Epidemiology of Fasciola Hepatica Infestation in Dairy Cattle at Dorado, Puerto Rico[1]

J. Chiriboga, D. de León, and J. Rodríguez-Frías[2]

ABSTRACT

Eighty-six percent (230 out of a total 268) of the cows on a Dorado dairy farm were found positive to *F. hepatica* on coprological examination.

The highest infestation rate of 95.4% was determined in the older animals in the herd (9 to 12 yr old) while the lowest rate of 71.3% was in the younger (3 to 3½ yr old) animals.

L. cubensis, the predominant snail intermediate host for *F. hepatica* on this particular farm, is a relatively dynamic organism that has a potential life-cycle (egg to egg) of 21 days.

The number of snail habitats, the number of snails, and the percentage of *F. hepatica*-infested snails noticeably increased during the winter months of December, January, and February, when there was high average monthly rainfall of 145 mm and low average monthly temperature of 24°C.

Cattle in the Dorado area acquire *F. hepatica* infestation only during certain months of the year, that is, from November to February. This finding disproves the assumption of year-round infestation.

An increase in the quantitative serum glutamic oxalacetic transaminase analysis (SGO-T) of cattle in an endemic area is important since it may indicate that the animals may have been exposed to *F. hepatica* infestation.

No effective control of fascioliasis in dairy cows in Dorado is recommended except sanitation and good nutrition.

INTRODUCTION

Fasciola hepatica infestation known by such terms as fascioliasis, hepatic-distomiasis, liver-fluke disease, and "flukey-liver," is one of the most widespread liver diseases throughout the world (19). It is a serious problem in the livestock industry and among humans in many parts of the world (1, 2, 4).

In Puerto Rico, fascioliasis is the most important parasitic disease of dairy cattle. An accurate determination of loss to the livestock industry due to fascioliasis is not easy to make. However, direct loss due to condemnation of infested livers at time of slaughter is available. USDA records reveal that the livers of 26% of cattle killed at the nine slaughter-houses on the Island during 1974 were condemned because of fluke infestation (5). This represents a direct loss to the livestock industry of over a million dollars. The value of other direct losses, such as deaths

[1] Manuscript submitted to Editorial Board December 28, 1978.

[2] Scientist, Environmental and Biomedical Science, P.R. Department of Health U.P.R. School of Medicine, Medical Sciences Campus, Río Piedras, P.R.; Associate Parasitologist, Agricultural Experiment Station, Mayagüez Campus, University of Puerto Rico, Río Piedras, P.R.; and former Fellow in Parasitology of the Pan American Health Organization.

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from fascioliasis, cost of medicines and of treatment, etc., is harder to estimate. Losses due to indirect causes such as reduction of milk production, poor feed conversion, low meat quality and others are even more difficult to evaluate.

F. hepatica seems to have existed in Puerto Rico long before it was mentioned by Van Volkenberg (23) in 1939. The first report on the occurrence of the liver-fluke in the different municipalities of the Island was published in 1952, when Rivera-Anaya and Martínez-de Jesús (17) studied the incidence of liver-fluke infestation in the different slaughterhouses in operation at the time. Almost all the municipalities of Puerto Rico were reported infested with *F. hepatica.*

In 1971, De León et al. (7), in their coprological examination survey of fascioliasis in dairy cattle in the Dorado-Toa Baja-Toa Alta area of the Río La Plata basin, reported that nine of the 11 farms in Dorado had fascioliasis, with 11 to 56% of the animals positive for the infestation. All five farms in Toa Baja were positive for liver-fluke, with infestation rates of 18 to 80%. The three farms in Toa Alta also were positive with infestation rates of 5 to 79%. The overall infestation rate for 1,229 cows was 37%.

The incidence of fascioliasis in dairy cattle is influenced by such factors as topography, season, presence of the snail intermediate hosts (*Lymnaea* spp.) and management practices.

The Island of Puerto Rico is located in the Caribbean Sea at latitude 18° 30′ W. Its climate is definitely tropical and its area is 3,500 sq. miles. The prevailing land and sea winds, and also northeasternly trade winds modify the temperature, especially in the northern coastal area. Most of the precipitation is orographic in nature and the least amount of precipitation falls on the southwestern part of the Island, due to its location south of the Central Mountain Range.

Dorado is in the Río La Plata basin in the central northern part of Puerto Rico. According to Ewel and Whitmore (12), the study area is known as the sub-tropical Moist Forest Life Zone, according to Holdridge's Classification of Life System. The zone is delineated by mean annual rainfall of 1000 to 2200 mm and a mean biotemperature between 18° and 24° C. This zone has been deforested at one time or another primarily because of the climate conditions. Pastures seems to be the predominant land use today, but sugarcane and coffee occupy large areas.

Lymnaea columella and *L. cubensis* are known vectors of fascioliasis in Puerto Rico. The biological potentials of *L. columella* have been studied by De León (10), but no such studies for *L. cubensis* have been reported previous to the experimental data presented in this article.

Diagnosis of fascioliasis in cattle is usually by coprologic techniques. These techniques are erratic, and often repeated stool examinations are

required; even so, eggs are never observed (14) in many cases of fascioliasis. The wandering about of juvenile flukes through the liver parenchyma causes tissue necrosis, which triggers the release of the serum glutamic oxalacetic transaminase enzyme (SGO-T) into the blood stream. This phenomenon was deemed worthy of investigation in order for researchers to know whether quantitative determination of SGO-T could prove of use in the diagnosis of fascioliasis in cattle.

Transmission of fascioliasis in Puerto Rico is assumed to be year-round because no pronounced drought occurs in the northern part of the Island. However, no data concerning transmission of *F. hepatica* are available.

Control of fascioliasis in dairy cattle in directed towards the destruction of the snail intermediate host together with such management practices as sanitation, drainage, and animal nutrition.

FIG. 1.—Map of Puerto Rico indicating study location marked by arrow and areas reported to be highly infested with *F. hepatica*.

MATERIALS AND METHODS

The dairy farm used in this study is located in the village of Mameyal, Dorado (fig. 1). It has a total area of 350 hectares, and is bordered along its entire western side by the Río La Plata which flows into the Atlantic Ocean. Pangola grass (*Digitaria decumbens*) and Para grass (*Panicum muticum* Forsk) are cultivated in most of the areas. The farm is flat, low, and uses a system of hand-dug canals for drainage. Drainage is poor because the farm is almost at sea level and has a high annual rainfall. The canals were clogged most of the time by weeds. As a side observation, many land crabs (*Cardisoma quanhumi*) and (*Aratus pisonii*) were noticed in and around the canals.

The cow population in 1973 was 450, mostly Holstein-Friesians, born and raised on this farm. The present survey, done from April to July

1973, used 268 milking cows. Fecal samples from each cow were collected after the morning milking. Two grams of feces were concentrated in the laboratory by a formalin-ether sedimentation technique using a buffered alcoholic medium (16) with adaptation for cow feces. Microscopic slide preparations were made with a drop of a 0.5% solution solution of methyl green (2′) which stained the debris but not the eggs. The 268 cows were classified according to their date of birth into the following age-groups: 3 to 3½ years, 4 to 4½, 5 to 5½, 6 to 6½, 7 to 7½, 8 to 8½, and 9 to 12.

Some of the oldest cows were culled and recommended for slaughter; a few of these were examined at the Río Piedras abattoir.

FIG. 2.—Overflowing drinking trough with the resultant mud formation and algal growth, a very favorable snail habitat on a dairy farm in Dorado.

Snail surveillance was officially started on this farm in August 1973. The known snail habitats were visited and the farm was surveyed monthly for new snail colonies. The permanent colonies were generally found around the drinking troughs (fig. 2), and of small extent, 20 to 30 ft^2.

Because most of the recommended methods for counting the snail intermediate hosts of schistosomiasis seemed not applicable to our purpose, the technique described below was developed. With a tally hand counter, the number of snails was counted in each habitat for a span of 3 min at a time, and recorded. Twenty snails from the different areas of the habitat were collected, brought to the laboratory and examined for

rediae and cercariae of trematodes. Differentiation was made between radiae and cercariae of *F. hepatica* and *C. cotylophorum* in the snail following a technique reported by De León and co-workers (9).

Climatologic data were obtained from the Dorado weather station of the National Oceanic and Atmospheric Administration, U. S. Department of Commerce.

Clutches of *L. cubensis* were incubated in Petri dishes to determine the egg-to-egg cycle, growth rate, egg production, reproduction and life spans. Newly hatched snails were transferred singly to water glass aquaria with and without a bottom layer of mud with an algae over-growth (mostly *Oscillatoria*). A blended form of Cerophyl, wheat germ, "Gaines Meal"[3] dog food and powdered milk (4:2:2:1) was used as a supplement or complete diet.

In August 1973, sixteen 1 ½ year-old, apparently healthy and fascioliasis-free replacement heifers were introduced into the herd. These heifers were born and raised in elevated cages on the farm and fed with fodders from *Fasciola*-free pasture. Initial and monthly fecal examinations of the 16 heifers were recorded. In December, 4 mo after the replacement heifers were brought into the herd, blood samples from the jugular vein were taken from the replacement heifers to study the group's blood biochemical constants with the aid of a Simultaneous Multiple Analyzer (SMA-12) manufactured by Technicon Corporation, New York.

RESULTS AND DISCUSSION

INFECTION RATE OF FASCIOLIASIS

A total of 230 out of 268 cows from the Dorado Farm were found positive for fascioliasis on coprological examination. The average infestation rate was 86%. According to Dorsman's findings, cited by Pantelouris (14) the actual prevalence of fascioliasis in the Dorado farm was probably higher than this figure. Dorsman demonstrated that diurnal fluctuation occurs in the number of eggs in the feces of cattle infected with *F. hepatica* with progressive increases of egg counts during the morning to a peak near mid-day, and with decreases thereafter, to a low during the night. Correspondingly, the number of eggs in the feces examined during this investigation may have been relatively low, yielding an undeterminable number of "false negatives" because all feces were collected very early in the morning.

[3] Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

RESISTANCE TO THE EFFECTS OF FASCIOLA—INFESTATION AND RE-INFESTATION

Figure 3 shows the relationship of age to infestation rate of fascioliasis in cows on a Dorado dairy farm. The highest infestation rate, 95.4% was observed in the 9 to 12 year age-group, the oldest group; and the lowest infestation rate, 71.3%, in the 3 to 3½ year age-group, the youngest group.

All infested cows did not exhibit clinical signs of fascioliasis, indicating

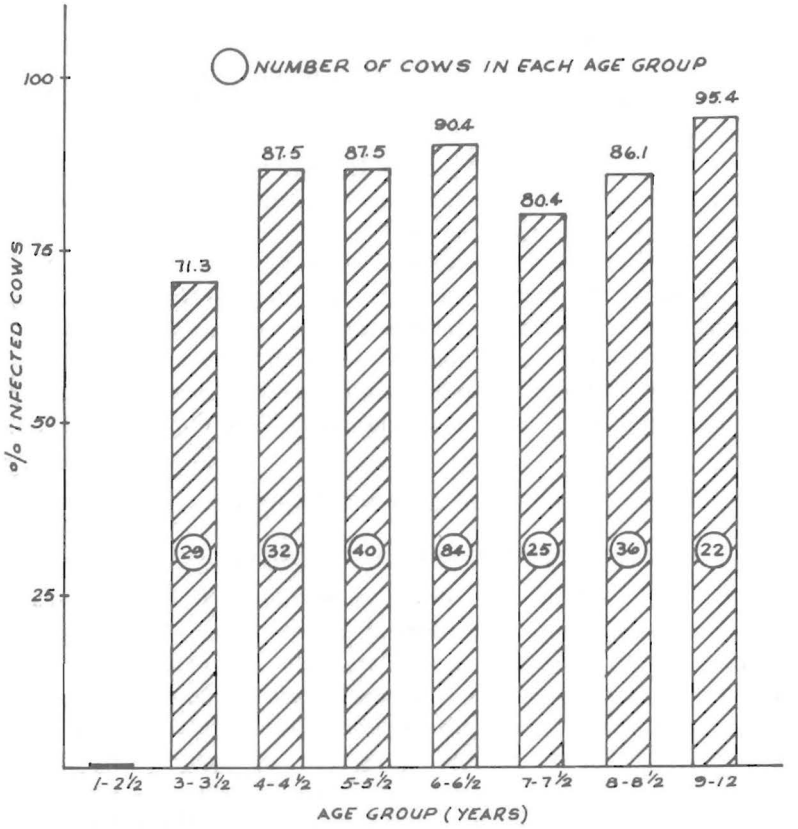

FIG. 3.—The relationship of age to infection rate of fascioliasis in cows on a Dorado dairy farm.

that the cows had developed resistance to the effects of *F. hepatica* infestation. However, these findings specifically showed that cows in the oldest age-group did not develop immunity to reinfestation of *F. hepatica*. The high EPG of cows in the 9 to 12 year age-group substantiates the assumption that the flukes were young, and the infestation, recent (fig. 4). The *F. hepatica* infestation in the oldest-age group of cows may not

represent the first infestation of liver-flukes affecting this group-age because the patent period of *F. hepatica* is only 9 mo as reported by Dixon (11). These observations are contrary to the report of Boray (3), that cattle after a single infestation with *F. hepatica* are practically protected against re-infestation.

Three of the cows from the 8 to 8½-year and 9 to 12-year age-groups, which were later culled and slaughtered, showed massive cirrhosis of the ventral portions of the livers, calcifications of the bile ducts with dark brown mucoid substance and groups of liver flukes. The number of liver-flukes recovered varied from 13 to 22 per liver.

The high incidence of fascioliasis in the dairy cattle in Dorado, Puerto Rico, may suggest that the cows in that area get low-level infestation,

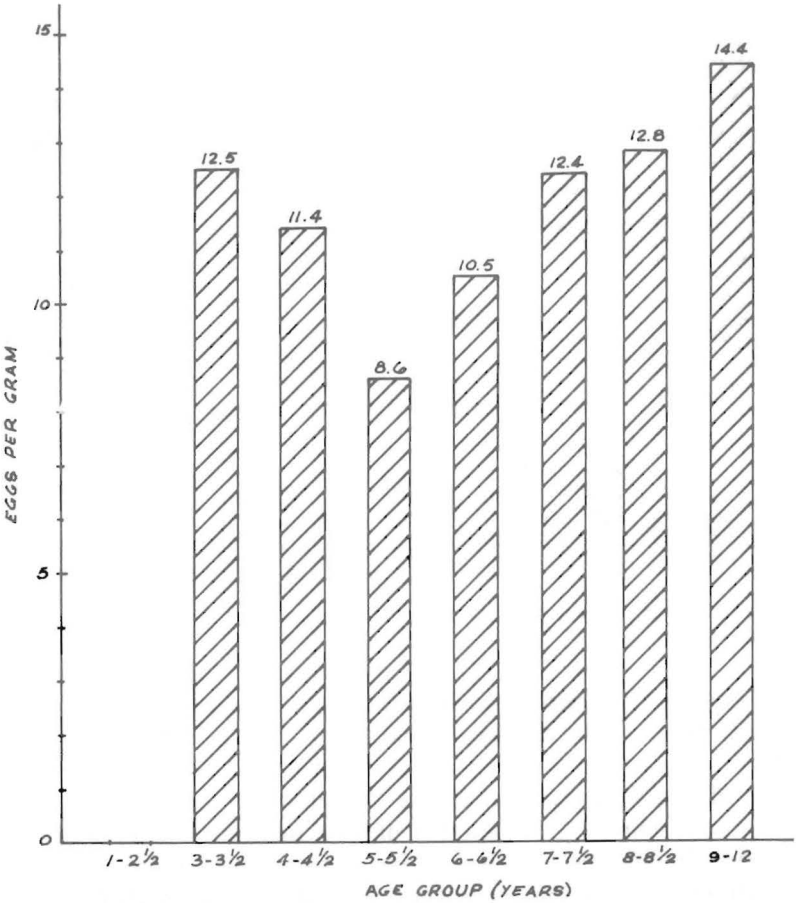

FIG. 4.—The relationship of age to the EPG of *F. hepatica* in cows on a Dorado dairy farm.

and perhaps, only during certain months of the year. Ross (18) claimed that low-level infestation with *F. hepatica* metacercariae has a higher percentage "take" than high level infestation. It is interesting to report that we found calcification of the bile ducts and extensive cirrhosis of the infested livers of the 8 to 8½-year and 9 to 12-year age-groups. Perhaps the extensive pathology in the infested livers was the result of continuous low-level infestation through the years with *F. hepatica* and not by a single massive or high level infestation. If the first exposure of cows to *F. hepatica* were massive, the liver pathology should have prevented further migration and development of new liver-flukes. If so, then the liver-flukes collected from the old cows' livers would represent the original flukes and therefore, the life-span of the parasite in cattle would be longer than the 9 mo reported by Dixon (11).

SNAIL SURVEILLANCE

L. cubensis is the predominant snail species in the Dorado dairy farm. A few *L. columella* as well as many *Physa cubensis, Aplexa marmorata,*

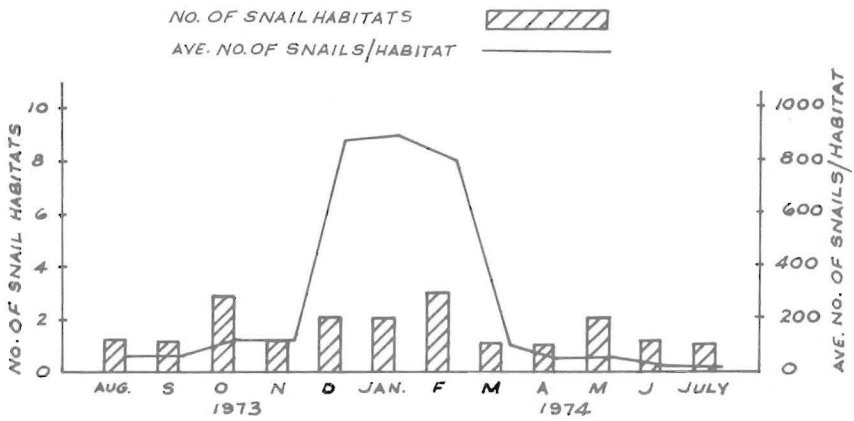

FIG. 5.—Number of snail habitats and average number of snails per habitat at the Dorado dairy farm.

and *Marisa cornuarietis* was observed. No *L. cubensis* or any other snails was noted in the drainage canals.

Figure 5 shows the number of *L. cubensis* habitat and the average number of snails per habitat at the Dorado dairy farm. In December, January and February, the number of snail habitats was increased with a corresponding increase in the number of snails per habitat. Also, during these winter months, the percentage of snails infested with *F. hepatica* was high (fig. 6). The increase in the number of snail habitats, the number of snails per habitat and the percentage of snails infested with *F. hepatica*

were directly related to the high average monthly rainfall (145 mm) and low average monthly temperature (24° C), during the winter months (fig. 7).

By late April the test areas started to become progressively drier with corresponding decreases in the snail population. The ground became cracked and "bone dry" five months later, at which time no snails could be found in the majority of the snail habitats, which depend chiefly on rainfall. Under such conditions, adult snails die, egg-masses dry out, and only the small snails (1-2 mm long) survive by undergoing cryptobiosis or aestivation (6) to surge as the nucleus of the snail population in the next rainy season.

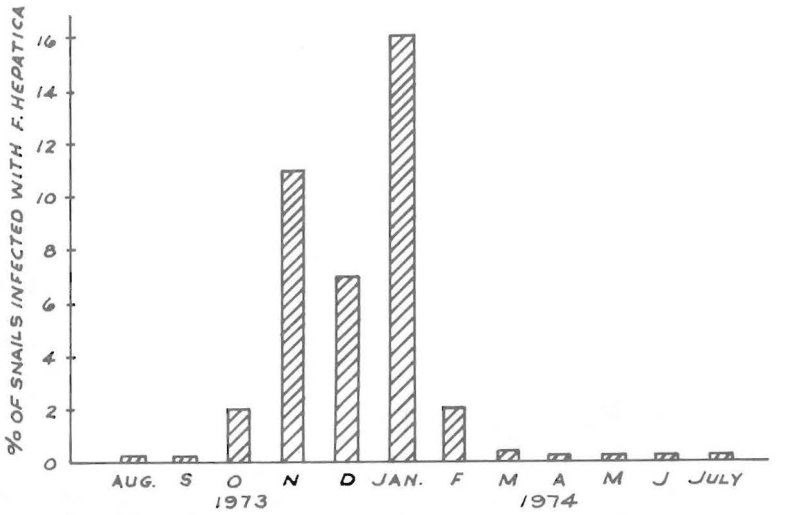

FIG. 6.—Percentage of snails infected with *F. hepatica* at the Dorado dairy farm.

Neither Physa cubensis, which was reported in Cuba (24) as a snail intermediate host of *F. hepatica*, nor *A. marmorata*, which was found in association with *L. columella*, was found to serve as intermediate hosts of *F. hepatica* in Puerto Rico (8). Similarly, *M. cornuarietis* (13), which was introduced to the Island for biological control of *Biomphalaria glabrata* and reported in New Zealand to shed some kind of cercariae, does not serve as intermediate host of *F. hepatica* (21).

LABORATORY STUDIES ON *L. cubensis*

L. cubensis is a relatively dynamic organism (15). Under laboratory condition it has a potential egg-to-egg cycle of 21 days. It can reach a near-maximum size in 3 weeks. Ova production at constant high level

was observed throughout the whole egg production stage. The maximum reproductive and life spans are relatively short, 4 to 5 months.

The absence of *L. cubensis* and other snails from the drainage canals in the Dorado dairy farm was not fully understood. It was observed that sea water refluxes into the drainage canals during high tide; water sampled from different areas of the canals had an average salinity of 0.076% (NaCl). However, no snail fatality was observed when 10 *L. cubensis*, collected from the farm, were kept in each of 10 tall glasses filled with canal water for 24 and 72 hours. The possibility of the constant clogging of the drainage canals by cattle feces, feed and miscellaneous

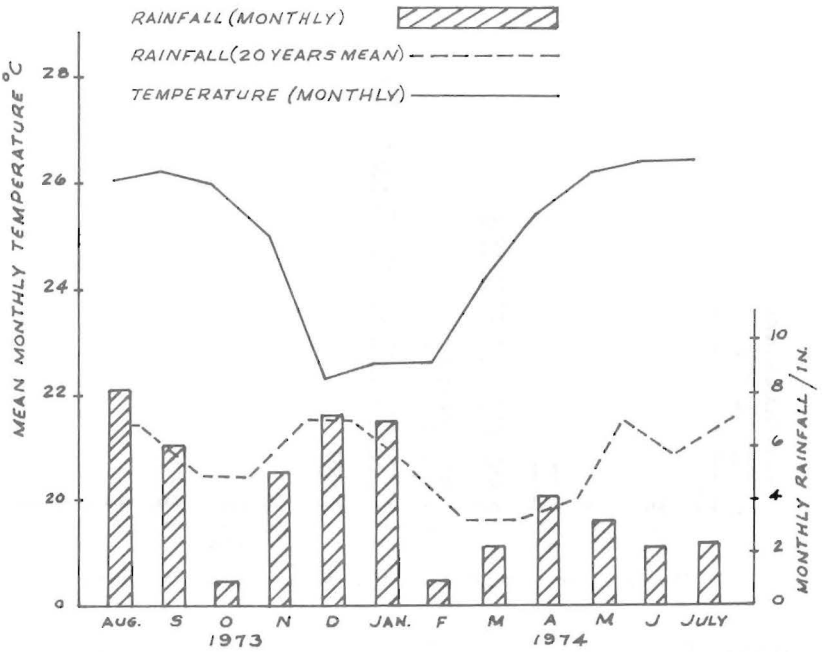

FIG. 7.—Mean monthly temperature and rainfall at Dorado, P.R.

detritus from the barn, and that such materials might prevent the growth of sufficient amount of algae and other microbiota essential to snail development, could be an explanation to the absence of *L. cubensis* and other snails in the ditches.

SERUM GLUTAMIC OXALACETIC TRANSAMINASE

Four of the 16 replacement heifers were found to be *F. hepatica*-infested by coprological examination only 2 mo after their addition to the herd in December 1973; five other heifers were also found positive after

2 additional months. Serum of blood samples from the nine positive and seven negative heifers was analyzed for constants, using SMA_{12}. No significant differences in hematological values were found. However, all heifers had elevated SGO-T values. The seven negative heifers had a relatively higher average level of SGO-T, 143.42 ± 27.19 MU while the nine *Fasciola*-infected had lower 127.41 ± 11.54 MU.

The relatively high SGO-T in the negative heifers probably indicates that they had been exposed to liver-fluke infestation and were sustaining hepatic tissue necrosis consequent to migration of juvenile flukes through the liver.

The relatively low SGO-T levels in the known *Fasciola*-infected heifers indicate perhaps that many of the flukes had already reached the bile ducts and there was less tissue damage. In fact, in March 1974, 8 mo after the replacement heifers were introduced into the herd, six of the seven heifers that had been negative for fascioliasis by coprological test were positive by the same test.

Ross (18) also observed elevated SGO-T values in calves given low and high levels of *F. hepatica* metacercariae. In cows with chronic fascioliasis, Valcarenghi and Molinary (22), did not observe elevation in either SGO-T or SGP-T. The value of the analysis of SGO-T in the diagnosis of fascioliasis in cattle is important. In areas where the disease is endemic, the SGO-T values may give a hint that the cow under examination might have been exposed to *F. hepatica*.

TRANSMISSION

It is assumed that infestation of cattle by *F. hepatica* in Puerto Rico occurs throughout the year because no pronounced drought is observed in northern Puerto Rico. Contrary to such assumption, the present field studies indicate that transmission of fascioliasis usually occurs from November to February when temperature and rainfall are favorable for the growth and development of the snail and of the infective stage of the liver-fluke.

In the laboratory at the Agricultural Experiment Station where the snail culture boxes were exposed to ambient temperature and the humidity controlled by addition of water to the culture boxes, the only time the snails grew well and produced large number of *F. hepatica* metacercariae was from November to February.

Figure 8 shows the monthly infestation index of the 16 replacement heifers in the Dorado dairy farm. In October, 2 mo after the heifers were introduced into the herd, 4 or 25% were positive for *F. hepatica* by coprological examination. In December, 9 or 56% were positive and in March, 15 or 94% were positive. The peak of transmission of fascioliasis in Puerto Rico is in December and January.

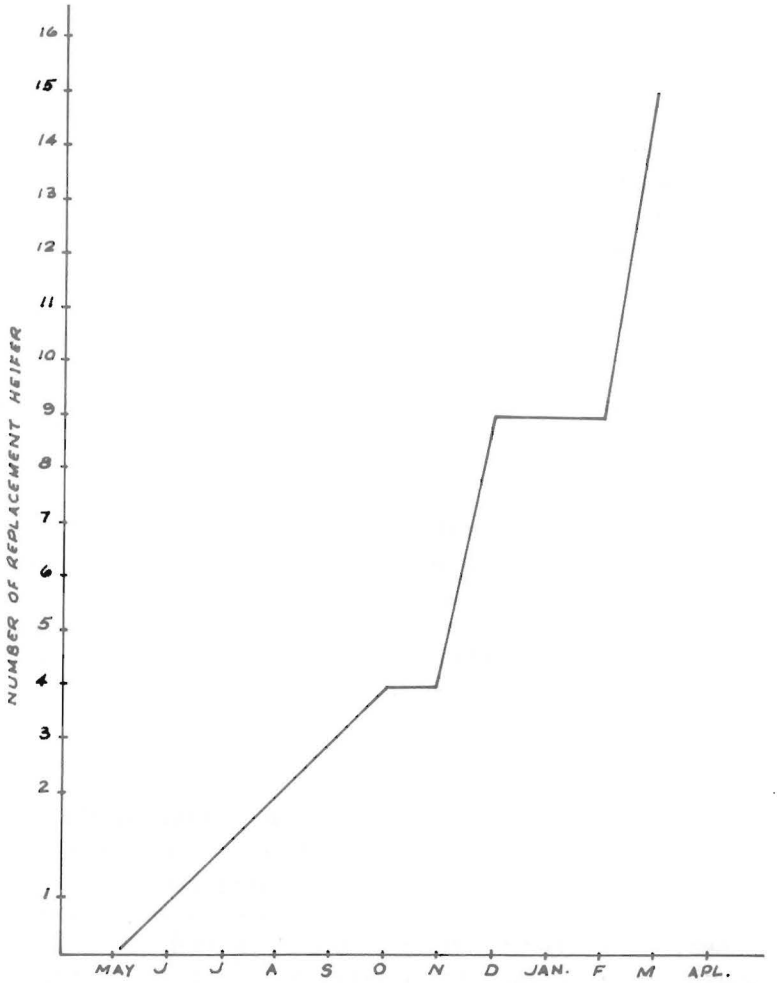

FIG. 8.—Monthly infection index of replacement heifers on the Dorado dairy farm.

CONTROL OF *F. HEPATICA*

Control of fascioliasis in dairy cattle in Puerto Rico is not easy. Hexachloroethane, the only drug approved by the Food and Drug Administration for treatment of fascioliasis, is effective only against mature liver-flukes. Furthermore, the drug is discharged through the udder which makes milk produced by hexacholorethane-treated cows unfit for human consumption. Also, because cows do not go "dry" all at one time, treatment of a few at a time is inconvenient, time-consuming, expensive, and actually ineffective.

Destruction of the snail intermediate host, *L. cubensis*, and its habitats is very discouraging. The flat and poorly drained pasture land lets water

accumulate during the rainy season. Molluscicide application, drainage and fencing of the snail habitats are expensive and impractical. Additionally, the ability of the snails to undergo cryptobiosis or aestivation constitutes a problem not easy to solve.

High quality forage and well-balanced concentrate may help correct the stress produced by fascioliasis in dairy cows until the time when highly effective drugs or vaccines are available.

RESUMEN

El 86% de las vacas (230 de un total de 268) de una lechería en una finca de Dorado tenían *Fasciola hepatica* cuando se hicieron exámenes croprológicos.

La infestación mayor fue de 95.4%, ésta se determinó en ganado de 9 a 12 años de edad, mientras que la infestación menor fue de 71.3% en animales más jóvenes (3 a 3½ años).

El caracol *Lymnaea cubensis* que sirve de hospedero intermedio a *F. hepatica*, era el más abundante en esa finca. Este es un organismo muy dinámico relativamente, con un ciclo de vida potencial de 21 días (de huevo a huevo).

La variabilidad y número de hábitats del caracol, el número de caracoles y el porcentaje de caracoles infestados con *F. hepatica* aumentaron significantivamente durante los meses de diciembre, enero y febrero, cuando el promedio de lluvia mensual fue de 145 mm y hubo temperaturas bajas con una media mensual de 24°C.

El ganado en el área de Dorado se infesta cone *F. hepatica* solamente durante los meses de noviembre a febrero. Este descubrimiento contradice la creencia de que las infestaciones occuren durante todo el año.

Un incremento en el número de análisis cuantitativos con el suero SGO-T (''serum glutamic oxalacetic transaminase'') del ganado en áreas endémicas es muy importante, ya que indicaría si los animales han sido expuestos a infestaciones de *F. hepatica*.

No hay control químico eficaz de la fasciolasis en el ganado lechero en Puerto Rico. Un control adecuado se puede obtener por medio de un programa sanitario y de buena alimentación.

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