced into pieces of approximately 1 cm thick and rinsed in water to remove flukes lodged in small bile ducts. Flukes were carefully removed and preserved in 50 ml tubes containing 70% ethanol. To ascertain the provincial distribution and infection rate of animals with the liver flukes, information of the provincial origin of infected animals was obtained from the abattoir records. Liver flukes were identified based on morphological features as described by Periago et al. (2008). The specimens were identified based on length, arrangement of shoulders and cephalic cones and counted by species. Morphological identification was confirmed following published protocols (Mucheka et al., 2015; Shoriki et al., 2016). The intensity of infection was calculated as the total number of liver fluke species (either *F. gigantica* or *F. hepatica*) in a single infected host.

2.5 *Data Analysis*

Infection rate was calculated as the percentage of cattle infected with *F. gigantica* or *F. hepatica* per number of cattle examined based on the abattoirs. Infection rates were also calculated based on province of origin, age, sex and intensity of infection. The Chi-squared test was employed to determine the relationship between these various rates and the BCS.

3. **Results**

3.1 *Liver fluke infection rate*

Of the 673 slaughtered cattle, 57 (8.5%) were found to be infected with *Fasciola* spp. The highest number of infected animals were recorded in Gauteng (n=27; 37%), and the least reported in

the Eastern Cape (n=1; 4.5%). For specific abattoirs, results further highlighted that the highest number of infected cattle were recorded from GPC abattoir (Gauteng province) (n=12; p=24.5%), followed by LPV (Limpopo province) (n=9; p=9.1%), GPZ (Gauteng province) (n=8; p=100%), LPP (Limpopo province) (n=8; p=5.7%), GPS (Gauteng province) (n=7; p=43.8%), MPBA (Mpumalanga province) (n=4; p=4.2%), KZE (KwaZulu-Natal province) (n=4; p=3.1%), KZG (KwaZulu-Natal province) (n=3; p=10%), MPBE (Mpumalanga province) (n=1; p=16.7%) and ECM (Eastern Cape province) (n=1; p=4.5%). No evidence of infection was recorded in three abattoirs from Mpumalanga (MPM), Free State (FSH), and KwaZulu-Natal (KZM) provinces (Table 1). There was a statistically significant difference ($P < 0.05$) between the number of infected individuals among abattoirs surveyed in the Gauteng province. However, no significant differences were observed in infection rates in animals from abattoirs of the other four provinces ($p > 0.05$) (Table 1).

Young cattle had the highest number of infections (29/57; 54%) as compared to adult cattle (28/57; 46%). In terms of sex categories, females (54%) had a higher infection rate than males (46%). These differences were not statistically significant ($P > 0.05$).

3.2 Animal tracing and geographical distribution

10 of the 13 surveyed abattoirs slaughtered cattle from the provinces of origin (Fig. 1, Table 1). During the sampling period, abattoir KZG (KwaZulu-Natal) slaughtered cattle originating from Eastern Cape. Furthermore, two abattoirs (GPC and KZE) slaughtered cattle from more than one province. Cattle slaughtered in GPC abattoir originated from Free State, Mpumalanga and Gauteng, whilst those from KZE abattoir originated from Free State and Gauteng.

The highest prevalence was recorded in Gauteng (32.8%) and the lowest prevalence of 0.0% reported in KwaZulu-Natal province (Table 2). Based on the proportion of infected animals, results showed that majority of the infected animals originated from Gauteng (n=20). The lowest proportion of infected animals originated from Eastern Cape (n=4) and Free State (n=4) provinces.

3.3 Body condition score and intensity of infection

Most of the infected cattle had moderate BCS (47%), and only a few cattle with poor BCS were among the infected (21%). Furthermore, most cattle harboured 1-10 flukes per animal. Cattle with moderate BCS had the highest infection intensity (Table 3).

3.4 Identification of *Fasciola* spp and species distribution

From the total number of liver flukes collected, *Fasciola hepatica* contributed 74% (710/960) and *Fasciola gigantica* specimens constituted 26% (250/960) (Table 2). Additionally, a mixed infection in one animal in Limpopo province (LPV) was recorded, where four *F. hepatica* specimens and 18 *F. gigantica* specimens were confirmed.

4. Discussion

The overall *Fasciola* spp. infection rate was 8.5% (57/673). This was higher than the 0.09% recorded in Botswana (Mochankana and Robertson, 2016), 3.8% in Sudan (Kheder and Mohamed, 2021), and 6.7% in Tanzania (Mwabonimana et al., 2010) but lower than the 37.1% recorded in Zimbabwe (Pfukenyi and Mukaratirwa, 2004). Our results also showed that the lowest provincial infection rate was recorded in KwaZulu-Natal, where none of the slaughtered animals originating from this province were infected. However, Gauteng province recorded the highest infection rate, which was higher than the 26.4% previously recorded in the North-West province (Olaogun, 2022) and the 16.33% (Ndlovu et al., 2009) in the Eastern Cape province. Results of no infections recorded for the KZN province were unexpected as previous studies reported that both *Fasciola* species were endemic (Mucheka et al., 2015). It is possible that the cattle in our study might have been exposed to treatment for liver flukes or might have been subjected to intensive farming with pen feeding. Moreover, the cattle might have been prevented from having access to grazing in contaminated water sites rather than extensive management with access to transmission water sites. However, such information was not available at the time of our study.

According to Aregay et al. (2013), various ecological, climatic, and management factors may contribute to differences in prevalence of infection rates of *Fasciola* spp from different geographical areas. These include survival of intermediate hosts which influences transmission of the parasite to the vertebrate host (Jaja et al., 2017). Howell et al. (2012) reported that in Uganda, animals at low altitudes, subjected to free grazing recorded a high prevalence of fasciolosis as compared to animals at high altitude which were fed leaves. Gauteng province is in the highveld with a high-altitude grassland and its northern part is more subtropical and mostly has a dry savanna habitat (Pfab et al., 2017). While the highest infection rate recorded in Gauteng may be biased due to a small sample size, the slaughtered animals came from small-scale farms, which allowed the cattle open grazing. Furthermore, an assessment of watering for cattle during our study showed the presence of snail species which serve as intermediate hosts of *Fasciola* spp.. These included *Pseudosuccinea columella*, which act as the intermediate host of both *F. gigantica* (Malatji et al., 2019) and *F. hepatica* (Pino Santos et al., 2018).

Use of animal tracing makes it easier to link climatic conditions associated with the origin of infected animals. This was observed in our study in the animals slaughtered in the Free State province, where tracing of infected animals revealed that they were all from Gauteng and KwaZulu-Natal provinces and there were no cases from the abattoirs surveyed in this province during the study period. Furthermore, tracing and recording movement of cattle for slaughter before introduction into a new province/area, which is a responsibility of the provincial veterinary services, is vital. This practice will assist in elucidating disease epidemiology, of which like fasciolosis might imply zoonotic risks (Fèvre et al., 2006).

Cattle with moderate, followed by poor BCS were associated with higher burden of flukes compared to those with good BCS. Several authors previously reported high infection rates in cattle

with poor BCS (Assefa et al., 2015; Moje et al., 2015), however, results from our study cannot unquestionably confirm that the poor to moderate BCS was due to fasciolosis as we did not compare the BCS of the infected groups with the non-infected animals. To note also is the fact that several reports have indicated a positive correlation between fasciolosis and weight loss in cattle (Wondwosen et al., 2012; Dawa et al., 2013) which might be the case in our study.

In our study, females had a high infection rate, which corroborated with previous findings in Ethiopia (Betebo 2017). While there is no direct explanation why females are more susceptible to infections, it is however presumed that the impact of hormones and stress may lead to immune suppression which could be associated with this occurrence (Karim et al., 2015). Other studies in central Ethiopia and northern Uganda found that sex had no significant impact on the occurrence and prevalence of fasciolosis since both genders are equally exposed to infection as a herd during grazing (Opio et al., 2021; Oljira et al., 2022).

Young animals (weaned and growing) had higher proportions of *Fasciola* infections as compared to adults and this is consistent with previous studies in South Africa (Jaja et al., 2017) and Ethiopia (Betebo, 2017) which reported significantly higher infection rates in young animals. However, our results differed from Opio et al. (2021) and Zewde et al. (2019) in northern Uganda and Ethiopia respectively, who reported significantly higher infection rates in adult cattle than young animals. This was attributed to longer grazing periods, which increases the probability of exposure to fluke metacercaria. According to Jaja et al. (2017), the low prevalence in older cattle may be due concomitant immunity to the parasite, which assists in stimulating acquired immunity in this age group (Khan et al., 2010).

Morphological identification of the liver fluke specimens from this study, showed the occurrence of both *F. hepatica* and *F. gigantica* in South Africa and this is in agreement with reports by Mucheka et al. (2015) and Chikowore et al. (2019). Results showed that the highest proportion of specimens identified were *F. hepatica*. This was not surprising as Chikowore et al. (2019) also documented that this species occurred in abundance amongst the specimens identified in the Belfast area of Mpumalanga province and occurred as the only species in the highveld of Mpumalanga and KwaZulu-Natal provinces. Results further reaffirmed the unexpected wider geographical expansion of *F. hepatica* in South Africa, which was recorded in all sampled provinces, while *F. gigantica* was only recorded in the Limpopo province. *Fasciola hepatica* was previously reported in humans and horses in Gauteng, in kudu (*Tragelaphus strepsiceros*) in Limpopo (Van Wyk and Boomker, 2011) and cattle in Mpumalanga and KwaZulu-Natal provinces (Mucheka et al., 2015; Chikowore et al., 2019). The co-occurrence of both species has also been documented in KwaZulu-Natal and Mpumalanga provinces (Haridwal et al., 2021), however, this is the first study confirming overlapping distribution and co-infections of the two *Fasciola* species in Limpopo province. The co-occurrence poses a significant risk of hybridization, especially in a country where the occurrence of this phenomenon has been suspected (Haridwal et al., 2021).

Ethics approval

The protocol for this study was approved by the University of KwaZulu-Natal Ethics Committee (reference number AREC/020/020PD), the South African National Biodiversity Institute National Zoological Gardens Animal Research Ethics and Scientific Committee (reference number SANBI/RES/P2021/10) and the Department of Agriculture Forestry and Fisheries (Section 20 permit reference number 12/11/1/1/18 (1866)).

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Credit authorship contribution statement

S.M., M.P.M. and S.N: Conceptualization, S.N. and M.P.M: Methodology, S.N., M.P.M, M.I.S, P.I.N., I.N., D.T., and I.S.N: Data curation, S.N. and M.P.M: Writing- Original draft preparation, S.M., S.N., M.P.M, M.I.S, P.I.N., I.N., D.T., I.S.N., M.C: Visualization, Investigation. S.M., M.P.M., M.C: Supervision.: S.N., M.P.M.: Software, Validation.: S.M., M.P.M, M.I.S, P.I.N., I.N., D.T., I.S.N., M.C: Writing- Reviewing and Editing,

Declaration of Competing Interest

The authors declare no conflict of interest.

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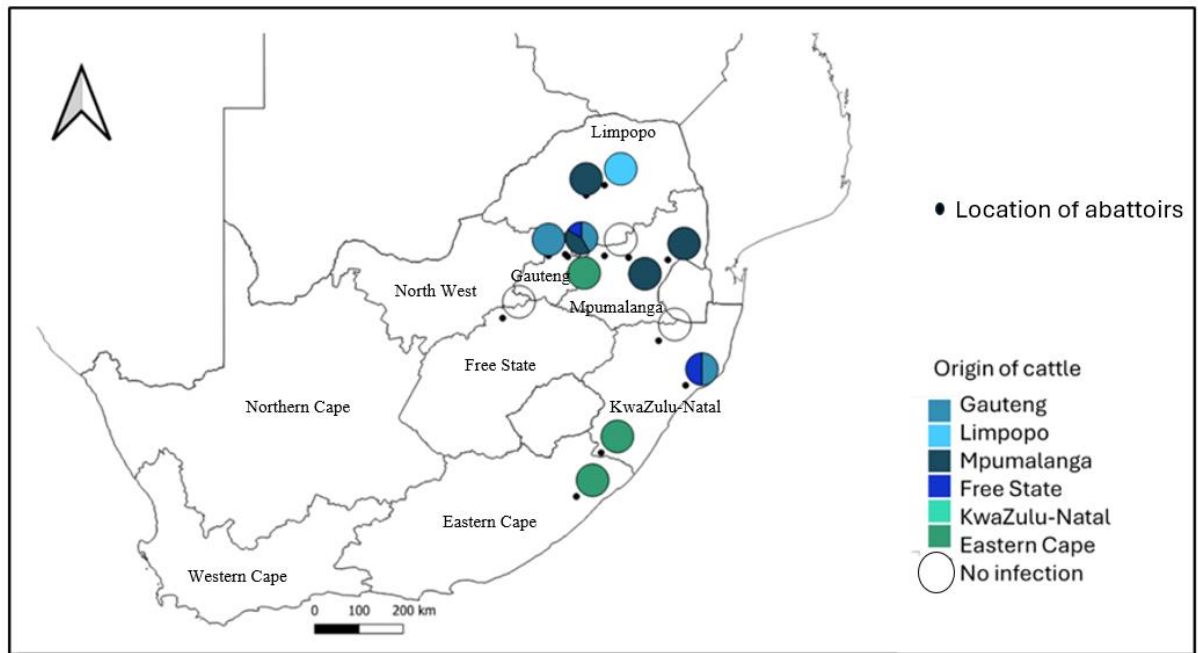


Fig. 1. Map of South Africa showing the location of abattoirs (small dots) visited in six provinces and the origin of the cattle sampled. The large colored circles show the provinces where the cattle originated with each color coding the province of origin (as shown on the legend). ○ No infection = abattoirs where sampled cattle livers were not infected.

Table 1. Infection rates of fasciolosis in cattle slaughtered at 13 abattoirs located across six selected provinces of South Africa.

Collection province	Abattoirs Codes	Origin	Throughput ¹	No. Slaughtered	No. Infected (n)	% Infection Rate (IR)	P - value
Gauteng (GP)	GPZ	Gauteng	LT	8	8	100	0.000
	GPC	Free State, Mpumalanga, Gauteng	HT ²	49	12	24.5	
	GPS	Gauteng	HT	16	7	43.8	
Limpopo (LP)	LPV	Limpopo	HT	99	9	9.1	0.317
	LPP	Limpopo	HT	140	8	5.7	
Mpumalanga (MP)	MPS	Mpumalanga	LT	14	0	0	0.403
	MPBE	Mpumalanga	HT	6	1	16.7	
	MPBA	Mpumalanga	HT	96	4	4.2	
KwaZulu-Natal (KZ)	KZE	Free State, Gauteng	HT	128	4	3.1	0.074
	KZM	KwaZulu-Natal	HT	40	0	0.0	
	KZG	Eastern Cape	LT	30	3	10	
Eastern Cape (EC)	ECM	Eastern Cape	HT	22	1	4.5	1.000
Free State (FS)	FSH	Free state	HT	25	0	0	
Total				673	57	8.5	

¹HT = High Throughput abattoir; LT = Low Throughput abattoir.²HT = Animals originated from multiple provinces

Table 2. Bovine fasciolosis infection rates based on cattle province of origin.

Province	No. of abattoirs	No. of cattle slaughtered	No. infected	Infection rate (%)	No. <i>Fasciola</i> specimens	<i>Fasciola</i> ¹ species
Limpopo	1	99	17	17,2	254	Fh (4) Fg (250)
Mpumalanga	5	261	10	3.83	44	Fh
Gauteng	4	61	22	36.1	558	Fh
Free State	3	160	4	2,5	72	Fh
KwaZulu-Natal	1	40	0	0	0	Fh
Eastern Cape	2	52	4	7.7	32	Fh
Total	16	673	57	8.5	960	

¹ Fh = *Fasciola hepatica*; Fg = *Fasciola gigantica*

Table 3. Intensity of *Fasciola* infections based on body condition score (BCS).

BCS	N	Intensity of infection			
		1-10	11-50	51-100	>100
Poor	12	3	8	1	0
Moderate	27	11	13	2	1
Good	18	18	0	0	0
Total	57	32	21	3	1

Author Agreement

All authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere

Declaration of Competing Interest

The authors declare no conflict of interest.

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Highlights

- Fasciolosis is prevalent in cattle slaughtered at abattoirs from six provinces of South Africa.
- Cattle movement for slaughter distorts the true prevalence at provincial level.
- Cattle movement may contribute to introduction of infections to new areas.
- *Fasciola hepatica* was recorded in all six provinces.
- *Fasciola gigantica* co-occurred with *F. hepatica* in the Limpopo province.