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Prevalence and Classification of Amphistomes in Cattle and Buffaloes

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Abstract

Amphistomes are snail-borne trematodes infect rumens and reticulums capable of causing acute and chronic disease in cattle and buffaloes. A total of 897 of cattle and buffaloes were examined by faecal examinations and by postmortem examinations in Giza and Garbia governourates. The collected Amphistomes were morphologically and histologically classified. We found that the incidence of Amphistomes in totally examined animals was 4.9%. The incidence was higher in the oldest animals(than young), in the spring (than other seasons) and in Garbia (than Giza). But the incidence was the same in males and females. The collected Amphistomes were classified as *Paramphistomum microbothrium*, *Paramphistomum cervi* and *Carmyerius gergaerius*. We concluded that Amphistomes are prevalent among the examined cattle and buffaloes in Giza and Garbia governorates..

Keywords: Paramphistomum; Carmyerius; Incidence; Classification; Bovine.

Introduction

Amphistomes are considered to be one of the most important trematodes of ruminants which produce high economic loss to the livestock industries through morbidity and mortality particularly in the young stock. Older animals can develop resistance but may still harbor numerous adult flukes in the rumen and reticulum without showing overt disease, however damage to the rumen due to heavy infection has been recorded and may be responsible for unthrifitness, emaciation, lower feed conversion rate, decrease milk yield and reduction of fertility (Kamaraj *et al*., 2010; Sanchis *et al*., 2012).

Paramphistomum cervi and *Paramphistomum microbothriuum* are considered to be one of the most common species of *Paramphistomum* since they have a wide –host range including cattle and buffaloes with cosmopolitan distribution (Rangel-Ruiz *et al*., 2003 and Magdy *et al*., 2009).

In Egypt, the fresh water snails including *Bulinus truncates* and *Bulinus forskalii* are prevalent and play role in the distribution of Amphistomes (Elsokkary *et al*., 2009) .The intermediate hosts (fresh water snails) living under some particular conditions including the presence of vegetation, humidity, frequent rainfall and mild temperatures (Pinedo *et al*., 2010).

Therefore, the present study was conducted to determine the prevalence of Amphistomes under field conditions in living animals as well as slaughtered cattle and buffaloes in Giza and Gharbia governorates in Egypt .

Materials and methods

Animals

A total of 897 of cattle and buffaloes of different ages, sexes, and breeds (native, foreign and mixed) belonging to Giza and Gharbia governorates were examined for paramphistomiasis. They include 545 animals from Giza governorate (340 cattle and 205 buffaloes) and 352 animals from Gharbia governorate (204 cattle and 148 buffaloes) .

Seven hundreds ninety four slaughtered cattle and buffaloes at Warak and Alsanta abattoirs were

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examined during postmortem examination for detection of Amphistomum flukes in their rumens and reticulums and other 103 living animals were examined coprologically for the presence of Amphistomum eggs in their faeces. Then they were classified into Amphistomum infected and free cattle and buffaloes.

The morbidity parameters of Amphistomes infected animals were recorded including body condition scores, mucous membrane of conjunctiva and faeces consistency.

A body condition score ranged from 1 to 5 and was given to each animal where (1) emaciation, (2) very thin, (3) thin, (4) good, (5) very well. The colour of the conjunctiva was classified into pale and normal. the fecal consistency (diarrhea score) was scored from (1) normal to (2) soft or (3) watery as previously carried out by Dorny *et al* (2011).

Samples

Living worms from rumens and reticulums of the slaughtered cattle and buffaloes were collected at Warak and Alsanta abattoirs. These worms used for identification and classification.

Faecal samples were collected from cattle and buffaloes at Giza and Gharbia governourates by back racking, and then each sample was put in separate covered cup until examined.

Parasitological techniques

Flukes recovered from each of the infected animals during the examination were counted and morphologically identified according to (Sey, 1977) Fresh living flukes were collected from the rumen and reticulum after slaughtering.

The collected flukes were morphologically identified and kept in formalin 10 % for histological examination for their classification.

Fecal samples were examined by direct smear and concentration sedimentation techniques and the eggs of amphistomes were identified by their morphology according to Rolfe and Boray (1987). Classification of the collected worms was done according to Nasmark (1937) and Eduardo (1985):

The flukes were classified depending on their anatomical morphology and their muscluture organs by median sagittal sections stained with H and E stain (pharynex, acetabulum, and genital atrium), male and female reproductive organs.

Histology (median sagittal section) was done according to Nasmark (1937), Eduardo (1985) and Dube and Aisien (2010).

Results

Results of clinical examination of the Amphistomes infected cattle and buffaloes were recorded in Table 1.

Table 1. The morbidity parameters of Amphistomes infected animals.

Morbidity parameters	Body condition score						Colour of conjunctiva			Fecal consistency			
Species	Emaciation	Very thin	Thin	Good	Very good	Total	Normal	Pale	Total	Normal	Soft	Watery	Total
Cattle	0	2	10	9	6	27	19	8	27	11	14	1	27
Buffaloes	-	-	-	-	-	-	10	7	17	8	8	1	17
Total	-	-	-	-	-	-	29	15	44	19	22	2	44

Table 2. The prevalence of Amphistomes in the examined cattle and buffaloes

	Cattle	Buffaloes	Total
Number of animal examined	544	353	897
Number of infected animal	27	17	44
Percentage of infected animal (%)	3.01	1.89	4.9

Results of the total prevalence of Amphistomes are shown on in Tables 2-6 and Figures 1-4.

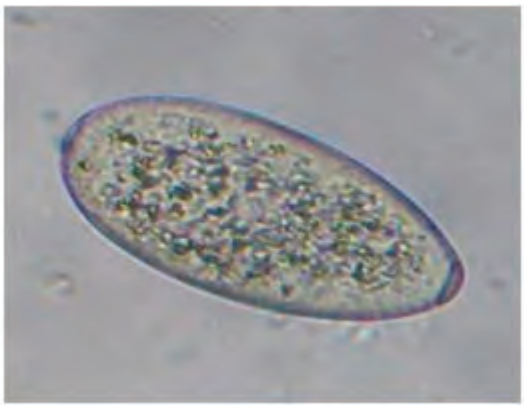

Fig.1. *Paramphistomum* egg (100 X)

Morphological findings of investigated Amphistomes

The adult flukes of *Paramphistomum* (Fig. 5) was conical in shape with posterior broad end (acetabulum), pink in colour when fresh, body dimensions were 13.9 mm measured from (12.8–14.9mm) length and 4.9 mm (4.4 -5.9 mm) width and the body was nearly rounded in cross section. While the morphology of *Carmyerius*, the adult flukes (Fig. 8) was elongated in shape and red to reddish brown in colour when fresh, the body dimensions were 12.8 mm (11.1–14.5 mm) length and 4.6 mm (4.2 -5.1 mm) width and the body is nearly rounded in cross section

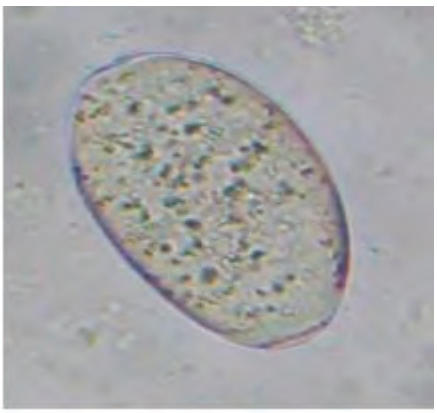

Fig. 2. *Carmyerius* egg (100 X)

Table 3. The sex-wise prevalence of Amphistomes in the examined cattle and buffaloes

Age	Cattle				Buffaloes				Total (infected)	
	Infected		Non infected		infected		Non infected			
	No	%	No	%	No	%	No	%	No	%
< 1.5 years	3	0.33	110	12.26	1	0.11	66	7.36	4	0.44
1.5-2 years	3	0.33	104	11.6	2	0.22	52	5.8	5	0.56
2.5-3 years	3	0.33	84	9.36	2	0.22	50	5.57	5	0.56
3.5-4 years	4	0.45	65	7.24	2	0.22	60	6.69	6	0.67
4.5-5 years	5	0.56	82	9.14	4	0.45	54	6	9	1
> 5 years	9	1	72	8.02	6	0.67	54	6	15	1.67
Total	27	3.01	517	57.64	17	1.89	336	37.46	44	4.9

Table 4. The age-wise prevalence of Amphistomes in the examined cattle and buffaloes

		Cattle	Buffaloes	Total
Number of the totally examined animals	Male	347	180	527
	Female	197	173	370
Number of the infected animals	Male	15	7	22
	Female	12	10	22
entage of the infected animals (%)	Male	1.67	0.78	2.45
	Female	1.34	1.11	2.45
	Total	3.01	1.89	4.9

Histological findings of investigated Amphistomes

The histology of Paramphistmum was characterized by the vertical (tandem) location of the testes and the ventral pouch was absent. The examination of pharynx, acetabulum and genital atrium) revealed two types of *paramphistomum*:

Paramphistomum microbothrium that had acetabulum of *Paramphistomum* type according to new classification of Edurdo (1985) and subterminally located Pharynx is of calicophoron type pear shaped broad posteriorly and tapered anteriorly

Paramphistomum cervi that had acetabulum of *Paramphistomum* type according to new classification of Edurdo (1985) and acetabulum is subterminally located with wide external diameter (1-2.8 mm) ratio to body 1:4 to 1:5 and

Pharynex was of liorchis type with ratio to body length 1:6–1:10 with regular lumen.

The histology of *Carmyerius* revealed a horizontal location of the oval testes and the presence of ventral pouch which extending near the acetabulum in sagital section (Nasmark, 1937 and Sey, 1977). Results of the histological findings were shown in Table 7 and in figures 9-13.

Discussion

The pathogenesis of Amphistomes could be summarized as the immature Amphistomes excyst in the duodenum or mid-to-proximal jejunum. As they migrate they attach firmly to the mucosa and may penetrate as far as the muscularis mucosa. Damage is related to the numbers of migrating flukes and increases in intensity from localized enteritis through patches of villous atrophy to severe destruction of the mucosa. Clinical and production effects are dependent upon the extent of the lesions as some compensation for functional deficiency can take place in the un-damaged lower small intestine. The presence of mature flukes in the rumen does not usually elicit any significant response but

Table 5. The rate of infection of Amphistomes in the examined cattle and buffaloes according to season

Season	Cattle				Buffaloes				Total(infected)	
	Infected		Noninfected		infected		Noninfected			
	no	%	no	%	no	%	no	%	no	%
Autumn	5	0.56	118	13.1	3	0.33	76	8.47	8	0.89
Winter	6	0.67	125	13.93	4	0.45	77	8.6	10	1.11
Spring	9	1	146	16.27	6	0.67	103	11.5	15	1.67
Summer	7	0.78	116	12.93	4	0.45	80	8.9	11	1.23
Total	27	3.01	517	57.64	17	1.89	336	37.46	44	4.9

Table 6. Comparison between the rate of Amphistomes infection in Giza and Garbia governorates.

	Giza				Garbia				Total (Infected)	
	Infected		Non –infected		Infected		Non –infected			
	No	%	No	%	No	%	No	%	No	%
Cattle	10	1.11	330	36.79	17	1.9	187	20.85	27	3.01
Buffaloes	8	0.89	197	21.96	9	1	139	15.5	17	1.89
Total	18	2	527	58.75	26	2.9	326	36.35	44	4.9

Table 7. Histological classification of the collected Amphistomum

	Testes	Acetabulum	Pharynx
Paramphistomum microbothrium	Tandem in position and lobulated	*Paramphistomum* type	*Calicophoron* type
Paramphistomum cervi	Tandem in position and lobulated	*Paramphistomum* type	*Liorchis* type
Carmyerius gergaerius	Horizontal in position and Slightly lobulated	*Paramphistomum* type	*Paramphistomum* type

Fig. 3. rumen of a buffalo severely infected with adult *Carmyerius* flukes

Fig. 4 Adult *Paramphistomum* rumen flukes among papillae of anterior dorsal sac of rumen.

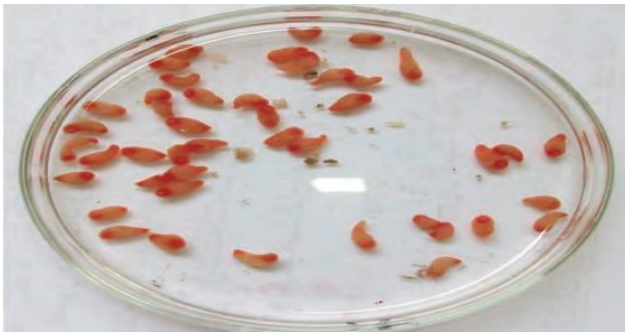

Fig. 5. Freshly collected *Paramphistomum* flukes

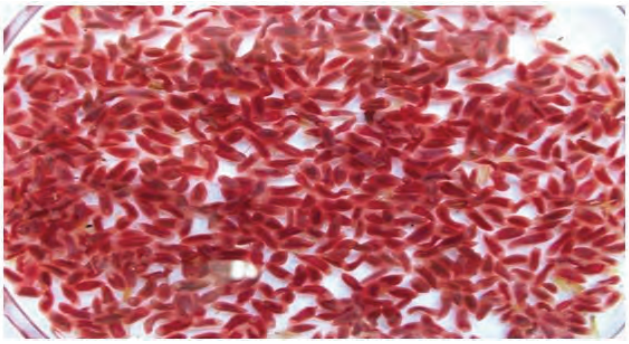

Fig. 6. Freshly collected adult *Carmyerius* flukes.

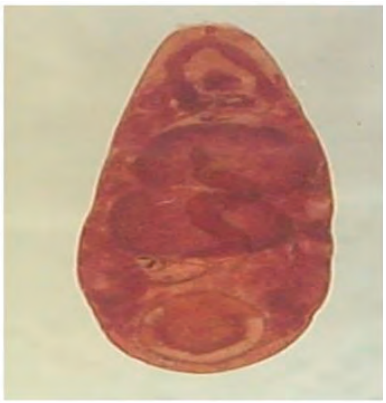

Fig. 7. Compressed *Paramphistomum* fluke stained in alcoholic acid carmine stain (3x)

Fig. 8. Compressed *Carmyerius* fluke stained in alcoholic carmine stain (3X)

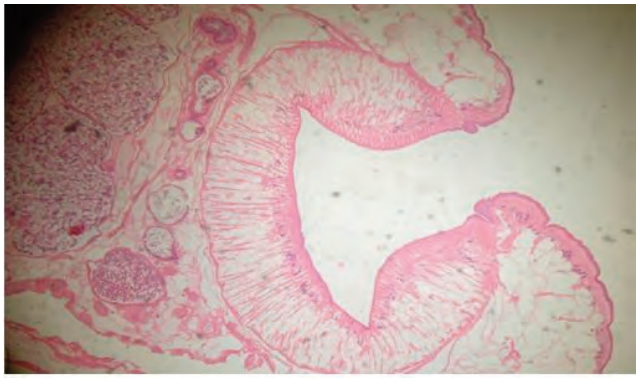

Fig. 9. Acetabulum located sub-terminal of *Paramphistomum* type (H and E stain) (4X).

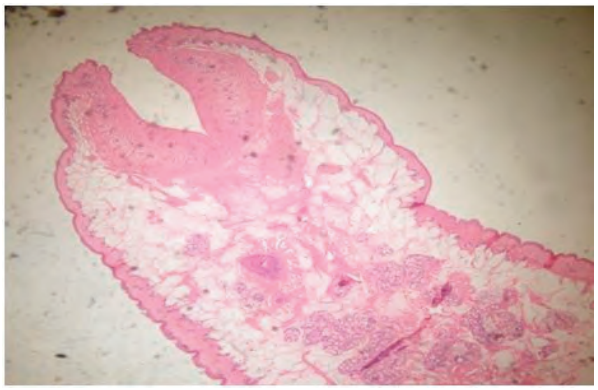

Fig. 10. Pharynx is of calicophoron type pear shaped broad posteriorly and tapered anteriorly (H and E stain) (4X).

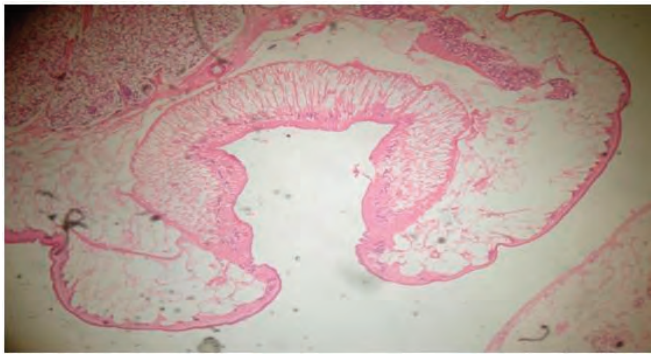

Fig 11. acetabulum located sub-terminal of *paramphistomum* type (H and E stain) (4X).

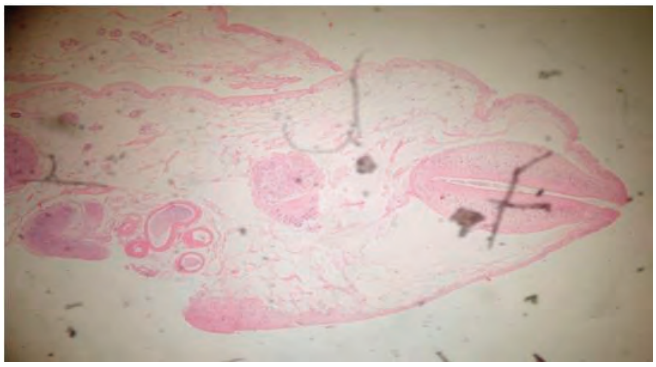

Fig. 12. Pharynx is of liorchis type ratio to body length 1:6 –1:10 with regular lumen (H and E stain).

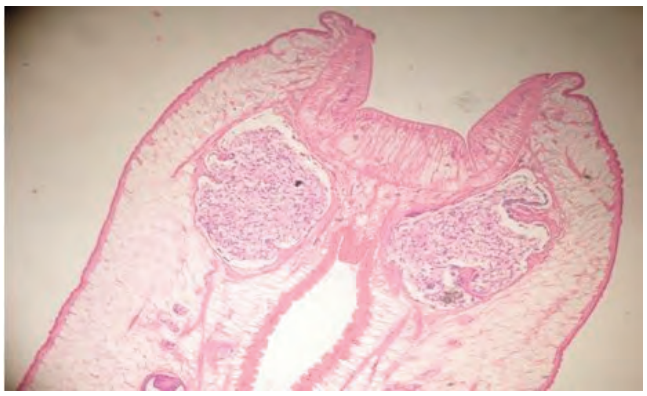

Fig.13. Testis are horizontally located and the ventral pouch extending near the acetabulum in *Carmyerius species* (H and E stain).

in massive infections papillae are short and red, becoming fused into aggregations with ruminal contents adhering firmly to the surface (Radostits *et al*., 2010).

The most observable clinical sign in the amphistomes infected cattle and buffaloes was emaciation that was resulted from the reduction of feed digestibility and depression of appetite (Dorny *et al*., 2011). Also the anemia was recorded that was caused by blood sucking due to the hematophagous activity especially for immature stages of Amphistomes (Diaz *et al*., 2006). The acute phase of Amphistomiasis caused by immature flukes infection was not detected in our study because it needs certain rare conditions in which there is huge number of Amphistomes metacercariae contaminating forages ingested by animals. That only occur in rare conditions where the snail population expands rapidly due to the heavy rains or the digging of a new pond or the flooding of a pasture (Howell, 2011).

In our study, we found the equal rate of infection of amphistomes in female (2.45%) than male (2.45%) that means the sex of animal has no effect on the susceptibility of animal to Amphistomes.

The occurrence of adult Amphistomes was more frequently recorded in adult cattle and buffaloes (older than 5 years ,1.67 %) than young animals (younger than 1.5 years, 0.44%) our finding in agreement with (Khani *et al*., 2008), they reported that cattle can develop resistance after exposure to the parasite which protect them against massive infections of mature flukes that cause the clinical form of the disease

The difference in infection rate among different seasons is not significantly but the higher rates of infection were in the spring (1.67%) and summer (1.23%) that could be interpreted by in the winter, the rains causes snails expanding then with warm climate in the spring and summer the snail produce metacercariae that ingested with barseem over long time and form adult Amphistomes in the infected animals (Howell, 2011).

The rate of infection of Amphistomes was higher in the Garbia (2.9%) than Giza (2%) governourates in both cattle and buffaloes, this may be explained by the Amphistomes snail (intermediate host) may be more prevalent in Garbia than Giza governorates (Howell, 2011).

In our study, three species of Amphistomes were classified from the examined cattle and buffaloes and recorded including *Paramphistomum microbothrium*, *Paramphistomum cervi* and Carmyerius. The *Paramphistomum microbothrium* was the most prevalent species that is agree with the previously reported by Dinnik (1964) and Butler and Yeomans (1962), *Paramphistomum microbothrium* was highly prevalent in Africa. That disagree with what reported by Elsokkary *et al*. (2009), the species recorded in Egypt were *Paramphistomum microbothrium*, *Paramphistomum cervi*, *Carmyerius* gregarius and *Cotylophoron cotylophrum*

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