The Geographical Distribution of Common Liver Flukes (the Genus *Fasciola*) with Normal and Abnormal Spermatogenesis

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Abstract. The geographical distribution of the common liver flukes (of the genus *Fasciola*) was investigated on 916 specimens collected at 34 localities in 22 countries of the world. The flukes were divided into two types, the normal and the abnormal spermatogenic, by the number of spermatozoa in the seminal vesicle. Both *Fasciola hepatica* and *F. gigantica* from Europe, South and North Americas, Oceania, and Africa belonged to the normal spermatogenic type which had numerous spermatozoa in the vesicle. In Asia, not only the normal but also the abnormal spermatogenic type which has a few or no spermatozoa in the vesicle were found. Especially in Japan and Korea, only the abnormal spermatogenic type was observed.

Many papers reported cytological studies of the common liver flukes [2-5, 21, 23, 27], but none of them dealt with the abnormal spermatogenesis of the flukes. Recently, Sakaguchi [11], Sakaguchi and Nakagawa [16], and Sakaguchi and Yoneda [20] found that there were two chromosome types of the common liver flukes (the genus *Fasciola*) in Japan: one was the diploid type with 20 chromosomes, and the other was the triploid type with 30 chromosomes. Moriyama et al. [10] found another type which had both 20 and 30 chromosomes within a single fluke. Kayano and Cho [6] clarified that both diploid and triploid types of the Japanese common liver flukes performed meiotic division without pairing of homologous chromosomes, and especially a spermatocyte was divided unevenly and no fertilization

occurred. Moriyama and Terasaki [9] observed the process of the spermatogenesis in those types of the flukes in Japan using a differential phase contrast microscope, and recognized their abnormality in spermatogenesis.

In this study, the common liver flukes from various countries were observed to confirm the presence of spermatozoa in the seminal vesicle, and the world-wide geographical distribution of the flukes with normal or abnormal spermatogenesis was figured.

Materials and Methods

The materials (common liver flukes) used are shown in Table 1. Nine hundred and sixteen flukes were collected at 34 localities in 22 countries.

Eight hundred and eighty-eight flukes were used for mounted specimens, and most of them were

Table 1. Common liver flukes (*Fasciola* spp.) on which the present study is based and existence of spermatozoa in the seminal vesicle

Locality	Host	Infection*	Conditions of specimens†	No. of examined specimens‡			Spermatozoa§			Imma- ture speci- mens	Indis- tinct speci- mens	Total
				Mount.	Sec.	Ext.	++	+	—			
JAPAN												
Akita	Cattle	Nat.	A	4					3		1	4
Nagano	Cattle	Nat.	B	35					24	11		35
Kanagawa	Rabbit	Expt.	A	3					3			3"
	Sheep	Expt.	A	2					2			2"
Hyogo	Rabbit	Expt.	A	6				1	5			6#
Hiroshima	Cattle	Nat.	B	41	1			1	41			42
Fukuoka	Cattle	Nat.	B	320				6	310	4		320
	Swine	Nat.	B	4					4			4
KOREA												
Seoul	Cattle	Nat.	C	50	1			2	49			51
TAIWAN												
Tai-Pei	Cattle	Nat.	C	1					1			1
?	?	Nat.	A	4			2		1		1	4
PHILIPPINES												
Manila	Buffalo	Nat.	D	39	1	1	36	3	2			41
Leyte	Buffalo	Nat.	D	5			5					5
VIETNAM												
Haiphong	Cattle	Nat.	E	1		3	3		1			3
	Buffalo	Nat.	E	3			3					3
THAILAND												
Bangkok	Buffalo	Nat.	B	19		2	15	4				19
BURMA												
Rangoon	Cattle	Nat.	F			2	2					2
NEPAL	Buffalo	Nat.	F	34	3	4	2		11		28	41
INDIA												
Darjeeling	?	Nat.	A		1		1					1
Madras	Goat	Nat.	C	29	2		27	2	2			31
PAKISTAN												
Karachi	Buffalo	Nat.	A	3			2			1		3
	Sheep	Nat.	A	1							1	1
Quetta	Sheep	Nat.	C	13			13					13
Rawalpindi	Cattle	Nat.	B	15			15					15
	Goat	Nat.	B	1			1					1
U. S. S. R.												
Vladivostok	Cattle	Nat.	E & H	30	2		27				5	32
EGYPT												
Alexandria	Buffalo	Nat.	D	5			5					5
ETHIOPIA												
Addis Ababa	Cattle	Nat.	G	6			6					6
	Sheep	Nat.	G	3			3					3
KENYA												
Nairobi	Cattle	Nat.	A & F	26	1		17				10	27
ENGLAND												
	Rabbit	Expt.	A & F	5	1		6					6
	Goat	Expt.	A	1			1					1
	Sheep	Expt.	A	2			2					2

GERMANY										
Hamburg	Cattle	Nat.	D	13			13			13
HUNGARY										
	Sheep	Nat.	C	6			5		1	6
AUSTRALIA										
	Rabbit	Expt.	A & F	22			11		11	22
NEW ZEALAND										
	Cattle	Nat.	B	6			6			6
U. S. A.										
Washington	Cattle	Nat.	B	68	1	2	70		1	71
Texas	Cattle	Nat.	D	2			2			2
MEXICO										
Mexico city	Horse	Nat.	D	21			21			21
PERU										
Cajamarca	Swine	Nat.	A	1			1			1
	Sheep	Nat.	C	28			28			28
Tingo Maria	Cattle	Nat.	B	10	1	1	8		4	12
Total				888	15	13	359	19	459	916

*: Nat.; Natural infection (the flukes were collected from slaughter houses). Expt.; Experimental infection

†: A; Flattened, stained, and mounted. B; Alive. C; Flattened and fixed in alcohol. D; Flattened and fixed in formalin. E; Fixed in alcohol. F; Fixed in formalin. G; Flattened and fixed. H; Fixed in Carnoy solution.

‡: Mount.; Mounted specimens. Sec.; Sectioned specimens. Ext.; Extracted specimens of seminal vesicle.

§: ++; A large number of spermatozoa were observed. +; A few spermatozoa were observed. —; No spermatozoa was observed.

": The shape resembled *F. gigantica* (Length/Breadth=about 5.7).

#: The shape resembled *F. hepatica* (Length/Breadth=about 1.7).

fixed with 70% alcohol and stained with alumcarmin. Fifteen flukes were embedded in paraffin sectioned 10 μ m thick, and stained with hematoxylin-eosin. Thirteen flukes were dissected to remove their seminal vesicles.

Observation was made under the light microscope. Sectioned specimens were the most suitable for the observation of spermatozoa of the three—mounted, sectioned, and dessected specimens. Testes, sperm ducts, uteri, and ootypes of these specimens were observed in addition to seminal vesicles.

Results

Fig. 2 shows the seminal vesicles in the mounted (A, B, and C) and sectioned specimens (D and E), the contents of seminal vesicles in the dissected ones (F and G), the uterus (H) and the sperm ducts (I) in the mounted ones. Round germ cells called "rosettes" (E and G), broken cells, or no matter (B) were found in the seminal vesicles of some flukes without spermatozoa. A small number of spermatozoa (C) were found in the seminal vesicles of some flukes,

but they might not be functioning [6, 9, 22].

The specimens were divided into three types according to the number of spermatozoa in the seminal vesicle (Table 1), namely:— denoting those with no spermatozoa (Fig. 2, B, E, and G), + with a small number of spermatozoa (Fig. 2, C), and ++ with many spermatozoa in the vesicle (Fig. 2, A, D, and F).

Fig. 1 is a world map showing the localities listed in Table 1. Open part in the circles shows the ratio of the flukes with a few (+) and no (—) spermatozoa. The common liver flukes with many spermatozoa in the seminal vesicles were distributed in U. S. A. (Washington and Texas), Mexico, Peru, England, Germany, Hungary, Egypt, Ethiopia, Kenya, Pakistan, Burma, U.S.S.R. (Vladivostok), Australia, and New Zealand. Most of the specimens from Japan and Korea contained no spermatozoa and the remaining specimens contained a few sper-

Fig. 1. The geographical distribution of the common liver flukes of normal and abnormal spermatogenic types. Figures in parentheses show the number of specimens examined. Open part in each circle shows the ratio of abnormal spermatogenic specimens.

matozoa in the seminal vesicles. In India (Madras), Nepal, Thailand, Vietnam, the Philippines, and Taiwan, not only the flukes with many spermatozoa but also those with a few or no spermatozoa in the seminal vesicle were distributed.

Comparatively many spermatozoa were usually found in the uterus of the flukes which contained many spermatozoa in the seminal vesicle (Fig. 2, H). Some of the flukes from India (Madras) and Thailand which contained many spermatozoa in the seminal vesicle also contained many spermatozoa in the uterus. Other flukes from these areas contained no spermatozoa in the uterus. The fluke specimens collected in Japan and Korea, had no spermatozoa in the uterus, though they had a few or no spermatozoa in the vesicle.

Some of the fluke specimens with many spermatozoa in their seminal vesicles contained spermatozoa and/or round germ cells in their sperm ducts. Some of the flukes that had no or a few spermatozoa in their seminal vesicles contained round germ cells

or no matter in their sperm ducts, as observed in the specimens from Japan and Korea.

Almost all the flukes examined in this study contained no spermatozoa in the ootype, and only a few flukes contained some spermatozoa in it.

Discussion

The common liver flukes from Japan perform abnormal spermatogenesis [6, 9]. In this study, 416 flukes from Japan were observed, and 390 out of 400 flukes, which excluded immature flukes and the unclear mounted specimens, contained no spermatozoa. The remaining 10 flukes contained a few spermatozoa in the seminal vesicle. Sakaguchi et al. [11, 14] reported that the common liver flukes from Korea performed abnormal spermatogenesis. In the present material, 49 out of 51 flukes from Korea contained no spermatozoa and the remaining contained a few spermatozoa in the seminal vesicle. The flukes collected in Puerto Rico, Australia, and England were

reported to perform normal spermatogenesis [11, 13, 15, 17, 18]. The flukes from Australia and England used in this study contained many spermatozoa in the seminal vesicle.

These facts show that the flukes with a few or no spermatozoa perform abnormal spermatogenesis, while those with many spermatozoa in the seminal vesicle perform normal spermatogenesis.

We propose to call the common liver flukes, which perform normal spermatogenesis and contain many spermatozoa in the seminal vesicle, "normal spermatogenetic type", and those that perform abnormal spermatogenesis and contain a few or no spermatozoa in the vesicle, "abnormal spermatogenetic type".

Spermatogenetic type cannot be determined only by the observation of spermatozoa in the uterus, sperm duct and ootype but must be done by observing the spermatozoa in the seminal vesicle. Usually, most of the flukes with many spermatozoa in the vesicle contained many spermatozoa in the sperm duct also, but some contained only round germ cells in the duct. On the other hand, the flukes without spermatozoa in the vesicle contained no matters or round cells in the duct. Some specimens collected in such areas as India (Madras) and Thailand, where both the types of the flukes are distributed, occasionally contained many spermatozoa in the uterus, though no spermatozoa were found in the seminal vesicle. Those spermatozoa in the uterus may come from another fluke of the normal spermatogenetic type by copulation. The spermatozoa in the uterus, furthermore, were difficult to observe when many eggs existed in it. The seminal receptacle could not be located under the light microscope, and spermatozoa were rarely found in the ootype.

As shown in Fig. 1, all the specimens from Europe, South and North Americas, and Oceania, where mainly *F. hepatica* is considered to be distributed, and from Africa, where *F. gigantica* is dominant, belong to the normal spermatogenetic type. Both the normal and abnormal spermatogenetic types, however, are considered to be distributed in Asia. Much controversy exists among parasitologists in the classification of the *Fasciola* fluke from Asia. Especially in Japan and Korea, only the abnormal spermatogenetic type is known to be distributed.

Sakaguchi and Ueno [19] and Sakaguchi et al. [12, 13] reported that the normal spermatogenetic type was distributed in Puerto Rico, the abnormal spermatogenetic type in U. S. A. (Hawaii), and both of the types in India (Assam) in addition to the areas shown in Fig. 1. Sakaguchi et al. [13] showed the distribution of the normal spermatogenetic type in Thailand and India (Madras), where we found both of the types.

Kendall [7] and Bergeon and Laurent [1] found the distribution of *F. hepatica* in the high altitude areas of Pakistan and Ethiopia respectively. In the northern colder area of Asia such as Vladivostok in U. S. S. R., all the flukes had a body shape of *F. hepatica* and belonged to the normal spermatogenetic type. Only two flukes were obtained from Burma and they were the normal spermatogenetic type, but if more flukes could be observed, the abnormal spermatogenetic type flukes might also be found.

The common liver flukes of the genus *Fasciola* have been morphologically studied by many parasitologists for many years. Several specific names have been advocated to the genus *Fasciola* and some of them have been sunk as synonyms. Kendall [8] considered the two species of *Fasciola*, *F. he-*

patica and *F. gigantica*, were valid. It is very difficult, however, to discriminate between these two species accurately because there are too many variations in the morphological characters used for classification. As far as the Asian materials of *Fasciola* are concerned, the intermediate type is usually observed between those two species. Many Japanese researchers would avoid to give an definite specific name for the *Fasciola* specimens from Japan, and call them *Fasciola* sp. because of the difficulty in specific identification [24–26].

Watanabe and Iwata [26] divided *Fasciola* sp. from Japan into three morphological types: *gigantica*, *hepatica*, and *indica* types. In the present study, the *gigantica* type flukes were selectively used among the flukes collected in Kanagawa, Japan, and the *hepatica* type ones among the flukes collected in Hyogo, Japan. All the types of flukes collected from the other areas in Japan and Korea were also used for this study. What was common to all the flukes collected in Japan and Korea, regardless of their types, was that they perform abnormal spermatogenesis although they contained enough eggs in their uterus. The study of karyotypes of Japanese common liver flukes by Sakaguchi [11] is also concluded that all the flukes collected in Japan perform abnormal gametogenesis.

In such area as the south-eastern part of Asia, on the other hand, the flukes of abnormal spermatogenetic type were distributed sympatric with *F. hepatica* or *F. gigantica* which perform normal spermatogenesis, but those flukes are considered to be reproductively isolated from normal *F. hepatica* and *F. gigantica* because they perform parthenogenesis [6, 9, 11, 22]. It is clear that the abnormal spermatogenetic type of flukes belongs to a strain different from those to which the normal spermatogenetic type of *F. gigantica* or *F. hepatica* belongs.

At present, we cannot assert that to what taxon of classification these abnormal spermatogenetic type of flukes must be assigned. However, the study of the karyotype of the common liver flukes succeeded in dividing them into the diploid and the triploid types [11, 14, 20], and a further morphological study must be carried on the basis of the karyotype of the flukes. Such study is necessary for the classification of the abnormal spermatogenetic type of the liver fluke.

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要 約

精子形成異常を示す肝蛭の地理的分布: 寺崎邦生・赤羽啓栄・波部重久 (福岡大学医学部寄生虫学教室), 森山信子 (広島大学医学部寄生虫学教室)——日本産肝蛭の分類学的位置は未だ明確にされていない。そこで肝蛭の分類を再検討するために, 22 カ国 34 地方から集めた肝蛭の貯精嚢内の精子の状態を圧平標本, 切片標本, および摘出した貯精嚢の標本について観察し, 肝蛭を 2 型に分類した。1 つは貯精嚢内に多数の精子を認めるものであり, 他の 1 つはその中に精子を認めないか認めてもわずかしかないものである。前者を精子形成正常型, 後者を精子形成異常型とした。ヨーロッパ, 南北アメリカ, オセアニア, およびアフリカからの *Fasciola hepatica* や *F. gigantica* はいずれも精子形成正常型であった。一方アジアからの肝蛭は精子形成正常型のほかに精子形成異常型も認められ, 特に日本と韓国からの肝蛭はいずれもすべて精子形成異常型であった。

Explanation of Figures

Fig. 2. Existence of spermatozoa in genitalia of the common liver flukes.

A: Seminal vesicle with many spermatozoa in a whole mounted specimen. B: Seminal vesicle without spermatozoa of a whole mounted specimen. C: Seminal vesicle with a few spermatozoa in a mounted specimen. D: Seminal vesicle with many spermatozoa in a sectioned speci-

men. E: Seminal vesicle with "rosettes" instead of spermatozoa in a sectioned specimen. F: Removed seminal vesicle with many spermatozoa. G: Removed seminal vesicle with "rosettes". H: Uterus with many spermatozoa in a whole mounted specimen. I: Sperm duct with round germ cells in a whole mounted specimen.