HAWAII AGRICULTURAL EXPERIMENT STATION
HONOLULU, HAWAII

Under the joint supervision of the
UNIVERSITY OF HAWAII
and the
UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN NO. 80

OBSERVATIONS ON THE LIFE HISTORY OF FASCIOLA GIGANTICA, THE COMMON LIVER FLUKE OF CATTLE IN HAWAII, AND THE INTERMEDIATE HOST, FOSSARIA OLLULA

By

JOSEPH E. ALICATA, Parasitologist

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CONTENTS

INTRODUCTION

Fascioliasis is one of the outstanding parasitic diseases of cattle in the Hawaiian Islands. Knowledge of the presence of liver flukes in this region dates back at least to 1892 when Lutz (*7*)[1] made observations on the presence of flukes in cattle and pointed out that infestation existed on the islands of Kauai, Oahu, Maui, and Molokai. Fluke infestation is now also found on Hawaii.

The investigations herein described were undertaken principally to

[1] Reference is made by number (italic) to Literature Cited p. 20.

discover facts concerning the development and biology of the fluke and its intermediate hosts which might lead to practical methods of controlling the parasite in the field. At the start of this investigation, the late Dr. M. C. Hall, at that time Chief of the Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture, came to Hawaii at the request of the Hawaii Agricultural Experiment Station to make a preliminary survey of the liver-fluke situation and to give recommendations as to laboratory and field methods[2].

HISTORICAL RÉSUMÉ, GEOGRAPHICAL DISTRIBUTION, AND HOSTS OF FASCIOLA GIGANTICA

Fasciola gigantica was first described by Cobbold (*5*) in 1855; the specimens were taken from a giraffe in a traveling menagerie in England. In 1895, flukes taken from cattle slaughtered at St. Louis, Senegal, Africa, were described by Railliet (*12*) under the name of *Fasciola hepatica angusta.* These flukes were regarded by Blanchard (*3*) as identical with *F. gigantica.* A year later, liver flukes from cattle and buffalo in Egypt were described by Looss (*6*) under the name of *Distomum hepaticum* var. *aegyptiaca;* this subspecies is also now regarded as identical with *F. gigantica.*

F. gigantica has a wide geographical distribution. In addition to its occurrence in Hawaii, it has been reported from the following continents: Africa (Kenya, Northern and Southern Rhodesia, Senegal, Tanganyika); Asia (Assam, Ceylon, China, Formosa, India, Indo-China, Philippines); and Europe (Spain).

The final hosts from which specimens of *F. gigantica* have been taken, according to Brumpt (*4*), include the following: Buffalo, cattle, giraffe, goat, man, sheep, and zebra. In addition, as will be noted below, flukes were collected from a horse in Hawaii by A. R. Rowat in 1894; guinea pigs, rabbits, and a pig have been experimentally infested in the course of the work described herein.

The intermediate hosts reported for *F. gigantica* are the following fresh water snails: *Physopsis africana* and *Limnaea natalensis* in South Africa [Mönnig (*11*)], *Limnaea acuminata* in India [Bhalerao (*2*)], *Limnaea philippinensis, L. swinhoei,* and *Amphipeplea cumingiana* in the Philippines [Manipol (*9*)], and *Fossaria ollula* in Hawaii [Alicata and Swanson (*1*)].

SPECIES OF LIVER FLUKE FOUND IN CATTLE IN HAWAII

Lutz (*7, 8*), in 1892 and 1893, pointed out that the liver flukes found in Hawaii did not differ in shape or structure from the common liver fluke (*Fasciola hepatica*), and he believed them to be of that

[2] The writer wishes to express his indebtedness to the late Dr. M. C. Hall for many valuable suggestions throughout the course of this investigation.

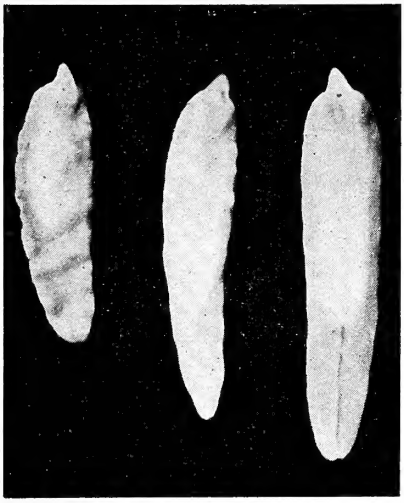

Fig. 1.—*Fasciola gigantica*, various sizes of adults; natural size.

species. However, among specimens collected by Alicata and Swanson (*1*) in 1937 some adult flukes measured 4 to 5 centimeters (fig. 1), and eggs, 156 to 197 microns in lengths. These measurements agree better with those of *Fasciola gigantica* (fig. 2, *a, b*). Several of the specimens were submitted to the Bureau of Animal Industry, Washington, D. C., and were identified by Mr. A. McIntosh and Dr. E. W. Price as *Fasciola gigantica*. In addition, the measurements of eggs of 1,000 flukes collected from cattle from the various islands of the Territory have all agreed with those of *F. gigantica*.

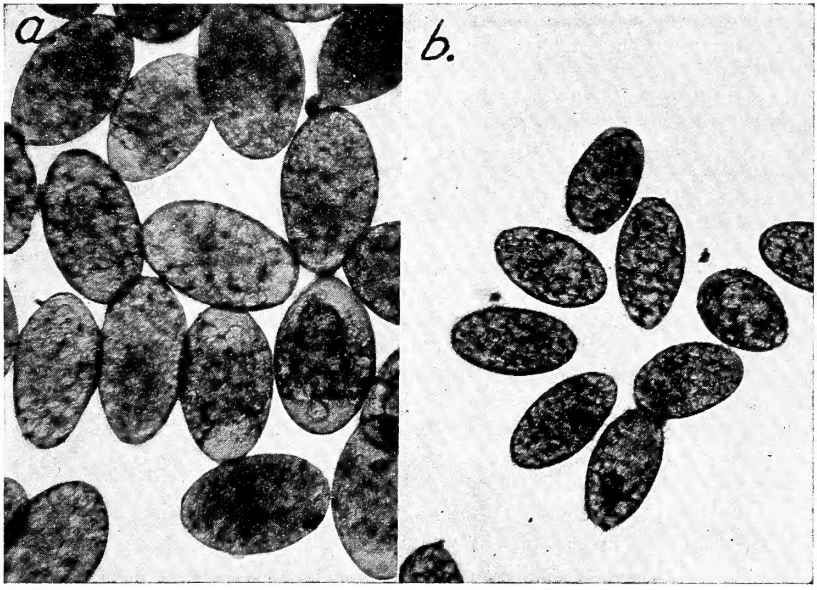

Fig. 2.—a, Eggs of *Fasciola gigantica;* and b, eggs of *F. hepatica*, showing comparative sizes. Photographed with same magnification.

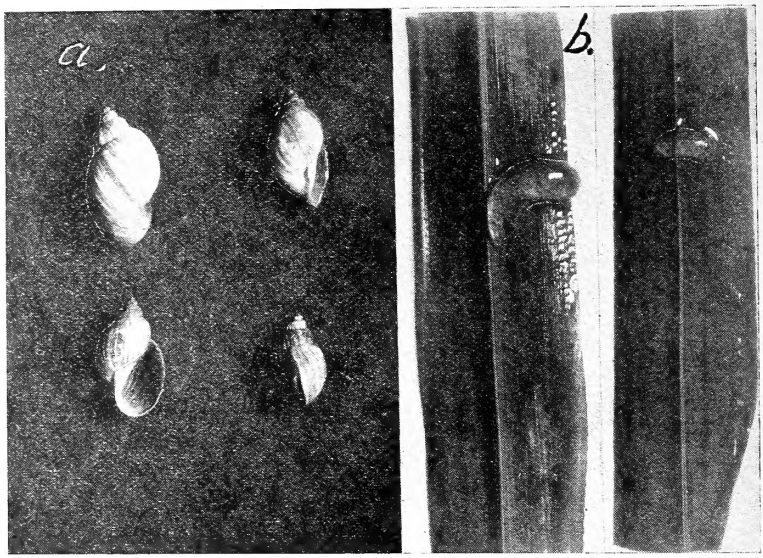

Fig. 3.—a, Various sizes of adult fresh-water snails, *Fossaria ollula;* and b, egg masses of the same species of snail attached to a blade of grass; slightly enlarged.

A. R. Rowat, in 1892 and 1894, respectively, forwarded to the U. S. Bureau of Animal Industry liver flukes collected from sheep and from a horse, from Honolulu; these flukes were then classified as *F. hepatica,* but a recent reexamination of the flukes by Mr. A. McIntosh of the Bureau of Animal Industry revealed them to be *F. gigantica.* It is believed that Lutz also erred in his original determination of the species of fluke prevalent in Hawaii.

SPECIES OF SNAIL SERVING AS INTERMEDIATE HOST FOR FASCIOLA GIGANTICA IN HAWAII

Lutz (1893) reported *Lymnaea oahuensis* as the snail commonly found infested with liver-fluke cercariae, and also considered *Lymnaea rubella* as a possible liver-fluke carrier on the island of Kauai. In reference to the validity of this latter species, Pease [quoted by E. R. Sykes in *Fauna Hawaiiensis,* edited by David Sharp (*14*)] believed that *L. rubella* was probably a variety of *L. oahuensis.*

During the present investigation, a number of fresh-water snails (7,497 dextral lymnaeids, 636 physids, and 5,027 melanids) were collected from 102 liver-fluke-endemic localities on the islands of Kauai, Oahu, Maui, and Hawaii. Many snails of each group and from each locality were isolated in individual glass vials in order to determine which snails were liver-fluke hosts. In each vial was placed a blade of grass on which emerging cercariae might encyst. Of the snails thus

studied, 90 dextral lymnaeids shed liver-fluke cercariae; subsequently, the cercariae obtained from each of 10 infested snails, after encystment, were fed to a rabbit. In all cases liver-fluke infestation was produced. The adult flukes recovered from the rabbits at necropsy, 3 months after experimental infestation, were identified as *F. gigantica.* In no case was it is noted that a physid or melanid snail shed liver-fluke cercariae.

Subsequent to the above finding, about 5,000 of the dextral lymnaeid and 1,000 of the physid and melanid snails were submitted to Dr. J. P. E. Morrison and Dr. Paul Bartsch of the U. S. National Museum, Washington, D. C., for determination. These specimens included the lymnaeids that shed liver-fluke cercaraie used in infesting the rabbits, and, in addition, a number of representatives of each of the 102 localities studied. After a careful morphological study, the snails were determined as follows: All dextral lymnaeids—*Fossaria ollula* (fig. 3, *a*); the physids—*Physa compacta;* and the melanids—*Melania indefinita* and *Melania mauiensis.* Dr. Bartsch, in a personal communication, expressed the opinion that *Limnaea oahuensis,* reported by Lutz from Hawaii in 1893, was probably identical with *Fossaria ollula.*

Following the above determinations, experiments were conducted to infest physid and melanid snails with the miracidia of *Fasciola gigantica.* At dissection, from a few days to several months later, no developing liver flukes were noted in these snails.

OBSERVATIONS ON THE DEVELOPMENTAL STAGES OF FASCIOLA GIGANTICA

PREPARASITIC STAGES IN THE LIFE CYCLE

1. The egg.

Morphology and development.—In a series of measurements of 100 eggs recovered from the gall bladder of infested cattle, eggs varied from 156 to 197 microns in length and from 90 to 104 microns in width. The eggs are usually light golden brown in color and elliptical or oval in shape (fig. 2, *a*); when first eliminated with the feces, each contains an undivided germ cell surrounded by a large number of yolk cells.

In two experiments, eggs recovered from the bladders of infested cattle, washed and kept in tap water at room temperature (78° to 82° F.) were found to develop rapidly, and hatched in large numbers 14 days after the culture was begun. The progressive development of the egg and formation of the miracidium is shown in figure 4, *a* to *i.*

2. The miracidium.

Morphology and behavior.—The newly hatched miracidia of *F. gigantica* (fig. 4, *i*) are, in general, similar in structure to other fascioloid miracidia. In normal swimming position they measure from 160 to 190 microns in length and 45 to 52 microns in maximum width.

Soon after hatching, the miracidia become very active and are strongly

phototropic. In one experiment, miracidia which hatched at room temperature (80° to 82° F.) at about 9 a.m. were still swimming actively when again observed at 9 p.m. of the same day. By 8 o'clock the

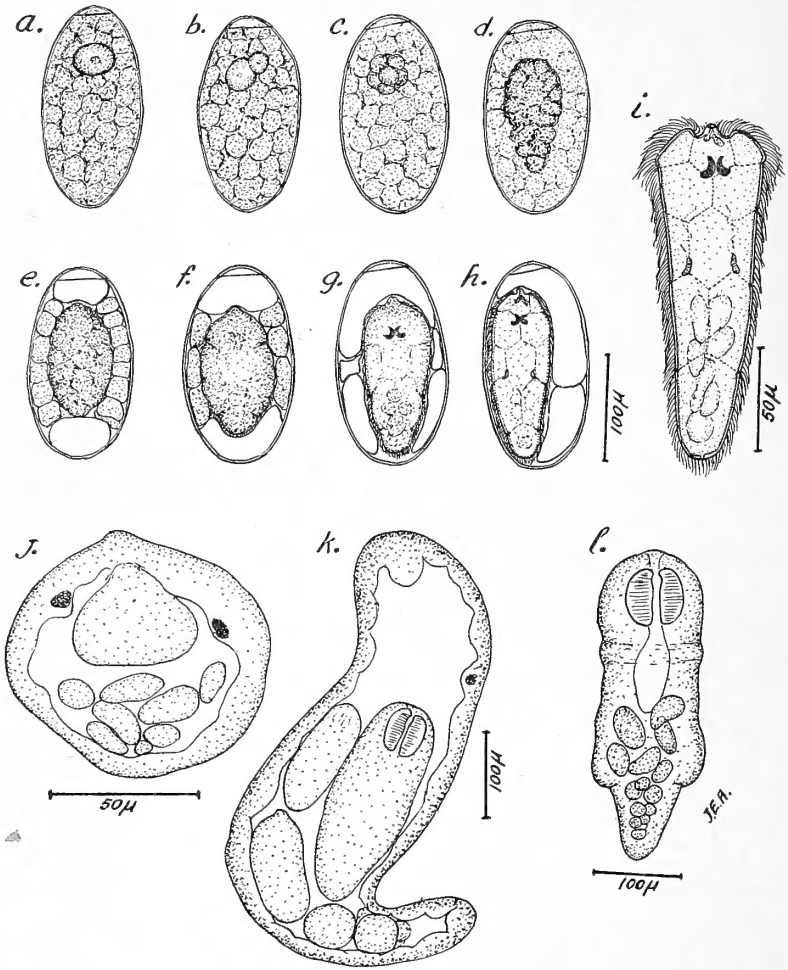

Fig. 4.—Development of the egg of *Fasciola gigantica* in water at a temperature of 78° to 82° F. and formation of the sporocyst and mother redia in the snail, *Fossaria ollula*.

Egg: a, Newly recovered; b, after 1 day; c, after 3 days; d, after 6 days; e, after 9 days; f, after 10 days; g, after 11 days; h, after 13 days; i, miracidium, after 14 days, newly emerged from the egg.

Sporocyst: j, Recovered from a snail 24 hours after experimental infestation; k, 4 days after experimental infestation, showing developing mother rediae.

Mother redia: l, Young form, 5 days after experimental infestation.

following morning most of the miracidia were dead, but a few showed sluggish movements.

Temperature is apparently a factor in the longevity of the miracidia, since those kept in a refrigerator (38° F.) for 48 hours were active soon after they were removed to room temperature.

Effects of copper sulphate and sea water on the miracidia.—Approximately 100 newly hatched miracidia were transferred to each of various concentrations of copper sulphate in water and dilutions of sea water, with results as indicated below:

Concentrations of copper sulphate	*Effects on the miracidia*
1:1,000	All dead after 6 minutes
1:2,000	All dead after 8 minutes
1:4,000	All dead after 15 minutes
1:8,000	All dead after 30 minutes
1:32,000	All dead after 4 hours
1:120,000	Still active after 6 hours.
Dilutions of sea water	
None	All dead after 3 seconds
1:1	All dead after 1 minute
1:3	20 dead after 20 minutes; 50 dead after 5 hours; others alive
1:9	All active after 5 hours
Control (tap water)	All active after 5 hours.

The above results indicate that, under field conditions, neither copper sulphate at the rate of 1:500,000, commonly used for destroying snails, nor water of slight salinity would effectively control miracidia.

DEVELOPMENTAL STAGES IN THE INTERMEDIATE HOST

Miracidia hatched in the laboratory were placed in a dish containing water and laboratory-raised snails. In most cases the miracidia, although observed to swim actively around the snails for a short time, attacked fairly promptly; a few did not attack for 1 or 2 hours.

1. Sporocyst.

The miracidia, in the process of penetrating the intermediate host, shed the ciliated epithelial coverings; after penetration they gradually transform into sporocysts. Snails dissected 1 day after experimental infestation showed several early-forming sporocysts in various parts of the body; at this stage the sporocysts were somewhat spherical and about 80 microns in diameter (fig. 4, *j*). In most cases the eye spots and germinal cells were clearly visible.

Snails dissected 2 days after infestation showed sporocysts slightly elongated, about 270 microns long and 140 microns wide; the eye

spots were still present, and no conspicuous changes were noted in the germinal cells.

Sporocysts recovered 3 days after infestation were about 300 microns long by 60 microns wide and showed distinct growth of the germinal cells. A sporocyst 4 days old was 610 microns long by 190 microns wide and enclosed developing mother rediae showing well formed pharynges (fig. 4, *k*).

2. Mother redia.

Mother rediae, escaped from the sporocyst, have been noted within snails as early as 5 days following infestation. These rediae have been found to measure about 350 microns in length by 95 microns in width; they possess well developed pharynges, small enterons, and groups of germinal masses in the posterior halves of the bodies (fig. 4, *l*). On the other hand, mother rediae measuring 600 microns in length have been noted still within the sporocyst 8 days after experimental infestation.

As with related species of liver flukes, the redia of *F. gigantica* possesses a conspicuous collar near the anterior third of the body and a pair of locomotor appendages near the posterior third. The development of the mother redia continues; one well developed redia, 14 days following experimental infestation of the snail, was found to be 2.05 millimeters long and 250 microns wide, and contained well formed daughter rediae (fig. 5, *b*).

3. Daughter redia.

In general morphology, the daughter redia resembles the mother redia. Young forms about 250 microns long, still within the body of the mother, show well developed pharynges and enterons, and possess several germinal cells. After emergence from the mother, the daughter redia continues to grow within the snail. Snails dissected 31 days after experimental infestation showed daughter rediae from 2 to 3.5 millimeters long and 260 to 400 microns wide, with well developed and motile cercariae (fig. 5, *c*). A daughter redia examined 37 days after experimental infestation of the snail was 4.5 millimeters long by 450 microns wide and contained several motile cercariae.

4. Cercaria.

Snails kept in tap water at a temperature varying from 76° to 80° F. were first noted shedding cercariae 39 days following experimental infestation. After emergence, the cercariae swam actively in the water; at the end of periods varying from a few to about 30 minutes, they were noted encysting on vegetation or other objects, usually near the level of the water.

The cercaria of *F. gigantica* (fig. 5, *d*) is, in general, similar to other fascioloid cercariae; the body is from about 320 to 380 microns long by 269 to 317 microns wide, the oral and ventral suckers are ap-

proximately 52 and 57 microns in diameter, respectively, and the tail is from 800 to 950 microns long.

5. Metacercaria.

The encysted metacercariae (fig. 6, *b*, *c*), referred to in this paper

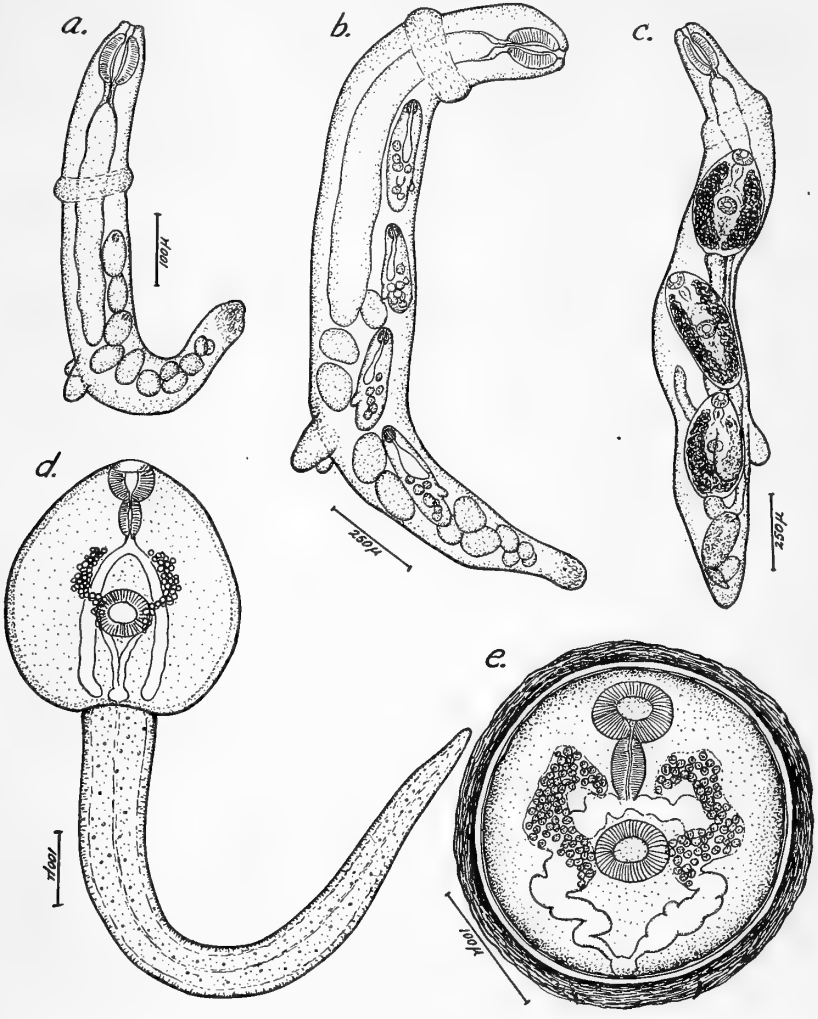

Fig. 5.—Various stages in the development of *Fasciola gigantica* in the snail, *Fossaria ollula*.

Mother redia: a, Eight days after experimental infestation; b, 14 days after experimental infestation, showing developing daughter rediae.

Daughter redia: c, Thirty-one days after experimental infestation, showing well developed cercariae.

Cercaria: d, Liberated from the snail 39 days after infestation.

Metacercaria: e, Encysted.

as "fluke cysts," are enclosed by hard, rough, outer cyst walls and by somewhat elastic inner cyst walls (figs. 5, *e* and 6, *a*). The diameter of the outer cyst wall has been found to vary from 238 to 268 microns; this diameter was larger than that of the corresponding stage of *F. hepatica* which, in 10 specimens available, varied from 180 to 206 microns. The inner cyst wall of *F. gigantica* was found to vary from 225 to 250 microns.

One feature of particular interest is the thinness of the outer cyst wall at the point of attachment to the plant (fig. 6, *a*)[1]. It is possible that this thin wall allows interchange of moisture between the plant and the encysted larva, thus guarding the cyst from desiccation.

LONGEVITY AND RESISTANCE OF FLUKE CYSTS TO VARIOUS ENVIRONMENTAL CONDITIONS

Marek (*10*) pointed out that cysts of *F. hepatica* survived on moist hay and were able to infest animals eight months after the hay was harvested. Ross and McKay (*13*), working with the same parasite, found the cysts viable after being kept for 4 months in water. These writers also noted that cysts were infestuous to rabbits after having been desiccated in shade at room temperature (71.6°-80.6° F.) for 17 days. In view of the above observations, experiments were conducted to determine longevity of cysts of *F. gigantica* under various conditions.

In the following tests, leaves or whole plants of honohono (*Commelina diffusa*) were experimentally infested with fluke cysts. Small plants about one foot long, carefully pulled from the ground to prevent much injury to the roots, were placed in glass dishes containing water and a large number of snails collected from "fluky" areas. Plants on which metacercariae encysted were placed in separate dishes containing water for at least 2 days before being used in experiments. When infested honohono was replanted in sunny or shady areas, no difficulty was encountered in keeping the replants alive. Fluke cysts which had been exposed to various experimental conditions were fed to laboratory-raised guinea pigs; the guinea pigs were killed 25 days later to test the viability of the cysts. Results are shown in table 1.

These experiments would indicate that fluke cysts attached to honohono leaves and exposed indoors were viable after 20 days but not after 30 days. Cysts attached to whole plants and kept in sunny areas were viable after 15 days but not after 42 days. During the 15-day period there were approximately 10 noncloudy days, and during the 42-day period, approximately 32 noncloudy days. The temperature during the hours of sunshine ranged from about 77° to 87° F. Showers were common in the locality where the infested plants were reset, so the cysts were moist during part of the time.

[1] The writer is indebted to Mr. W. B. Storey of this station for sectioning the encysted metacercaria.

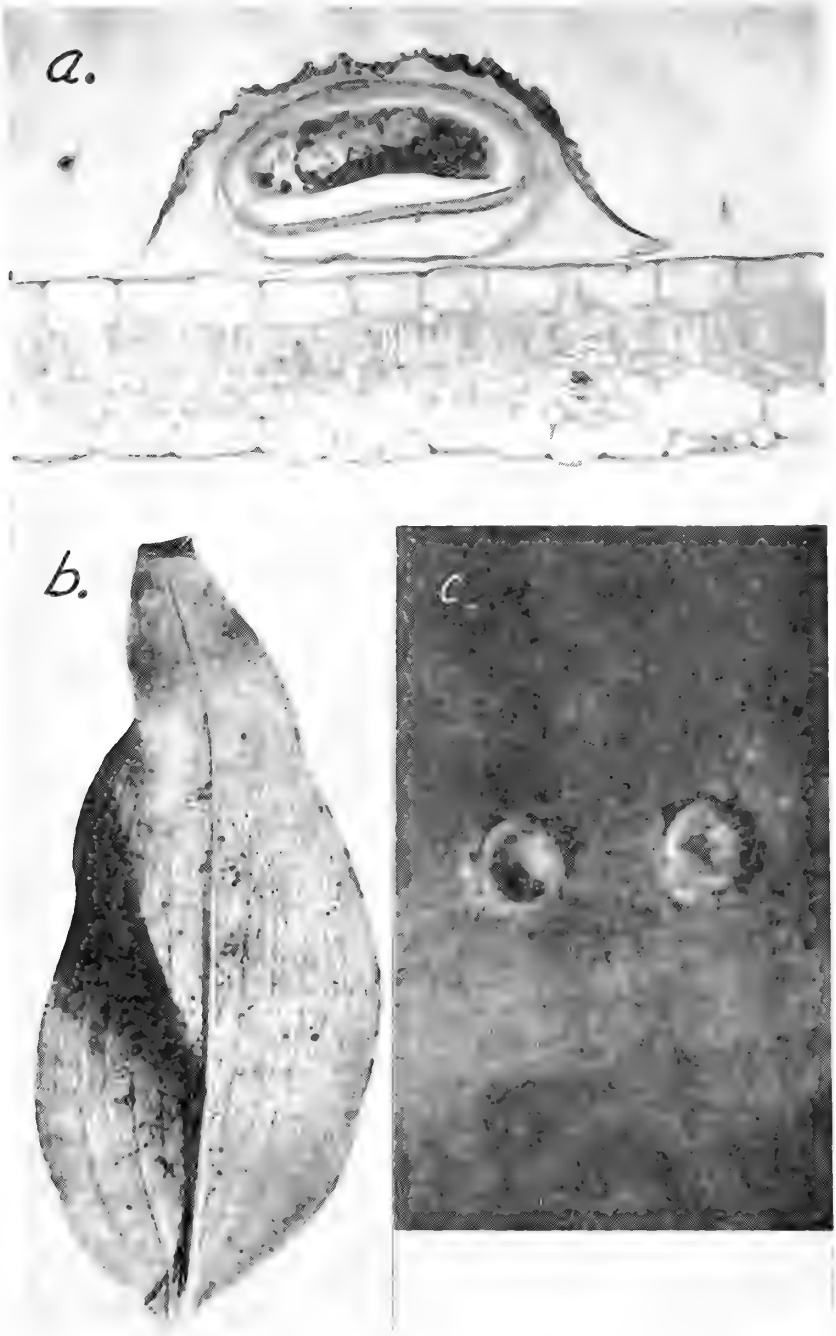

Fig. 6.—Metacercariae of *Fasciola gigantica* encysted on blades of honohono (*Commelina diffusa*), enlarged. a, Cross section of cyst and blade, 1 week after experimental infestation; b, several cysts attached to blade; c, appearance of cysts 106 days after experimentally infested honohono had been planted outdoors in a shady area. Note collapse of cyst walls indicating desiccation.

Table 1.—Longevity of fluke cysts attached to honohono (*Commelina diffusa*)

Method of exposure of cysts on plants	Period of exposure in days	Number of cysts fed to each guinea pig	Number of guinea pig	Lesions on liver and flukes found in the guinea pig 25 days after infestation
	Under dry conditions			
Detached, infested leaves of plant kept indoors (temperature 72°-75° F.)	5	10	21	5 fluke burrows, 1 fluke found
do	20	75	36	3 fluke burrows, 1 fluke found
do	30	50	37	No lesions or flukes found
Whole plant reset in the ground and exposed to sun from about 9 a.m. to 3 p.m. whenever the sun was shining.	3	82	14	Several lesions and flukes found (no count made)
do	15	215	17	Many fluke burrows, 32 flukes found
do	42	74	23	One 2 mm lesion on liver resembling, to some extent, a fluke burrow; no flukes found
do	75	155	24	No lesions or flukes found
Whole plant reset in ground in shady area	13	131	19	Many fluke burrows; 18 flukes found
do	75	130	25	No lesions or flukes found
do	106	75	28	No lesions or flukes found

Table 1 (continued)

Method of exposure of cysts on plants	Period of exposure in days	Number of cysts fed to each guinea pig	Number of guinea pig	Lesions on liver and flukes found in the guinea pig 25 days after infestation
	Under submerged conditions			
Whole plant reset in a layer of soil at the bottom of a water-trough and submerged in water which was kept running continually	30	50	21A	Many fluke burrows; 14 flukes found
do	61	90	15	Many fluke burrows; 10 flukes found
do	90	72	26	Many fluke burrows; 7 flukes found
do	122	115	27	4 fluke burrows; 2 flukes found
Detached infested leaves kept in 1-quart bottle. The water in the bottle was unchanged throughout the experiment. The bottle was plugged loosely with cotton	33	25	6	2 fluke burrows; 2 flukes found
do	63	65	16	7 fluke burrows; 3 flukes found
do	95	45	22	No lesions or flukes found
do	109	100	17A	No lesions or flukes found

In experiments involving infested plants kept under running water, some of the cysts were viable after 122 days; however, the fact that only a light infestation was produced indicates that not all of the cysts were viable. It was also noted that cysts did not produce an infestation when kept in stagnant water for 95 days.

THE EFFECT OF ENSILAGE ON FLUKE CYSTS

It is well known that, in ensilage, air is excluded from the fodder. At first a certain amount of fermentation takes place, which is later checked by the accumulation of chemical substances—notably lactic

acid and the poison carbonic acid gas—which saturate the forage and tend to preserve it from decay [F. H. Storer, (*15*)]. To test the possibility that this exclusion of air and the presence of fermentation and poisonous gas would kill fluke cysts, the following experiments were conducted. From a large number of fluke cysts collected, some were fed to two control guinea pigs and proved to be infestuous. Other cysts from the same group were then stored for 3 months under the conditions described below to determine their viability.

Experiment I.—A 50-gallon metal drum was half-filled with panic grass (*Panicum* sp.), and blades of grass carrying several hundred viable fluke cysts were placed thereon. The remainder of the drum was then packed tightly with chopped grass and covered. In order to facilitate recovery of infested grass at the end of storage, long strings were tied to each infested blade. Three months later the drum was opened, and 200 cysts were recovered; 100 cysts were fed to each of 2 guinea pigs. After 25 days the guinea pigs were killed and were found to be free from flukes.

Experiment II.—This experiment was similar to experiment I, but a mixture of hydrochloric and sulphuric acids, as outlined by A. J. Virtanen (16) for preserving fresh fodder, was sprinkled on the grass. The grass in this case was stored in a 50-gallon wooden barrel. Three months later 300 cysts were recovered and fed in equal numbers to 2 guinea pigs. There were no flukes or fluke lesions noted when the guinea pigs were killed 25 days later.

The above experiments indicate that the same results may be obtained in large or regular-size silos, and that ensilage may have a practical significance in preventing fluke infestation when vegetation from swampy areas is to be used for livestock feeding.

DEVELOPMENT OF FASCIOLA GIGANTICA IN THE FINAL HOST

Observations were made on the development of flukes in 5 guinea pigs, 10 rabbits, a pig, and a calf, after experimental feeding of fluke cysts. In order to determine when fluke eggs were first eliminated, the feces of the rabbits and of the calf were examined, beginning on the fiftieth day following experimental infestation, and continuing twice a week thereafter. All of the guinea pigs had been killed before fecal examination of the other animals commenced, and five of the rabbits were killed within 75 days after infestation. No eggs had as yet been eliminated with the feces of any of the rabbits. The appearance of fluke eggs in the feces of the remaining five rabbits and the calf is recorded in table 2, as well as data on flukes and fluke lesions secured at necropsy of all the animals included in this experiment.

The above results show that *F. gigantica,* in addition to developing in the calf, which may be considered the normal host, also developed in

Table 2.—Experiments on the development of *Fasciola gigantica* in final hosts

Hosts	Number of cysts fed	Number of days from experimental infection to first appearance of fluke eggs in feces	Period from feeding of cysts to necropsy in days	Number of flukes recovered at necropsy and stage of development	Size of flukes in millimeters and locality in which flukes were found
Guinea pigs					
1	20	[1]	9	1 immature	0.73 x 0.35; in liver tissue
2	30	[1]	19	6 do	2 to 2.5 x 0.4 to 0.5; in liver tissue
3	22	[1]	21	5 do	2.5 to 3 x 0.55 to 0.8; in liver tissue
4	50	[1]	31	Several immature	3 to 5 x 0.8 to 1.2; in liver tissue
5	36	[1]	40	18 immature	6 to 8 x 2.5 to 3; in liver tissue
Rabbits					
1	25	77	81	1 do 5 mature	Immature, 20 x 6; mature, 26 to 30 x 6 to 8; in bile duct near gall bladder
2	25	[2]	75	3 immature	16 to 19 x 5; 2 in liver duct, 1 in peritoneal cavity
3	25	77	81	5 mature	31 to 32 x 10; in bile duct near gall bladder
4	25	[2]	72	1 immature	18 x 5; in bile duct
5	25	77	81	6 mature	26 to 29 x 9; in bile duct near gall bladder
6	25	[2]	59	5 immature	15 to 17 x 4 to 5; 3 on surface of liver, 2 in liver
7	25	[2]	54	18 do	14 to 17 x 4 to 5; 12 flukes in liver, 6 in peritoneal cavity
8	25	[2]	76	2 do	16 to 20 x 6 to 7; in liver
9	25	77	119	4 mature	30 to 33 x 10; in bile duct
10	25	84	87	3 do	26 to 29 x 10 to 11; in bile duct
Pig					
1	300	[3]	64	82 immature	20 to 28 x 4 to 6; in liver tissue and bile duct
Calf					
1	200	84	216	3 mature	37 to 42 x 11; in bile duct

[1] Killed before fecal examination started
[2] Killed before eggs had appeared in feces
[3] Feces not examined

guinea pigs, rabbits, and a pig. In rabbits No. 1, 3, 5, and 9 the first fluke eggs were noted in the feces 77 days after experimental infestation, and in rabbit No. 10 and in the calf the eggs were noted on the eighty-fourth day.

DESCRIPTION, HABITAT, AND LIFE HISTORY OF THE INTERMEDIATE HOST, FOSSARIA OLLULA

MORPHOLOGICAL DESCRIPTION

Dr. J. P. E. Morrison (previously mentioned) has furnished the writer with the following description and remarks concerning this snail:

FOSSARIA OLLULA (Gould) 1859.

Synonyms:

Lymnaea ollula Gould, Proc. Bost. Soc. Nat. Hist., vol. 7, p. 40 (June, 1859).

Lymnaea pervius Martens, Ann. Mag. Nat. Hist. (3) XVII, p. 207, 1866; Mal. Blatt. 14, pp. 221-222, 1867.

Limnaea goodwinii E. A. Smith, Quart. Journ. Conch., vol. 1, pp. 125-126, 1875.

Limnaea pervia Kobelt, Fauna Extra Marin. Japon., p. 105, pl. XV, fig. 6.

Shell: Small, variable, turreted, globose ovate to elongate-ovate, thin to moderately thick; periostracum light horn, often streaked or spotted with white where eroded; surface dull, lines of growth fine, conspicuous, often with sub-equally prominent spiral striae; whorls 4-5, convex, indistinctly shouldered above, accentuating the suture; spire acute; nuclear whorls 1-1¼, with finely striate sculpture; the first whorl small, flattened, the second broader, regularly convex; body whorl 2/3 or more of the length of the shell, tumid; aperture ovate, evenly rounded below, little narrowed above; outer lip thin, sharp, inner lip broadly reflected over the moderately open umbilicus; columellar region a little excavated below the distinct parietal callus.

Jaw: Wide and low, with evenly rounded ends and a wide, rounded swelling on the ventral border.

Radula: Formula variable, 15—24+2+6+1+6+2+15—24, in ten individuals from one lot examined.
(15-24 marginal; 2 intermediate; 6 lateral + 1 central in a half row)

Genitalia: Penis sheath 2/3 length of praeputium; penis slender, simple (cf. F. C. Baker 1928, fig. 114); praeputium without sarcobelum.

Type: *Ollula* Gould, U.S.N.M. Cat. No. 831.

Type localities: *Ollula,* "Streams and marshes on Hong King Island (Wright)."

Range: China, Japan; and Hawaiian Islands (introduced).

Remarks: The United States National Museum collections

> contain specimens of *ollula* from "Prov. Canton, China", and from Foochow, Fukien, China, as well as the numerous fine sets you have sent from the Hawaiian Islands.
>
> The omission of this widespread form of lymnaeid from all of the earlier collections and published records clearly indicate its relatively recent introduction into the Hawaiian Islands. On the other hand, both its presence and its even great variability have served to further confuse our paucity of knowledge of the indigenous species of lymnaeids, which are of a clearly different facies if not generically distinct.

F. ollula is widely distributed and has been found by the writer on the islands of Kauai, Oahu, Maui, and Hawaii. This snail is commonly located in the lowlands on the windward side of each island, where there is considerable rainfall. It is amphibious in habit, and is usually found in shallow marshes, often crawling on damp mud just outside the water or on stems or leaves immediately above the water level. *F. ollula* is also occasionally found in swiftly flowing streams, clinging to the rocks in midstream, and in water troughs. In boggy areas containing thick vegetation the snails are most commonly found in isolated and scattered pools where vegetation is not thick; it is possible that this fact is influenced by the presence of sunlight.

LIFE-CYCLE STUDIES

Observations on the life cycle of *F. ollula,* involving period and frequency of oviposition and span of life, were conducted with the assistance of Mr. T .H. Hong, formerly a member of the staff of this station, and were, for the most part, under laboratory conditions. Individual egg masses from snails were isolated, placed in a receptacle containing stream water, and allowed to develop and hatch. Each newly hatched snail was transferred to a moisture-dish, 240 millimeters in diameter and 80 millimeters deep. The dish was about three-fourths filled with stream water and contained a bottom layer of soil about 1 centimeter thick. One or two rocks were placed in the dish, sufficiently large that a portion of the rock would slightly emerge above the water level. The rocks offered the snails an opportunity to come to the surface for aeration. One or two small pieces of fresh lettuce were placed in the dish about twice a week as food for the snail. Once a week most of the water in the dish was siphoned off and replaced with fresh water. The dish was covered with a fine wire screen.

The dishes, as prepared above, were kept outdoors, floating in a specially constructed water trough, 1½ feet deep, half-filled with fresh running water. The temperature of the water in the dishes containing the snails was found to vary from 68° to 75° F. throughout the experiment. The data on the development of the snails are summarized in tables 3 and 3A.

Table 3.—Observations on the development, size, oviposition, and span of life of snails, *Fossaria ollula*, hatched on Janary 4, 1936

No. of snail	Date, age, and size (length and width) of snail at first oviposition	Number of egg masses (EM) and eggs (E) deposited monthly by each snail; also size (S) of each snail in millimeters														Date and age at death	Total egg masses and eggs deposited	
		February			March			April			May			June				
		EM	E	S	EM	E	S	EM	E	S	EM	E	S	EM	E	S		EM	E
1	February 6 33 days 9x5 mm	23	598	9x5	14	378	10x5	15	315	12x6	4	18	13x7	....	.....	.....	May 12 128 days	56	1300
2	February 13 40 days 9x5 mm	13	323	9x5	9	265	10.5x6	16	323	12x6	35	640	12.5x6	15	372	13x6.5	June 17 164 days	88	1923
3	February 16 43 days 9.5x5 mm	20	520	9.5x5	22	523	10.5x6	13	400	12.5x6.5	....	.....	.....	....	.....	.....	April 8 114 days	55	1443
4	February 14 41 days 9.5x5 mm	19	447	9.5x5	22	751	9.5x5	24	788	11x6	16	398	11.5x6	....	.....	.....	May 31 147 days	81	2384
5	February 15 42 days 9x4.5 mm	13	209	9x4.5	10	223	9.5x5	8	197	10.5x5.5	4	48	11.5x6	....	.....	.....	May 7 123 days	35	677

Table 3A.—Observations on the beginning of oviposition of snails hatched May 14, 1936

Number of snail	Date of first oviposition	Age of snail Days
6	June 21	38
7	June 17	34
8	June 15	32
9	June 17	34
10	June 23	40
11	June 9	26
12	June 11	28
13	June 16	33

Oviposition, in 13 snails, began from 26 to 43 days following hatching. The size of the snails at the time (records on five snails only) ranged from 9 to 9.5 millimeters in length and from 4.5 to 5 millimeters in width. The observed life span of 5 snails varied from 114 to 164 days, and the total number of eggs deposited by each snail varied from 677 to 2,384. The individual egg masses observed (fig. 3, *b*) varied from 1 to 21 millimeters in length and from 1 to 3.5 millimeters in width, and contained from 1 to 92 eggs. The data obtained do not indicate that the number of egg masses or of eggs deposited by each snail showed any particular correlation with either the age of the snail or with the months during which observations were made. Snails reached a length of 13 millimeters under these laboratory conditions; this compares with the size of the largest snails collected under field conditions. Since the snails in the experiments survived less than half a year, it was not possible to determine whether oviposition was influenced by the seasons of the year. Under natural conditions, oviposition goes on throughout the year, as observations made by the writer have shown that egg masses are found in streams and marshes on Oahu during every month, and it is possible that, since the temperature from one season to another shows little variation in Hawaii, the rate of oviposition is about the same throughout the year.

SUMMARY

The common liver fluke infesting cattle in Hawaii is *Fasciola gigantica*. This determination is based upon the examination of more than 1,000 flukes collected from cattle on various islands of the Territory.

The common fresh-water snails found in fluke-endemic areas are: *Fossaria ollula, Physa compacta, Melania indefinita,* and *Melania mauiensis*. Of these snails only *F. ollula* was found naturally infested with cercariae of *F. gigantica,* and, when exposed to experimental infestation

with miracidia, only *F. ollula* acquired the infestation. This observation points out that the control of the molluscan carrier in Hawaii should be directed against *F. ollula.*

At a temperature of 78° to 82° F., eggs of *F. gigantica* required 14 days to develop and hatch as miracidia.

The various stages in the development of *F. gigantica* in the snail have been followed in detail, and motile cercariae have been found to escape from snails 39 days following experimental infestation.

Metacercariae encysted on honohono plants which were replanted and exposed in a sunny area were found viable after 15 days but not after 42 days; this indicates that sunlight or possibly a state of dryness exerts a lethal effect on the encysted metacercariae. Laboratory animals became infested as a result of being fed encysted metacercariae which had been in running water for 122 days. Metacercariae were not found infestuous after 3 months' exposure in ensilage.

The development of the fluke has been observed in the guinea pig, rabbit, calf, and pig. In rabbits, the fluke reached the egg-laying period in from 77 to 84 days, and, in a calf, 84 days following experimental infestation.

The snail *Fossaria ollula* has been noted most commonly in the lowlands and windward side of each island, where fluke infestation in cattle is most common, and in various localities such as marshes, flowing streams, and water troughs.

Snails have been found to oviposit when they are about 26 to 42 days old; they have reached a length of about 13 millimeters. The life span of five snails kept under laboratory conditions was found to vary from 114 to 164 days.

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