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Biochemical Responses of Yankasa Sheep to Experimental Fasciola gigantica Infection in Zaria, Nigeria

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Abstract: A study was conducted to determine the biochemical responses of Yankasa ewes to experimental Fasciola gigantica infection for a period of 10 weeks. A total of 12 Yankasa ewes were used in the experiment and were obtained from the Reproduction Unit of the National Animal Production Research Institute, Shika-Zaria, Nigeria. 2 weeks baseline pre-infection data were collected and the ewes were later divided into two groups of infected and controls. The six animals in the infected group were each orally inoculated with 1200 Fasciola gigantica metacercariae and monitored for a period of 16 weeks. The pathogenic effects of Fasciola gigantica infection began to manifest as of 9th week post infection through clinical symptoms of bottle jaw, emaciation, depression and pallor of mucus membrane among the infected group. Eggs were demonstrated in faeces of the infected animals at 12th week post-infection. The pathogenic effect was also evident based on fluke biomass, mean fluke length and width, degree of liver damage, serum AST, ALT, ALP and electrolytes responses. There was an increased level of AST from the 4th week post-infection indicating greater damage to the liver parenchyma while the levels of ALT began to rise at the 9th week post-infection among the infected group indicating epithelial damage in the bile ducts. The findings of this study reveals the diagnostic potentials of liver enzymes in the early detection of ovine fasciolosis.

Key words: Biochemical responses, diagnostic potentials, Fasciola gigantica, Yankasa ewes, Nigeria, pathogenic effects

INTRODUCTION

Fasciolosis which is an infection caused by either Fasciola gigantica or Fasciola hepatica is a major parasitic disease of livestock with over 700 million production animals at risk of the infection and world wide economic losses estimated at >US and 3.2 billion per annum (Spithill et al., 1999). In temperate regions, Fasciola hepatica commonly infects sheep and cattle while in tropical regions Fasciola gigantica infects sheep, cattle buffalo and goats.

In Nigeria, small ruminants represent a very important national resource. Sheep are reared primarily for meat and play significant socio-economic roles in the lives of rural dwellers (AbdulmoJeed and Isa, 2011). "Yankasa" breed is the predominant breed of sheep in the Guinea Savanna region of Nigeria (RIM, 1992) within which this study is been carried out.

The detection of fasciolosis infections in ruminants relies on the microscopic observation of fasciola eggs in the faeces of infected animals. However, early diagnosis by coprological examination is not possible because eggs are not found in the facces until 10-12 weeks after infection, when flukes reach maturity and when hepatic injury has been pronounced (Urquhart et al., 1996). Hence the need for diagnostic techniques other than faecal examination for the diagnosis of fasciolosis (Ahmed et al., 2006). Many sheep breeds are susceptible to fasciola infection including most sheep selected for enhanced production traits such as wool production in merino sheep (Raadsma et al., 2006). Similarly, research evidences exist that fasciolosis infection is highly prevalent in the study area among ruminant livestock (Schillhorn Van Veen et al., 1980; Ulayi et al., 2005). Therefore, this current study is aimed at determining the biochemical responses of Yankasa ewes to this disease condition which could be of tremendous assistance in the early detection of ovine fasciolosis in the study area.

MATERIALS AND METHODS

Experimental and animals: Twelve (12) Yankasa ewes obtained from Reproduction unit of the National Animal Production Resea Institute, Shika-Zaria, Nigeria, between 10-12 months were used. The experiment was conducted within a period of four months (April-July, 2010) at the Animal Reproduction unit of the National Animal production Research Institute, Shika-Zaria, Nigeria. The animals each received concentrate feed at 300 g per ewe per day (Akinbamijo et al., 1993). Hay, water and salt licks were given adlibitum. Baseline pre-infection data were collected and the ewes were ranked on the basis of live weight and body condition score (Ahmed et al., 2006) and randomly assigned to two treatment groups.

Isolation and preservation of infective materials: Fasciola gigantica metacercarine were obtained from naturally infected Lymnaea natalensis snails collected at A.B.U Zaria dam and other small streams in Zaria environs.

Collected snails were taken to Departmental parasitology laboratory, Faculty of Veterinary Medicine, A.B.U Zaria where they were crushed in water using petridishes and snail tissues removed. The swimming cercariae were viewed under a microscope and left to attach to the petridish; after which they were left in water in petridish for 3 days at room temperature to become infective (Ajanusi, 1987). Just before infections, metacercuriae were examined under stereomicroscope to ascertain their viability.

Animal infection: The 12 animals were randomly divided into two groups of six animals each representing infected and control group. Each of the animals in the infected group were inoculated orally with 1200 Fasciola gigantica metacercariae as described by instead of (Ajanusi, 1987).

Post infection monitoring:

Parasitology: 5 g faccal samples were collected from the rectum of each animal every week as from week 1 post-infection and fluke egg count was performed using sedimentation technique as described by Urquhart et al. (1996).

Live weight changes: Live weight changes were monitored bi-weekly for a period of 10 weeks after 2 weeks pre-infection monitoring.

Serum enzyme and electrolyte essay: 5 mL of blood was collected in test tubes not containing anticoagulants. There after, the serum was obtained for liver enzymes assay and for the determination of serum electrolytes.

Liver enzymes and electrolytes such as sodium, potassium, phosphorus, chloride, Bicarbonate, urea and albumin were assessed spectrophotometrically using (Bayer Express Plus Clinical Chemistry Analyzer).

Necropsy: To recover the immature flukes from the parenchyma, the liver was cut into small pieces and suspended in physiological saline at room temperature and it was then gently squeezed. The suspension was strained and the flukes recovered were counted and recorded. Ten intact worms were randomly picked from each of the dead sheep and their length and width was measured with transparent ruler (Eguale et al., 2009).

Statistical analysis: Statistical analysis was carried out using SAS (2002). The biochemical parameters of the infected group were compared with those of the controls. Data is expressed as mean± standard error of mean. Values of p<0.05 were considered significant.

RESULTS

Clinical observations and parasitology: Clinical symptoms such as bottle jaw, emaciation, depression and pallor of mucus membrane were seen as of 9th week post-infection in the infected group. The infected sheep started egg shedding at 12th week post-infection with a high mean Egg per Gram count (EPG) of 530±96.44 obtained at the 12th and 13th weeks post-infection respectively.

Mean live weight changes: The Cumulative changes in body weight over the duration of the experiment are shown in Fig. 1. All animals gained weight during the entire experimental period to a certain degree as a consequence of being on adhibitum feed intake and at a young growing age. However, there was a significant drop in the mean weight of the infected animals from the 6th week post-infection where as there was a progressive increase in the mean weight of the controls throughout the period of the experiment.

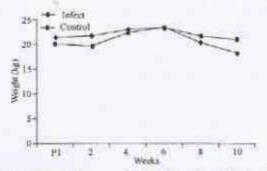


Fig. 1: Weight changes obtained from Fasciola gigantica infected yankasa sheep and their controls

1 Serum alanine amino transferase (ALT) levels obtained from Fauciola gigantica infected yenkasa cives and their controls

	Mean#S.E					
wildes (WK)	Infected	Control	p-value	p-value	LS	
Part I	12.58±0.79	11.33±0.48	1.35	0.211	NS	
	11.83+1.19	11.83±1.19	0.60	0.561	NS-	
	12.16±0.83	10.83±0.79	1.16	0.271	NS	
	15.16+1.35	11 #340.79	2.13	0.06	NS	
4	10.6740.33	10.33+0.33	0.71	0.49	NS	
	13.50±0.62	10.83±0.54	3.24	0.01		
	10.83±0.48	10.50±0.50	0.48	0.64	NS	
	10.50±2.13	10.3340.49	0.45	0.64	NS	
6	12.00±2.41	10.33+0.49	0.68	0.51	NS	
W.	15.33+0.49	10.47+0.65	6.30	<0.00)	**	
	16.83±0.60	10.50±0.56	7.69	<0.0001	.09	

² weeks pre-Infection record; =: Standard error of mean; * Level of significance at 5% (p<0.05), ** Highly significant (p<0.01); LS: Level of milicance; NS: Not Significant (p>0.05)

2 Serum aspartate amino transferage (AST) levels obtained from Fasciolo gigantica infected yankasa ewes and their controls

	MCERES E					
Burlables (WK)	Infected	Control	t-value	p-value	1.8	
74	74,25+3,48	74.58±2.31	- 0.08	0.94	2525	_
	75.33±3.28	76.67+5.20	-0.22	0.63	NS	
2	73.16±6.47	72.50+4.08	0.09	0.93	NS	
	93.67±3.06	89.00±4.97	0.08	0.44	NS	
	99 33±1 20	70.33+2.54	10.33	< 0.0001	**	
	99.13±0.40	70.67±4.08	6.94	<0.0001	**	
	99.33+0.88	70.67±2.59	10.48	<0.000	***	
	97.83±1.74	70.3342.88	8.17	<0.0001	: :	
	62.83+19.8	970.67±2.5	38.17	<0.0001	4.4	
	94.16±1.04	70 00±3 02	7.56	<0.0001	**	
m .	94.50±0.85	71.50±3.28	6.78	<0.000	100	

² weeks pre-infection record, *: Standard error of mean; *: Level of significance at 5% (ρ=0.05); **: Highly significant (ρ=0.01); LS: Level of militarice; NS: Not Significant (ρ=0.05)

sole 2. Serum alkaline phosphatase (ALP) levels obtained from Faectohr gigantica infected vankasa ewes and their controls

	Mean=S.E				
Variable:	Infected	Control	6-value	p-value	1.5
2	20.41 ± 3.05	21.33 ± 2.18	-0.24	0.81	NS
	20.83 ± 3.67	23.50±2.26	+ 0.62	0.55	N5
	20.00 ±3.34	19.16±5.32	0.13	0.89	N5
	24.16=1.45	18.33±0.95	3.36	0.007	1.0
6	26.60+1.77	20.67 (2.46	1.76	0.12	NS
	30.83±2.56	21.0012.50	2.75	0.021	*
	26.83±1.85	22.50±1.96	1.61	0.14	NS
	31.16= 1.10	22.00 ±1.80	4.32	0.001	**
	34.00 ±0.45	22.50+2.04	5.49	0.0003	**
	26.83±1.72	23.50+2.34	4.58	0.001	**
16	37.67± 1.89	23.674-2.26	4.25	0.0008	**

² weeks pre-infection record; 4: Standard error of mean; * Level of significance at 5% (p<0.05); ** Highly significant (p<0.01); LS: Level of entiremes; NS: Not Significant (p>0.05)

Table 4: Serum albumin levels obtained from Fasciola gigantica infected yankasa ewes and their controls

	Meuni/S.E				
Variable	Infected	Control	t-value	p-value :	LS
75	3.17±0.27	2.98±0.18	0.60	0.57	NS
10	3.10±0.41	2.93±0.24	0.35	0.73	N5
2	3.23±0.14	3.01±0.14	1.07	0.31	NS
3.	3:2040.18	3.38+0.11	-0.985	0.41	*
1	2.95+0.07	2.62±0.54	0.61	0.55	NS:
5	2.37+0.05	3.15+0.14	5.10	0.0005	
6	2.40+0.05	3.10±0.13	-4.79	0.0007	NS
7	2.52±0.10	3.13v±.12	- 3.73	0.003	**
3.	2.47+0.15	3.38+0.13	-4.62	0.001	***
9	2.30+0.04	3.33+0.15	-6.35	< 0.0001	**
102	17±0.06	3.02±0.12	- 11.45	<0.0001	**

P. 2 weeks pre-infection record, + Standard error of mean, * Lovel of significance at 5% (y<0.05), ** Highly significant (p<0.01); LS. Level of significance, NS. Not Significant (p<0.05)</p>

Table 5: Serum potassium (K) levels obtained from Fasciole gigantics infected yackass ewes and their controls

	Mean+S.E				
Variables (WK)	Infected	Control	1-value	p-value	LS
Po	4.56±0.27	4.70±0.27	-0.37	0.72	NS
1:	4.65±0.48	4.93±0.48	0.42	0.69	NS
2	4.47±0.35	4.47±0.18	0.00	1.000	NS.
3	4.60±0.24	3.90±0.42	1.45	0.18	NS
4	4.60±0.17	4.40±0.13	0.90	0.39	NS
5	4.63±0.44	4.31±0.18	0.67	0.62	NS
6	4.83±0.29	4.47±0.18	1.07	0.31	NS-
7	4.83±0.24	4.21±0.27	1.69	0.12	NS
1	5.18+0.27	3.5140.75	2.09	0.01	**
9	5.48±0.28	4,33+0.19	3.43	0.006	**
10	7.98±1.32	4.45±0.11	2.67	0.02	

P_x 2 weeks pre-Infection record, ±: Standard error of mean, *. Level of significance at 5% (p<0.05); ** Highly significant (p<0.01); LS: Level of significance; NS: Not Significant (p>0.05)

Table 6: Serum sodium (Na) levels obtained from Fasciola gigantica infected yankana ewes and their controls

Variables (WK)	Mean+S.E				
	Infected	Control	t-value	p-value	1.5
Po	146,75±3:24	153.33+2.25	-1.67	0.130	NS
1	146.83±5.06	149.50=6.08	-0.34	0.741	NS
2	146.67#2.20	157.17±3.68	-2.45	0.031	N5
3	148.50±2.63	146.33±2.20	0.63	0.540	*
4	143:50±2:14	147.67=1.11	- 1.73	0.120	NS
5	150:50+3.44	143.00=1.63	1.97	0.080	NS
6	145.83#2.37	146.17+2.12	- 0.10	0.920	NS
7	154.50±1.61	145.67±1.73	3.75	0.004	
6	150.50+2.46	142 33=1 89	2.63	0.030	
9	152.00±1.79	143:50±1:28	3.86	0.003	**
10	152.50±2.15	144.00±1.00	3.58	0.00%	**

P_y 2 weeks pre-Infection record; i: Standard error of mean; *: Level of significance at 5% (p<0.05); **- Highly significant (p<0.01); LS: Level of significance; NS: Not Significant (p>0.05)

Table 7: Serum bicarbonate (HCO₃) levels obtained from Fasciola ejeunica infected vankasa ewes and their controls

	Mean#S.E.				
Variables (WK)	Infected	Control	t-value	p-value	LS
Po	25.35±091	24,41+0.75	0.77	0.460	NS
1	22.83±1.38	24.83±1.17	-1.11	0.290	NS
2	27.83±1.94	24.00±0.93	1.78	0.120	NS
3	19.83a1.40	21.50±2.24	- 0.63	0.541	
4	21.33±1.58	25.67±2.04	- 1.68	0.121	105
5	19.17a1.17	21.83+0.91	+1.80	0.101	NS NS
5	20.17+2.44	27.00±3.46	-161	0.140	NS
7	20.1741.62	22.50±1.52	- 1.05	0.320	NS
	18.6741.49	22 33+1.56	- 1.69	0.120	NS
4	19 00±1 97	20.67±0.80	-0.78	0.450	NS
10	22.00±2.21	20.00±0.45	0.89	0.390	NS

P. 2 weeks pre-Infection record, #: Standard error of mean; *: Level of significance at 5% (p<0.05); **: Highly significant (p<0.01); 1.5. Level of significance, NS: Not Significant (p<0.05)

Table 8. Serum chloride (CI) levels obtained from Fanciola encontica infected vankasa ewes and their controls

	MeanaS.F.				
Variables (w.k)	Infected	Control	t-value	p-value	LS
Po	102.3340.86	100.58#1.74	0.90	0.390	NS NS
1	101.00+1.73	100.67a1.90	0.13	0.890	NS
2	103.67+2.28	100.50=2.06	1.03	0.320	NS
3	103.17±1.74	105.17=2.48	-0.66	0.520	NS
4	88.67±4.389	4.17±3.27	- 1.0	0.300	NS
5	107.67±5.39	103.17±2.16	0.77	0.400	NS
6	88.00±4.899	3.67±3.57	- 0.94	0.370	NS
7	117.67±2.86	102.50±1.20	2.95	0.014	NS
1	111.67±2.86	102 50±1 20	2.95	0.010	44
9	110.83±1.81	104.50±1.48	2.71	0.020	**
10	114.17±1.62	109 50+2.77	1.46	0.170	NS

F_a 2 weeks pre-Infection record; ±: Standard error of mean; *: Level of significance at 5% (p<0.05); **: Highly significant (p<0.01); LS. Level of significance, NS: Not Significant (p>0.05)

Table 9: Serum urea levels obtained from Fascaola gigantica infected yankasa ewes and their controls

variables (WK)	Mean±S E	While Am			
	Infected	Control	t-value	p-value	LS
	27.5f±1.42	27.00±2.67	0.22	0.83	NS
	30.83±1.83	30.50±2.01	0.12	0.91	NS
2	24.33±3.20	23.50±2.06	0.22	0.83	NS:
2	23.00±2.90	21.33=0.61	0.56	0.59	NS
	22.50+2.99	21.00±0.68	0.49	0.63	NS
1	1983±0.45	22.00+2.86	- 0.76	0.47	NS
	20.33+3.27	22.67+0.71	- 0.70	0.50	NS.
	21.83+2.23	23.33+1.12	-0.58	0.57	NS
	21.83±2.21	22 17±1 30	-0.31	0.89	NS
	23.00+2.31	23.17+1.89	+0.06	0.96	MS
12	23 17±2 30	23.17±1.89	0.00	1.000	NS

² weeks pre-Infection record, a: Standard error of mean; *: Level of significance at 5% (p<0.05); **: Highly significant (p<0.01); LS: Level of agrificance; NS: Not Significant (p>0.05)

Table 10 Total Protein (TP) levels obtained from furciola gigantics infected wasking ewes and their controls

Suriables (WK)	MeanaS F				
	Infected	Control	t-value	p-value	LS
Pa .	6.25±0.13	6.5610.30	0.92	0.38	NS NS
	6.27±0.14	6.68±0.26	1.33	0.21	NS
2	6.0+0.21	6.7±0.32	1.82	0.09	*
100	5.88±0.27	7.25±0.49	2.45	0.03	**
4	5.6+0.26	7.6±0.38	4.32	0.001	**
5	5.7A0.35	7.5+0.29	3.86	0.003	22
N.	5.58±0.22	7.53±0.49	3.58	0.005	44
	5.31±0.24	7.55±0.49	4.06	0.002	
B.	4.91+0.39	7.560.24	5.66	0.0002	**
9	45+0.55	6.61+0.40	3.17	10.0	
10	4.1A0.50	6.8±0.39	4.07	0.002	**
11	5.940.41	6.941.54	0.70	0.500	
12	2.7+1.20	7.2±0.40	3.52	0.005	NS
23	2.040.70	7.2±1.40	3.62	0.004	**
14	2.72±1.74	7.88±1.75	2.93	0.02	
15	2.65=1.68	7.93±0.1	3.14	0.01	*
16	2.01.26	6.980.49	3.67	0.04	

² weeks pre-lifection record, a Standard error of mean; * Level of significance at 5% (p=0.05); ** Highly significant (p=0.01); LS: Level of significance; NS: Not Significant (p>0.05)

Table 11: Besinophil counts obtained from fasciola gigantica infected yanknsa ewes and their controls

Variables (WK)	Mean±S.E.				
	Infected	Control	t-value	p-value	LS
196	1.16±0.60	1.75±0.30	0.88	0.4	**
1	2.5±0.34	0.16±0.16	6.14	@ 0001	**
2	4.1±1.02	1.0±0.41	2.71	0.02	
1	3.781.14	0.6s1.22	2.64	0.02	*
£ .	3.340.23	0.3+1.22	2.36	0.04	**
9	0.5±0.22	0.3±0.42	0.42	0.68	NS
5	4.5±0.21	0.5±1.03	3.93	0.002	**
7	3.8±0.62	1.5=0.1	2.40	0.03	
	2.0±1.83	1.1±1.11	0.80	0.44	N5
r.	0.00±0.01	1.0=0.61		0.14	NS NS
0	1.3±0.30	0.6±0.51	1.58	0.28	NS
ii.	0	0.5+0.5	1,00	0.34	NS
2	0		0	0	NS
3	0.00±0.80	0.8±0.8	0	0	N5
4	0	0	0	0	NS NS NS NS NS
5	ō	0	0	0	NS
16	0	0	0	0	N5

P. 2 weeks pre-Infection record; ± Standard error of mean; *: Level of significance at 5% (p<0.05); **: Highly significant (p<0.01); L5: Level of significance; NS: Not Significant (p>0.05)

Serum enzyme and electrolyte assay: The results of enzyme and electrolyte assay are shown in Table 1-9. The infected groups showed detectable increase in AST levels from the 4th week post-infection. Infected groups had significantly elevated levels of AST compared to the controls (p<0.01). Elevated level of ALT, as an indicator of epithelial damage in the bile ducts, began to rise significantly (p<0.01) from the 9th week post-infection

among the infected group compared to the controls. There was a significant (p<0.05) rise in the levels of ALP among the infected group from the 7th week post-infection period. All serum electrolytes except bicarbonate and urea showed elevated levels from the 7th week post-infection. Serum Albumin levels began to drop significantly among the infected group from the 7th week post-infection while mean Total Plasma Protein (TPP) had a significant (p<0.05) drop in the infected group from the 2th week to the end of the experiment (Table 10). Eosinophilia predominates at the early period of the experiment among the infected group (Table 11).

Necropsy: The mean total of immature worms recovered from the liver of the four infected animals was 1129±12 at 10th-12th week post-infection with a corresponding high percentage recovery ranging from 92 to 96%.

The mean length/width of flukes recovered from the livers of the infected dead sheep is shown in Fig. 2. Mean parasite length/width increase with time as the values obtained increased progressively from the 10th-12th week post-infection.

DISCUSSION

Earlier studies have shown that the pathogenic effects of Fasciola gigantica infection in some breeds of sheep began to manifest as early as 7-10 weeks of infection resulting in death even with few parasites (Ahmed et al., 2006; Ajanusi, 1987). This may be in contrast to other findings that Fascinla gigantica is slower to develop in ruminant hosts and takes greater than 14 weeks following infection to reach the bile ducts (Wiedosari and Copeman, 1990). In this current study, pathogenic effects of Fasciola gigantica infection began to manifest as of 9th week post-infection through clinical symptoms of bottle jaw, emaciation, depression and pallor of mucus membrane in some infected animals while mortalities were experienced between 10-12 weeks in four animals in the infected group. Similarly, there was a significant drop in the mean live weight of the infect group compared to the controls from the 6th week post-infection due to the effects of fluke burden.

Eggs were demonstrated in facces of the infected animals at 12th week post-infection. The high mean egg count (EPG) of 530±96.44 obtained in this study showed a good measure of host-parasite relationship (Spithill et al., 1997).

Based on fluke biomass, mean fluke length/width, degree of liver damage, serum AST, ALT, ALP and serum electrolytes responses, this experiment suggests that Fasciola gigantica infection among Yankasa sheep is evidently pathogenic in the first 10 weeks of infection. This is evident with the increased levels of AST from the 4th week post-infection indicating greater damage to the liver parenchyma (Meusen et al., 1995). The levels of ALT were beginning to rise at the 9th week post infection

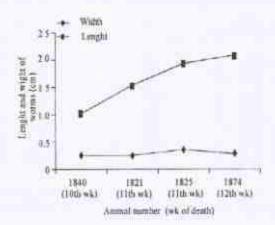


Fig. 2: Mean length/width of recovered worms from Fasciola gigantica infected yankasa sheep

indicating epithelial damage in the bile ducts (Chauvin et al., 1995). Serum ALP is known to be excreted via the bile duct. Its elevation which was observed to be significant as from the 7th week post infection may have been synchronized with the arrival of flukes to the bile ducts constraining biliary clearance (Ahmed et al., 2006). Elevated potassium, sodium and chloride levels of the infected sheep are suggestive of cellular damage (Eckert et al., 1977). A drop in the level of albumin from the 7th week post-infection which correlates with the low total plasma protein levels may be associated with liver damage (Cheung and Jickman 2002). Similarly, a high rise of mean circulating cosmophil numbers at the early period of the experiment also correlates with the elevated levels of the liver enzymes depicting a greater degree of damage to the liver tissues.

The high percentage recovery (92-96%) of immature flukes from the dead animals compared with the infective dose given, suggests a uniform viability of parasite establishment, an indication that yankasa sheep have demonstrated low resistance to Fasciola gigantica infection (Ajanusi, 1987). A slower maturation of Fasciola gigantica is also manifested by the finding of smaller, immature flukes in the liver parenchyma of dead sheep as from the 10-12% week post-infection (Spithill at al., 1999). The findings of this study revealed the potentials of liver enzymes in association with serum electrolytes being used as a measure of early warming signs in the detection of ovine Fasciola gigantica infection prior to the detection of eggs in the faeces of infected animals.

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