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MORPHOLOGICAL OBSERVATION ON LIVER FLUKE DETECTED FROM NATURALLY INFECTED CARABAOS IN THE PHILIPPINES

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Abstract

An attempt was made to clarify the species of liver flukes in naturally infected carabaos in the Philippines. The fluke collected from these carabaos were divided into three types which were similar to three established species, respectively, type A (like *F. hepatica*), type B (like *F. indica*), and type C (like *F. gigantica*), according to the shape and size of the adult. It was difficult, however, to distinguish one of the three types exactly from the others only by the differences in the shape and size of the adult and egg.

Introduction

Fascioliasis or distomatosis, otherwise known as liver rot, is common in the Philippines principally affecting cattle, carabaos, sheep and goats. This disease is caused by two species of the common liver flukes known as *Fasciola hepatica* and *F. gigantica*[1,9,12]. Recently, some workers have made it clear that the specimens in Japan and Taiwan differ from common European species, *F. hepatica*, rather resemble the African species, *F. gigantica*, an intermediate type between these two, and varieties of these three types. The intermediate type of the Japanese liver fluke is akin to *F. indica* described by VARMA in 1953[2-4,7,8,11,14,16]. It is required, therefore, to reconsider on the differentiation of species of the common liver flukes in the southeastern part of Asia.

This paper deals with the results of observation on the morphological difference between adult worms and eggs collected from naturally infected carabaos in the Philippines.

Materials and Methods

Specimens were collected from 5 carabaos naturally infected with liver flukes. They were pressed gently between two slides with two pieces of folded filter paper at both ends, and fixed in 10% formalin. Then they were stained with DELAFIELD's hematoxylin and examined for the shape of the adult and the arrangement and size of its internal organs. Macroscopically, all the specimens were divided into 3 types according to the length-breadth ratio of the body; that is, type A with a ratio of 1.7-2.0 : 1, type B with a ratio of 2.1-2.9 : 1, and type C with a ratio of 3.0-4.3 : 1.

Eggs were got from a fixed adult worm by cutting a part of the body immediately posterior to the ventral sucker, where the uterus contains mature eggs, and measured by a micrometer without using a coverglass.

Results

1. Size and outward appearance of the adult.

The adult worm of type A had a longer and broader body than that of type B or C. Its average length was 18.3 mm in 4 specimens. Its average breadth was 10.6mm. The length-breadth ratio of the body was 1.9 on the average. The shoulder was distinguishable. The breadth reached a maximum about the middle of the body and was reduced fairly abruptly toward both ends of the body.

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Table 1. Sizes of the external form, acetabulum, pharynx, ovary and testis of flukes

Type of flukes		Type A	Type B	Type C
No. of flukes examined		4	83	38
Body-size (mm)	Length	15.1-20.5 (18.3)	16.3-29.2 (22.1)	25.0-37.0 (31.2)
	Breadth	9.2-11.9 (10.6)	7.3-11.0 (9.1)	7.1-10.2 (8.5)
Body length/breadth ratio		1.7-2.0 (1.9)	2.1-2.9 (2.5)	3.0-4.3 (3.7)
Oral sucker (mm)		0.8-1.0×0.7-1.0 (1.0×0.7)	0.8-1.1×0.5-1.0 (1.0×0.8)	0.8-1.1×0.6-1.0 (1.0×0.8)
Ventral sucker (mm)		1.4-1.8×1.3-1.7 (1.5×1.4)	1.3-1.8×1.2-1.7 (1.5×1.4)	1.5-1.9×1.3-1.6 (1.6×1.5)
Ventral/oral sucker ratio		1.38-1.75 (1.54)	1.36-1.86 (1.58)	1.48-1.95 (1.64)
Pharynx (mm)	Length	0.51-0.92 (0.82)	0.47-1.03 (0.85)	0.67-0.99 (0.88)
	Breadth	0.40-0.73 (0.50)	0.39-0.83 (0.53)	0.37-0.86 (0.55)
Branching of ovary		8-15 (10)	8-19 (14)	13-27 (19)
Testis (mm)	Length	7.21-11.32 (8.25)	7.84-15.97 (10.68)	12.48-19.69 (15.46)
Testicular/body length ratio		0.38-0.62 (0.51)	0.40-0.66 (0.48)	0.44-0.78 (0.47)

( ) : Mean.

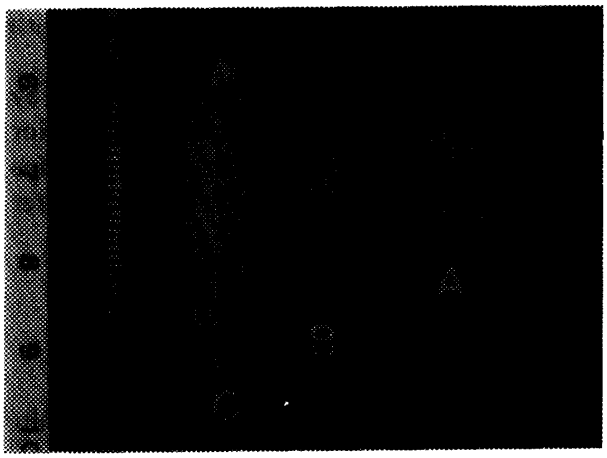


Fig. 1. Each type of specimens. A : Type A (like *F. hepatica*). B : Type B (like *F. indica*). C : Type C (like *F. gigantica*).

The adult worm of type B was 22.1 mm in length and 9.1 mm breadth, both being the average values for 83 specimens. The length-breadth ratio of the body was 2.5 on the average. The shoulder was comparatively distinguishable. The body was the broadest at the middle of the body and narrowed gradually toward the rundish posterior end.

The adult worm of type C had the longest body of all the types. Its average length was 31.2 mm in 38 specimens. Its body was the least broad, or 8.5 mm on the average, of all the types. The length-breadth ratio of the body was 3.7 on the average. The shoulder was not clearly recognized. Both sides of the body were nearly parallel to each other. The body was roundish at its posterior end. The worm was elongate in shape (Table 1 and Fig. 1).

2. Suckers.

The ventral sucker was larger than the oral sucker. Table 1 showns the sizes of the suckers and the ratio of the ventral sucker to the oral one. The average of this ratio was 1.54 in the group of type A, 1.58 in the group of type B, and 1.64 in the group of type C.

Table 2. Sizes of the eggs in the uterus of flukes

Type of flukes		Type A	Type B	Type C
No. of eggs examined		400	4,600	1,800
Egg-size (μm)	Length	113.3-163.3 (141.1)	105.0-201.3 (148.6)	125.0-171.7 (147.6)
	Breadth	65.0-113.3 (90.0)	58.3-146.7 (89.6)	75.0-133.3 (91.4)

( ) : Mean.

3. Pharynx and Oesophagus.

The pharynx was well developed and opened into the very short oesophagus. The average of the side of the pharynx was 0.82×0.50 mm in the group of type A, 0.85×0.53 mm in the group of type B, and 0.88×0.55mm in the group of type C (Table 1).

4. Intestine.

The general dendriform of the alimentary system was typical in no group of any type. In the group of type B, the intestine showed such a pattern as intermediate between those characteristic of the group of type A and C. Principal lateral branches were larger in number in the group of type C than in the group of any other type.

5. Ovary.

The ovary is deer horn-formed and is situated in the left side in front of Mehils' gland by ventrally view. The branching of the ovary were larger in number in the group of type C than in the group of any other type. Its average branching was 10, 14, and 19, in specimens of type A, B, and C, respectively (Table 1).

6. Testes.

The testes consist of anterior and posterior testis and both testes are much branched like tree and are located near the abdominal surface of digestive tracts. The testicular area, however, was much shorter in the specimens of type A, in which it occupied more than half the total length of the body. The ratio of testicular area to body length was 0.51, 0.48, and 0.47, on the average, in the specimens of type A, B, and C, respectively. The testes were racemose and very well developed in every specimen of each type (Table 1).

7. Eggs.

Eggs were collected from the uterus of the specimens. The number of eggs counted were 400, 4,600, and 1,800 in the groups of type A, B, and C, respectivly. The range of egg-size was $113.3\text{-}163.3 \times 65.0\text{-}113.3$ μm in the group of type A, $105.0\text{-}201.3 \times 58.3\text{-}146.7$ μm in the group of type B, and $125.0\text{-}171.7 \times 75.0\text{-}133.3$ μm in the group of type C. Its average egg-size was $141.1 \times 90.0\mu$m, $148.6 \times 89.6\mu$m, and 147.6×91.4 μm, in the group of type A, B, and C, respectively (Table 2).

Discussion

The common liver fluke is responsible for a serious disease in many parts of the world and is of particular importance in the Philippines[9,12]. ARANEZ[1] reported a higher incidence of the liver flukes both in cattle and carabaos. He found *F. hepatica* to occur in 4.73% of cattle and 11.5 % of carabaos while *F. gigantica* in 0.36% and 2.10 %, respectively, in the Philippines.

Up to date, numerous reports have been made on the genus of the parasite. HUANG et al.[3] classified the liver flukes collected from cattle in Taiwan and recognized the presence of *F. hepatica*, *F. gigantica*, and an intermediate type between these two. In Japan, WATANABE[14,15] mentioned that the Japanese liver fluke was divided into a few types on the basis of the shape of the adult; for example, one type similar to *F. hepatica*, another to *F. gigantica*, an third type intermediate between these two. The intermediate type of the Japanese liver fluke is

akin to *F. indica*. ITAGAKI and AKANE[4] classified the liver flukes found in Japan and recognized the presence of *F. hepatica*, *F. gigantica*, and *F. indica*, and their variation in the shape of the adult and in the size of the egg. OSHIMA et al.[10] studied patterns of variation in the liver flukes collected from cattle and goats in Japan. They recognized a single group showing such a wide range of variation in shape and size of worms and eggs that in covered the ranges of all the three species, *F. hepatica*, *F. gigantica*, and *F. indica*.

VARMA[13] described a new species of liver flukes which he had collected from goats, cattle, and buffaloes in Bihar, and named it *F. indica* n sp. He differentiated it from *F. hepatica* and *F. gigantica* by the external features, cuticle, acetabulum, intestine, and testes of the adult and the size of the egg.

In differentiating the three species, much emphasis has been placed on the shape and size of the adult body. JACKSON[5] stated, however, in his review of literature on *F. gigantica* that neither shape nor size of the adult body could be used for the differentiation of species because of the distortion of the body inevitably produced by maceration and fixation, unless there were distinct anatomical differences among the species. Nevertheless, his condensed description of *F. gigantica* contains the following point: body elongated and at least three time as long as broad. Prominent shoulders always absent. As a rule, both side of the body are roughly parallel. The posterior extremity of the body is bluntly rounded or, more rarely, bluntly pointed. Furthermore, he performed a comparative study with quite a large number of fresh spesimens, and revealed some points of specific importance in the system of gut-branching, cuticular armature, and eggs. KENDALL[6] considered the two species of *Fasciola*, *F. hepatica* and *F. gigantica*, were valid. It is very diffcult, however, to discriminate between these two species accurately because there are too any variations in the morphological characters used for classification.

As for the shape of the adult, the specimens of type C were distinguished from those of type A, since they were slender and tape-like in shape. The specimens of type A, B, and C were akin to *F. hepatica*, *F. indica*, and *F. gigantica*, respectively. Their eggs collected from the uterus of the specimens showed a wide variation in size. It was difficult, however, to distinguish these types by the differences in the shape and size of the adult and egg, though materials from each group can be classified macroscopically into the respective group from the shape of the adult.

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フィリピン産肝蛭の形態学的観察

木村　重・清水　晃・河野　潤一

要　　約

家畜に寄生する *Fasciola* 属の種類として *F. hepatica* と *F. gigantica* の2種が，古くから知られている。また，両種の亜種ないし中間種の存在についても検討され，*F. indica* が報告されている。近年，アジア諸国における肝蛭の種類について再検討が行われており，とくに日本産肝蛭では，前記3種の存在を肯定するもの，あるいは *F. hepatica* の存在を否定する報告があり，分類学的位置は未だ明らかにされていない。今回，Carabao から採集したフィリピン産肝蛭を形態学的に観察し，種類の同定を試みた。

肝蛭を分類するとき，体長と体幅の比率による区分が重要な指標とされている。今回の観察においても，その比率が2.0以下をA群，2.1—2.9をB群及び3.0以上をC群に区分して検討した。

各群の虫体の外形，内部器官の構造及び虫卵の大きさなどから，A群は*F. hepatica*，B群は *F. indica*，C群は *F, gigantica*にそれぞれ近似するものであった。しかし，一部の虫体と虫卵の外形または計測値は，互いに重複あるいは連続しているものもあり，明確に区分することはできなかった。