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MORPHOLOGICAL CHARACTERISTICS OF *DICROCOELIUM DENDRITICUM* (DIGENEA, DICROCOELIIDAE), PARASITIZING THREE HOST SPECIES IN THE CENTRAL REGIONS OF UKRAINE

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Morphological Characteristics of *Dicrocoelium dendriticum* (Digenea, Dicrocoeliidae), Parasitizing Three Host Species in the Central Regions of Ukraine. Kruchynenko, O. V., Mykhailiutenko, S. M., Klymenko, O. S., Kanivets, N. S., Korchan, L. M. — Distribution of trematodes of the species *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899 (Digenea, Dicrocoeliidae) parasitizing cattle (*Bos taurus* Linnaeus, 1758), sheep (*Ovis aries* Linnaeus, 1758) and goat (*Capra aegagrus hircus* Linnaeus, 1758) was studied in the Poltava and Kirovohrad regions of Ukraine. The prevalence of infection is 26.9 % in cattle, 28.42 % in sheep and 24.5 % in goat. *D. dendriticum* is the only species of the genus *Dicrocoelium* which infects domestic animals in the central regions of Ukraine. The trematodes obtained from cattle are significantly different from those isolated from sheep and goat by length, width and area of body, and length of vitelline ducts.
Key words: dicrocoelium, cattle, sheep, goats, morphological characters, prevalence.

Introduction

Dicrocoeliasis is a dangerous and widely distributed disease caused by trematodes of three species, *Dicrocoelium dendriticum* (Rudolphi, 1819), *Dicrocoelium hospes* (Looss, 1907) and *Dicrocoelium chinensis* (Sudarikov and Ryjikov, 1951), known in the genus *Dicrocoelium* (Digenea, Dicrocoeliidae) (Otranto et al., 2007; Maurelli et al., 2007). The trematodoses are associated with significant economic losses of animal farms, because the mature helminthes live in the liver ducts of ruminants. According to a number of reports, the most widely distributed *Dicrocoelium* species is *D dendriticum*. Its main hosts are ruminants. However, there are records of sporadic *D. dendriticum* infections in humans (Cengiz et al., 2010; Jeandron et al., 2011). There are reports of dicrocoeliasis of wild rabbits (Casanova et al., 1995), European hares and black jackrabbits (Liatis et al., 2017; Sergi et al., 2018), moose (Beck et al., 2014), roe deer (Liatis et al., 2017), wild boar and brown bears (Maslennikova, 2015), and in horses (Hazlett et al., 2018). In Slovakia, dicrocoeliasis has been recorded in mouflon and red deer (Iglódyová et al., 2017).

Scientists report the spread of dicrocoeliasis in cattle in Algiers (Chougar et al., 2019) and Nigeria (Elelu and Eisler, 2017). In Iran, these trematode infections have been found in cattle, sheep and goat (Arbabi et al., 2011; Khanjari et al., 2014; Mohamadzadeh et al., 2016; Majidi-Rad et al., 2018). The prevalence of dicrocoeliasis at sheep farms of Sardinia is 25.5 % in average (Scala et al., 2019). The pathogen has been found at the territory of the Russian Federation, with rates of infection ranging from 0.02 % to 20.6 % in cattle, and from 51.6 % to 84.1 % in Caspian red deer (Shmakova, 2019).

In Ukraine, only trematodes of the species *D. dendriticum* have been found by now. Monitoring surveys have regularly been conducted in cattle and small ruminants. Dicrocoeliasis is relatively common in cattle in Southern Ukraine (Soroka et al., 2015) and in sheep and goat in the zone of wood-and-steppe (Boyko, 2015; Korchan, 2015).

The determination of these parasites requires taking their morphological specifics in consideration. For example, *D. chinensis* was confirmed with a morphometric study in wild deer (*Cervus nippon centralis*) of Japan. The mean length of body of trematodes was 9.0 mm, and the mean maximum width of body was 3.0 mm. The mean outer diameter of oral sucker 439.0 μm, and that of ventral sucker was 559.6 μm (Taira et al., 2006).

Dicrocoelium spp. are helminthes of lancet shape, with weakly developed suckers of similar size in the anterior part of body. The mouth opens in the centre of oral sucker into the pharynx, which enters into a short thin esophagus with two straight branches of intestinal caeca, located alongside the body and lacking other openings. The trematodes are hermaphroditic. The uterus fills the trematode's hindbody and consists of descending and ascending branches (Shelyakin and Stepanov, 2013).

Hence, study the distribution and morphological characteristics of *Dicrocoelium dendriticum* in cattle, sheep and goat of Poltava and Kirovohrad regions of Ukraine is aimed to add new data on the variability of metric parameters of trematodes in various ruminant species.

Material and methods

The studies were conducted in 2018–2019 at the Laboratory of Parasitology and Veterinary-Sanitary Expertise of the Faculty of Veterinary Medicine of Poltava State Agrarian Academy. The prevalence of *Dicrocoelium dendriticum* were studied at livestock farming, sheep and goats farms of Poltava and Kirovograd Regions of Ukraine. The main indicators were the extensiveness and the intensity of infection (Ripolovskyi and Yuskiv, 2010). Trematodes were collected during helminthological investigation of the liver of dead or killed cattle, sheep and goats (Skrjabin, 1928). Flukes were identified according to Ivashkin & Mukhamadiev (1981) and Ivashkin et al. (1989).

The trematodes were obtained from livers during the killing of ruminants, and fixed in 70 % ethanol. Then they were mounted on slides in lactophenol and covered with cover slips. The specimens were examined and identified using the light microscope Zeiss Axio Imager M1 at the Schmalhausen Institute of Zoology (Kyiv, Ukraine) in interactive mode using ×4 objective. In total, 1103 trematodes were collected (379 specimens from cattle, 456 from sheep and 268 from goat). The morphometric parameters of *Dicrocoelium dendriticum* were studied on 30 specimens from each host species.

Statistical processing of obtained data was performed using software package STATISTICA 10 (StatSoft Inc., USA, 2011). For parameters of trematodes, mean and standard deviation (M± SD) were calculated. The Shapiro–Wilk test was used to test the distribution of parameters for normality. The morphometric parameters of *Dicrocoelium dendriticum* trematodes from various ruminant species were compared with one-way ANOVA, equality of variance in groups was compared with Leven's criterion (if equality was not found, Brown-Forsythe criterion was used). If the Gauss distribution of data were not confirmed, Kruskal-Walles criterion was used. Statistical significant of results was observed at $P < 0.05$. Risk factors were evaluated using the Pearson Chi-square test. A significant association between animal positivity to dicrocoeliasis and the sex, age, and season was determined if $P < 0.05$. All sampled cattle, sheep and goats were categorized into three age groups: < 2 years, 2–4 years, and > 4 years and finally the appearance of liver during the inspection. Confidence intervals of 95 % of the dicrocoeliasis in animals were calculated with Open Source Epidemiologic Statistics for Public Health, Version 3.01, updated 2013/04/06 (www.OpenEpi.com). Wilson's method was

used to determine the best estimate for frequencies and ratios, including for a small number of observations (Wilson, 1927) by the following formula:

$$\text{from} \quad \frac{p+\frac{z^2_{1-\alpha/2}}{2N}-z_{1-\alpha/2}\sqrt{\frac{p(1-p)}{N}+\frac{z^2_{1-\alpha/2}}{4N^2}}}{1+\frac{z^2_{1-\alpha/2}}{N}}$$

$$\text{to} \quad \frac{p+\frac{z^2_{1-\alpha/2}}{2N}+z_{1-\alpha/2}\sqrt{\frac{p(1-p)}{N}+\frac{z^2_{1-\alpha/2}}{4N^2}}}{1+\frac{z^2_{1-\alpha/2}}{N}},$$

where Z1 — α / 2 equals 1.96 for confidence interval of 95 %, N — number of observations, and p — frequency of periodicity of the parameter in sample.

Spearman's rank correlation coefficient (r_s) was used to estimate the dependence between variables: length of body (BL) / area of body (BA).

Results and discussion

The dicrocoeliasis infection was found in 20.2 to 40.51 % ruminants in Poltava and Kirovohrad Regions of Ukraine (table 1). The rate of infection was 18–104 helminthes per host in cows, 26–789 per one sheep and 23–495 per one goat.

Table 1. Prevalence of *D. dendriticum* in cattle, sheep and goat slaughtered in various regions in Ukraine

Regions	Cattle			Sheep			Goats		
	No. examined	No. of infected	Infected liver, %	No. examined	No. of infected	Infected liver, %	No. examined	No. of infected	Infected liver, %
Poltava	161	48	26.7	175	36	20.6	173	47	27.2
Kirovohrad	102	23	22.54	116	47	40.51	104	21	20.2
Total	263	71	26.9	292	83	28.42	277	68	24.5

Table 2. Summary of positive cases according to risk factors (seasons, sex, and age)

Risk factors	Cattle		Sheep		Goat	
	infection		infection		infection	
	+ (%)	– (%)	+ (%)	– (%)	+ (%)	– (%)
			Seasons			
Autumn	17 (6.5)	28 (10.6)	23 (7.9)	36 (12.32)	19 (6.8)	47 (16.9)
Winter	34 (12.9)	59 (22.4)	39 (13.4)	53 (18.15)	32 (11.5)	63 (22.7)
Spring	12 (4.6)	52 (19.8)	14 (4.7)	61 (20.9)	11 (3.9)	57 (20.6)
Summer	8 (3.04)	53 (20.15)	7 (2.4)	59 (20.2)	6 (2.2)	42 (15.2)
			Sex			
Male	28 (10.6)	111 (35.4)	42 (14.4)	115 (38.01)	51 (18.41)	129 (46.6)
Female	41 (15.6)	93 (42.2)	31 (10.6)	94 (33.6)	17 (6.14)	80 (28.9)
			Age (years)			
Aged (> 4)	42 (15.9)	61 (23.2)	51 (17.5)	63 (21.6)	39 (14.1)	58 (20.9)
Intermediate (2-4)	20 (7.6)	49 (18.63)	25 (8.6)	45 (15.41)	17 (6.1)	40 (14.4)
Young (< 2)	9 (3.42)	95 (36.1)	7 (2.4)	101 (34.6)	10 (3.6)	113 (40.8)

The factors (season, sex and age) are the same in the two study regions (Poltava and Kirovograd); therefore, the entire cattle, sheep and goat herd in the two regions was selected for the statistical study. The highest rates of infection were seen in all host species in autumn and winter. Significant relation was observed in cattle, sheep and goat between the rate of *D. dendriticum* infection and the season ($P < 0.01$) (table 2). We also saw the difference in rates of infection in female and male hosts. For cattle, the prevalence of infection was higher in females than in males ($P < 0.05$). There was no statistically significant difference between rates of infection in female and male sheep. In contrast, rates of infection were higher in male goats than in female goats ($P < 0.05$). We also found higher rates of infection in aged ruminants (older than four years) ($P < 0.001$).

According to our biometric studies, only *D. dendriticum* was found in cattle, sheep and goat hosts in Ukraine (fig. 1).

Metric examination of specimens revealed the highest mean length of body in trematodes obtained from catle was 6.2 ± 1.23 mm, with maximum length of 8.8 mm. Mean lengths of body in trematodes obtained from sheep and goat were almost the same, 5.7 ± 1.01 and 5.6 ± 0.94 mm, respectively. Frequency distribution of length of body in each host species is given on figure 2. The smallest trematodes were 4.2–4.3 mm in length.

The mean width of body was 2.0 ± 0.4 mm at most in studied specimens obtained from cattle. Maximum mean width of body was 1.9 ± 0.44 mm in trematodes obtained from sheep, 1.3 ± 0.25 mm for goat parasites.

We found the maximum body area in *D. dendriticum* trematodes obtained from cattle (8.45 ± 2.1 mm^2), smaller areas in trematodes from sheep (7.9 ± 1.5 mm^2) and goat (7.23 ± 1.6 mm^2).

Length of oral sucker was, in average, smaller than that of ventral sucker by 0.11 % in specimens from cattle, by 0.17 % in those obtained from sheep, and by 0.21 % in trematodes collected from goat (table 3).

The two testes are located directly posterior to acetabulum, slantwise to each other. They are round to weakly or strongly lobed in shape.

The ovary is located posterior to testes. The eggs are yellow to dark brown in color, without any significant mean fluctuations of metric parameters relative to host species. Vitelline ducts are formed by numerous small and merged large follicles, and located in the middle of liver fluke body. We measured the parameters of left and right vitelline ducts, which were the largest in *D. dendriticum* trematodes collected from cattle.

Twelve of 22 morphological parameters measured in trematodes of cattle, sheep and goat, were statistically different. According to results of ANOVA (table 3), there were differences ni length of body ($P = 0.047$), width of body ($P = 0.001$), area of body $F_{2.12} = 3.4$ ($P = 0.038$), oral sucker width ($P = 0.024$), pharynx length ($P = 0.003$), pharynx width ($P = 0.028$), anterior testis length ($P = 0.001$), posterior testis width ($P = 0.01$), posterior testis width ($P = 0.02$). It is also observed that the length of left and right testes were statisti-

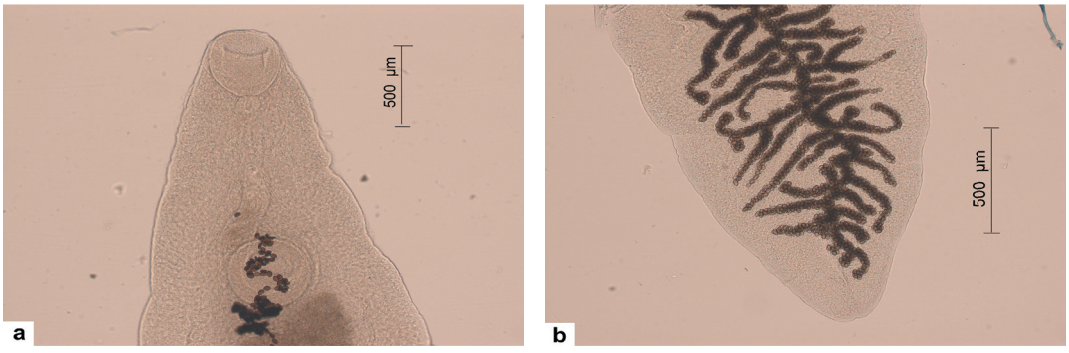

Fig. 1. Anterior and tail ends of *D. dendriticum* (a and b); x4.

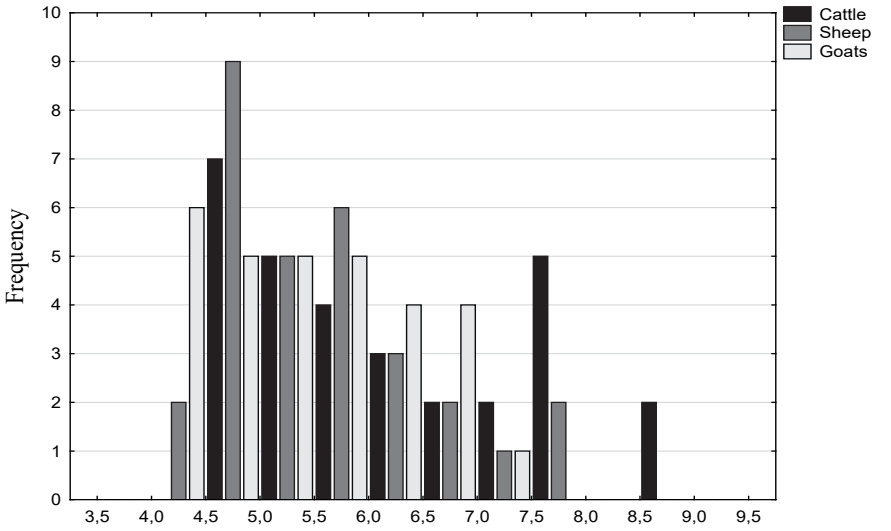

Fig. 2. Frequency distribution of body length of *Dicrocoelium* specimens from naturally infected cattle, sheep and goat (n = 30).

cally different.

We found the significant direct correlation between the length and area of body of lancet flukes ($P < 0.05$). The correlation coefficient r_s was 0.94 in trematodes from cattle, 0.9 in parasites from sheep and 0.93 in helminthes from goat.

We also observed the highest prevalence of infection in sheep (28.42 %), average in cattle (26.9 %), and smallest in goat (24.5 %).

Our data corresponds to the findings of other authors, who find the highest rates of infection in sheep (1.14 %) compared to cattle and goat (0.60 %) in Erak City of Iran (Arbabi et al., 2018 b). Majidi-Rad et al. (2018) report that in Guilan provincerom

Table 3. Morphometry of adult *D. dendriticum*, collected from naturally infected cattle, sheep and goat, x ± SD (min–max), n = 30

Characters	Cattle	Sheep	Goats	PValue
Body length, mm	6.2 ± 1.23 (4.9–8.8)	5.7 ± 1.01 (4.3–8.0)	5.6 ± 0.94 4.2–7.5)	0.047
Body width, mm	2.0 ± 0.4 (1.2–2.7)	1.9 ± 0.44 (1.2–2.5)	1.3 ± 0.25 (1.0–2.0)	0.001
Body area, mm^2	8.45 ± 2.1 (5.0–12.6)	7.9 ± 1.5 (5.9–11.9)	7.23 ± 1.6 (4.2–10.4)	0.038
Oral sucker length, μm	348.5 ± 77.1 (265.0–495.0)	330.8 ± 69.6 (214.0–480.0)	320.0 ± 61.8 (201.0–405.0)	0.55
Oral sucker width, μm	372.8 ± 77.4 (235.0–499.0)	330.2 ± 56.4 (207.0–480.0)	319.6 ± 60.3 (148.0–400.0)	0.024
Pharynx length, μm	151.8 ± 3.23 (145.0–157.0)	151.4 ± 3.2 (145.0–159.0)	149.03 ± 2.9 (145.0–155.0)	0.003
Pharynx width, μm	124.3 ± 18.8 (98.0–159.0)	130.2 ± 19.7 (100.0–154.0)	137.2 ± 2.9 (110.0–155.0)	0.028
Oesophagus length, μm	458.3 ± 45.3 (400.0–544.0)	480.3 ± 44.7 (410.0–540.0)	467.3 ± 42.9 (400.0–530.0)	0.21
Ventral sucker length, μm	389.6 ± 54.6 (312.0–499.0)	396.2 ± 42.9 (312.0–490.0)	404.7 ± 45.3 (329.0–498.0)	0.65
Ventral sucker width, μm	372.3 ± 53.5 (265.0–446.0)	390.1 ± 44.6 (268.0–440.0)	380.5 ± 41.8 (255.0–445.0)	0.39
Ovarium length, μm	149.2 ± 17.6 (115.0–193.0)	151.0 ± 20.9 (120.0–193.0)	153.5 ± 10.5 (130.0–170.0)	0.074
Ovarium width, μm	247.9 ± 14.3 (214.0 – 274.0)	250.4 ± 26.8 (212.0 – 310.0)	255.1 ± 26.8 (219.0 – 290.0)	0.35
Anterior testis length, μm	311.5 ± 155.6 (139.0 – 811.0)	479.1 ± 210.04 (221.0–903.0)	405.5 ± 135.1 (220.0 – 698.0)	0.001
Anterior testis width, μm	480.3 ± 92.9 (282.0–615.0)	465.4 ± 144.8 (275.0–858.0)	467.9 ± 72.6 (292.0–640.0)	0.36
Posterior testis length, μm	408.5 ± 226.5 (250.0–780.0)	504.6 ± 226.5 (269.0–905.0)	353.8 ± 115.8 (214.0–700.0)	0.01
Posterior testis width, μm	500.03 ± 118.7 (282.0–710.0)	551.6 ± 64.2 (470.0–685.0)	484.5 ± 86.4 (323.0–695.0)	0.02
Left vitelline length, μm	1408.7 ± 273.9 (990.0–1964.0)	1202.7 ± 179.04 (906.0–1620.0)	1315.8 ± 248.2 (970.0–1880)	0.003
Left vitelline width, μm	339.9 ± 101.1 (154.0–475.0)	281.8 ± 75.8 (164.0–420.0)	339.1 ± 83.7 (193.0–476.0)	0.037
Right vitelline length, μm	1391.9 ± 322.6 (995.0–2150.0)	1142.2 ± 210.9 (870.0–1679)	1311.7 ± 204.5 (904.0–1763.0)	0.001
Right vitelline width, μm	343.8 ± 80.9 (211.0–450.0)	290.3 ± 87.5 (182.0–480.0)	331.8 ± 75.9 (210.0–493.0)	0.025
Egg length, μm	39.9 ± 1.5 (37.0–45.0)	39.2 ± 1.6 (37.0–44.0)	39.7 ± 1.24 (37.0–43.0)	0.32
Egg width, μm	26.6 ± 1.6 (25.0–30.0)	26.7 ± 2.01 (24.0–30.0)	27.03 ± 2.05 (24.0–30.0)	0.71

Caspian Sea Littoral, Northern part of Iran sheep are more frequently infected with dicrocoeliasis compared to cattle. Khanjari et al. found higher prevalence of dicrocoeliasis in sheep compared to goat in Amol Abattoir, Mazandaran, northern Iran (Khanjari et al., 2014). Conversely, other scientists noted the higher prevalence of dicrocoeliasis in cattle (4.81 %) compared to small cattle (2.33 %) in western Iran (Shahbazi and Chalehchaleh, 2017). The prevalence of infection was as high as 25.5 % in Sardinia, Italy (Scala et al., 2019). In Algiers, the overall prevalence of dicrocoeliasis in cattle was 0.52 % (Chougar et al., 2019). At farms of Altai region, the prevalence was as high as 20.6 % in cattle (Shmakova, 2019).

We find that there are seasonal and age dynamics of dicrocoeliasis. For example, the highest prevalence of infection was observed in cattle, sheep and goat in autumn and winter. However, there are findings of winter to spring peak of prevalence of dicrocoeliasis in goat (Korchan, 2015). In warm climate, significant relation between the season and the prevalence of infection was not found (Arbabi et al., 2011 a; Chougar et al., 2019). In Iran, the distribution rates of dicrocoeliasis were much higher in summer compared to winter (Shahbazi and Chalehchaleh, 2017).

We noted that in ruminants the rates of infection increase with time, which is in agreement with a number of other reports (Beck et al., 2014; Korchan, 2015). It has been observed that in females, rates of infection are higher than in males (Chougar et al., 2019).

Our findings regarding the area of body of lancet flukes obtained from cattle, sheep and goat are in line with those of other scientists. Thus, the comparison of metric parameters of trematodes obtained from cow and sheep revealed that the mean body area of trematodes collected from cow was 7.8 mm^2, and that of trematodes obtained from sheep was 6.6 mm^2. The length of trematode body was 6.5 mm and 5.7 mm, respectively (Beck et al., 2015).

Other scientists have found that the mean length of body of *D. dendriticum* obtained from sheep was 7.2 mm, and the mean width of body was 2.5 mm (Kuchai et al., 2011). In our study, the maximum length of body in trematodes obtained from sheep was up to 8.0 mm, and the maximum width of trematode body was 2.5 mm. In their research, the scientists of Algiers have indicated significantly smaller metric parameters: the trematode body was no longer than 4.6 mm, and 1.2 wide (Chougar et al., 2019).

The scientists from Russian Federation have also considered the morphological and metric parameters of lancet flukes from various animal hosts. Their results are in line with the described variability of trematodes. According to their data, mean length of body of lancet flukes was 7.5 mm in cattle, and ranged from 5 mm to 7.0 mm in sheep and goat. The differences in width of body were not statistically significant. The authors also have established a high coefficient of direct correlation between the length of body and length of vitelline ducts in trematodes (Shelyakin and Stepanov, 2013). In our study, the correlation was found between the length of body and body area of trematodes.

Conclusion

Trematode species *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899 (Digenea, Dicrocoeliidae) infects cattle (*Bos taurus* Linnaeus, 1758), sheep (*Ovis aries* Linnaeus, 1758) and domestic goat (*Capra aegagrus hircus* Linnaeus, 1758) in the central regions of Ukraine. Local prevalence of dicrocoeliasis is 26.9 % in cattle, 28.42 % in sheep and 24.5 % in goat. Rates of infection are 18–104 trematodes per cow, 26–789 trematodes per sheep and 23–495 trematodes per goat. The peak of dicrocoeliasis infection is observed in autumn and winter, and the intensity of infection is higher of aged animals (over four years old). The significant variability of morphometric parameters of trematodes depends on the host species. Thus, the mean length and width of body, and length of vitelline ducts of *D. dendriticum* collected from cattle are higher than those of trematodes obtained from sheep and goat. A strong direct correlation is established between the length of body and area of body of lancet flukes ($P < 0.05$).

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