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# Natural and Experimental Infections of THELAZIA CALIFORNIENSIS Price (1930, Nematoda: Thelaziidae) with Descriptions of the Adult and Larval Parasites

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# NATURAL AND EXPERIMENTAL INFECTIONS OF *THELAZIA CALIFORNIENSIS* PRICE (1930, (NEMATODA: THELAZIIDAE) WITH DESCRIPTIONS OF THE ADULT AND LARVAL PARASITES.

by

Howard S. Burnett

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A Thesis in Partial Fulfillment
of the Requirements for the Degree
Master of Science in the Field of
Microbiology

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June, 1958

I certify that I have read this thesis and that in my opinion it is fully adequate in scope and quality as a thesis for the degree of Master of Science.

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# INTRODUCTION

An investigation of the eye worm, Thelazia californiensis, was made because of the increasing number of reports of the worms in the eyes of dogs and other animals throughout the state of California. The worm had been unknown prior to 1929, yet, in 1955, 26 years after its discovery, it had been reported from 23 counties. It was beginning to be recognized as a threat to the eyes of man and other mammals throughout a large portion of the state and it was suspected that the range of the parasite was far broader than the reports indicated. As early as six years after the worm was first reported, Kofoid and Williams (1935), in considering the danger to dogs alone, recognized the importance of the parasite from the veterinarian's standpoint.

The life cycle, incidence, and distribution of the worm had not been determined. In short, its biology was unknown. Although it had been found in the eyes of man, dog, cat, sheep, deer, coyote, and black bear, no one knew what other animals might be susceptible to the infestation.

In 1955, the College of Medical Evangelists at Loma Linda, California, received a grant of money from the United States Public Health Service to finance studies on the biology of the parasite. A research team under the directorship of Dr. Edward D. Wagner, head of the Section of Parasitology, began work on the problem in August of that year.

This study is a part of that work and was designed to: (1) provide a more detailed description of the worm than any previously given, (2) gain pertinent information regarding the behavior of Thelazia infested animals in their usual enrivonment, (3) attempt through field study to determine the extent of the reservoir and the distribution of the parasite, and (4) determine through a series of transmission studies the type of life cycle involved.

## REVIEW OF LITERATURE

The nematode, Thelazia californiensis (Spiruridea: Thelaziidae) is a parasite found in the conjunctival sac of mammals in California. Parmelee et al. (1956) state that it has been reported from the eyes of a deer in the adjoining state of Nevada and from a dog in Oregon, not far from the California-Oregon border.

The species was established by Price (1930) from material removed from the eyes of dogs in two separate areas in California. It had, however, come to the attention of Allerton (1929) the year before, who mistakenly reported it as Thelazia callipaeda Railliet and Henry, (1910b) a species found in the Orient. Allerton's material was from a mixed collie dog near Los Angeles.

Hyman (1951) states that the two genera, Thelazia Bosc, (1819), and Oxyspirura Drasche in Stossich, 1897, "are known as eye worms and are...parasites of domestic animals and man," and are the only members of the family Thelaziidae Railliet, 1916, of any great economic importance.

Worms of the genus Oxyspirura are parasitic under the nictitating membrane of birds (Yorke and Maplestone, 1926). Oxyspirura mansoni Cobbald, 1879, and Oxyspirura parvovum Sweet, 1910, are the only important members of the species. The life cycle of the former was elucidated by Fielding (1926-1928), who found the cockroach, Leucophaea surinameusis, to be the intermediate host.

Within the genus Thelazia, the more important of the two genera mentioned, are nine species known to be parasites in the eyes of domestic animals and man. The genus was established by Bosc (1819) on the basis of a brief unpublished description by Johannes Rhodes in 1818, of material obtained from the eye of an ox in France. Desmarets (1828) examined and described the worm, designating the species Thelazius Rhodesii (sic.). Railliet and Henry (1910a) re-described the parasite, and in conformity with present-day terminology, called it Thelazia rhodesi.

Since the early reports by Bosc (1819) and Desmarets (1828), T. rhodesi has been reported on four continents. A summary of data by Kofoid et al. (1937) indicates its presence in Europe, Asia, Africa, and the United States. Other thelazias have been reported in various parts of the world, Australia being the only continent on which they have not been found. Yorke and Maplestone (1926) list eleven species. Kofoid and his associates (1923) mention a total of twenty three. Of these, eight are parasitic in the eyes of domestic animals and fifteen in wild animals and birds. To the former category has been added Thelazia bubalis, found in the eye of a broad winged hawk in Georgia, and described by Hwang and Wehr (1957).

Table 1 is a revised summary of the occurrence

TABLE 1

Occurrence and distribution of *Thelazia* spp. in man and domestic animals.

| *Thelazia* species | Host | Distribution | Authority |
|---|---|---|---|
| *T. rhodesi* | Ox | Europe (France) | Bosc, 1819, etc. |
| | | Egypt | Railliet and Henry, 1910 |
| | | Rhodesia and Nyassaland | Griffiths, 1922 |
| | | China | Faust, 1927 |
| | | U.S.(California) | Kofoid et al. 1937 |
| | | Siberia* | Krastin and Ivashkin, (1946)* |
| | | Russia* | Klessov (1949)* |
| | Horse | East Indies | Smit and Noto-Soediro, 1930 |
| | | (?) India | Lingard, 1906 |
| | Buffalo | Sumatra | Railliet and Henry, 1910 |
| *T. gulosa* | Ox | Europe (France) | Railliet and Henry, 1910 |
| | | Siberia* | Krastin and Ivashkin, (1946)* |
| | | Russia* | Klessov (1949)* |
| *T. skrjabini* | Ox | Russia | Erschow, 1928 |
| | | Siberia* | Krastin and Ivashkin, (1946)* |
| *T. lacrymalis* | Horse | Europe | Gurlt, 1831, etc. |
| | | Brazil | Travossos, 1918 |
| *T. leesi* | Camel | India | Railliet and Henry, 1910 |
| | | (?) Africa | Edmonds and Walker, 1929 |
| *T. erschowi* | Swine | Russia | Oserskaja, 1931 |
| *T. callipaeda* | Dog | India | Railliet and Henry, 1910 |
| | | China | Fisher, 1919, etc. |
| | | Japan* | This paper* |
| | Man | China | Stuckey, 1917, etc. |
| | | India* | Friedmann, (1951)* |
| *T. bubalis** | Buffalo | India | Ramanujachari and Alwar* (1957)* |

* Not cited by Kofoid et al. (1937)

and distribution of Thelazia spp. in man and domestic animals as compiled by Kofoid et al. (1937), with the addition of data from four authors whose reports have appeared since that time. T. callipaeda in Japan was found in dogs by R. Nagata in 1956 and 1957 and sent to the author for identification. The summary by Kofoid and his coworkers is further revised to suit the requirements of this paper by the omission of data concerning T. californiensis, which will be considered separately.

Thelaziasis

Infestation by eye worms of the genus Thelazia is referred to as thelaziasis. There is no definite agreement among workers who have encountered the disease as to the severity of symptomatology or the extent of associated pathology. Kofoid et al. (1937) state that extensive scarification due to the active movement of the worms probably leads to total blindness. They state that in cattle, more extensive pathological changes may follow which lead to the complete destruction of the eye ball. On the other hand, Price (1931) states that "ordinarily the symptoms (in dogs) are relatively mild and disappear soon after removal of the worms." Faust (1928) notes that in cases of recurrent infestation, opacity of the cornea develops to such an extent as "to reduce the... vision to a marked degree."

It is difficult to determine from the literature how many cases, proportionately speaking, have been found with the more serious pathological changes described by Kofoid and his coworkers. This is made especially true by the fact that most of the reports deal with isolated cases in humans and dogs wherein the infestation was discovered and treated before extensive damage was done.

In contrast to these reports of isolated cases, however, mass infestations have been reported. Krastin and Ivashkin (1946), in reference to the disease in cattle in Siberia, mention "entire groups of animals showing clinical symptoms of thelaziasis." Krastin (1949), also in reference to the disease in Siberia, mentions a "wide distribution and damage done to cattle by bovine thelaziasis." In a later publication (1950) he refers to a "*Thelazia* infested farm." Klessov (1949) states that the disease, thelaziasis with conjunctivitis, in cattle in Russia leads to "negative productivity" (of milk?), and that the disease may lead to blindness.

In England, Mitchell and Hughes (1953) report that the disease is rather common in cows in that country, especially among Herfords. On one farm they found twenty cows with keratitis associated with thelaziasis (spp. unknown).

There have been fifteen cases of thelaziasis reported in humans. A recent paper by Lee and Parmelee (1958) summarizes twelve previously reported cases and describes three new ones. Six cases have occurred in China, two in Korea, one in India, and six in California.

In most of the cases, the disease in humans has been associated with only moderate symptoms and minimal pathological changes. This is not always true, however. In a case reported by Trimble (1917), the patient complained of great pain in the affected eye with excessive lacrimation. A marked ectropoin of the lower lid had developed as a result of paralysis of the face muscles on that side. Examination revealed two worms of the species T. callipaeda. The pain and lacrimation stopped with the removal of the worms and a few weeks later the muscle tone in the paralyzed area had improved fifty per cent. Trimble voiced the opinion that the facial paralysis was due to the constant eye irritation caused by the worms over a period of about three months.

Little has been accomplished toward the determination of the life histories of most of the economically important thelazias. Workers in the United States and Russia have conducted life cycle studies with varying degrees of success. Attempts by Faust (1928) to infest various species of filth flies; the dog fly, Hippobosca francilloni; and the cockroach, Blatella germanica, with larval forms of T. californiensis were unsuccessful. Kofoid et al. (1937) were equally unsuccessful in attempts to infest cockroaches with the larval forms.

Workers in the U.S.S.R. report more success, however. Krastin (1949) determined the life cycles of three of the thelazias in Siberia. He found the fly, Musca *convexifrons* (Diptera: Muscidae) to be the intermediate host of *T. rhodesi*. Later (1950), he found *Musca amica* to be the intermediate host of *T. gulosa* and (1952) *T. skrjabini*. Klessov, (1951), working in the Ukraine, found *Musca larvipara* and *Musca autumnalis* to be intermediate hosts of *T. rhodesi* in that region. He also found that *M. larvipara* was capable of fulfilling the function of intermediate host for *T. gulosa*.

Work by Burnett et al. (1957), though not conclusive, has cast suspicion upon *Fannia canicularis*, the lesser house fly, and a related species, *Fannia benjamini*, as intermediate hosts of *T. californiensis*.

## THE PROBLEM OF THELAZIASIS IN CALIFORNIA

Evaluation of the problem of any parasitic disease depends upon a broad knowledge of three basic factors: (1) the nature of the disease and its effects upon the host; (2) the occurrence and distribution of the parasite; and (3) a knowledge of its life cycle. Although there is still much that must be learned concerning thelaziasis in California, information gained during the last decade has materially aided in the approach to the problem of the disease in this state.

Generally speaking, the effects of T. californiensis upon the eye and its adnexa appear to be comparable to the effects caused by related species. Again, however, as in the case of thelaziasis in other countries, workers have placed varying degrees of emphasis upon the severity of the disease. Many of the workers who have encountered the worm in California have reported its effects as benign and temporary. Price (1931) states that ordinarily the symptoms are relatively mild and soon disappear after the worm is removed. In general, this may be true in the case of domestic animals and humans, in which the worms are probably detected and removed before any great damage has been done. Most of the publications of thelaziasis in this country have, in fact, dealt with such cases.

Reminiscent of the statement by Klessov (1949) regarding blindness in cattle, however, is a case encountered in a chow dog by Wagner (1958) in which both eyes were severely affected by keratitis and conjunctivitis. Corneal ulcers in the left eye had rendered it permanently sightless (Plate 1). The right eye was still partially functional. It is not known if the damage to the eyes was a sequela of thelaziasis or a coincidental condition of unknown cause. However, in light of the fact that Douglas (1938) also reports a case resulting in blindness in a dog, the first possibility cannot be completely discounted.

Even in man the worm may effect serious pathological changes. In a case reported by Lee and Parmelee (1958) the eye had been so affected that the upper and lower tarsal conjunctiva revealed a condition described as marked follicular hypertrophy. The same report states that in all, six cases have been reported in man in California.

Somewhat comparable to the report of infested herds of cattle in England by Mitchell and Hughes (1953) is an account by Stewart (1940) of keratitis associated with thelaziasis in three bands of sheep in Sonoma County, California. He does not state how many of the sheep were affected but the nature of the article suggests that the infestation was general throughout the herds.

Members of the California Department of Fish and

PLATE 1

*Thelazia californiensis* in the eye of a dog.

Photo by Dr. Edward D. Wagner, 1957.

PLATE 1

Game have shown some concern regarding the disease among deer. Oberhansley (1940), Herman (1944), and Dixon and Herman (1945) have suggested that thelaziasis may be responsible for numerous inflammatory and suppurative eye conditions observed in that animal in certain regions.

The occurrence and distribution of the disease throughout the state is wide spread. Wagner (1958) has expressed the opinion that the occurrence is probably quite common among deer. This opinion is based upon data given him by personnel of the Department of Fish and Game, which indicates that 21 (88%) of 24 deer examined in Tulare County in 1948 were infested. Data from other counties indicate an infestation rate ranging from 2 per cent to 70 per cent.

A survey by Parmelee et al. (1956) located the parasite in forty of the fifty-six counties in California and indicates that it has been found in Washoe County, Nevada, in a deer, and Josephine County, Oregon, in a dog. Both of these counties are adjacent to California. The data given by Wagner implicates two counties not reported by Parmelee and his associates. Work done during this study located the disease in one more county, making a total of 43 counties in which the parasite is known to exist. The counties involved are listed in Table 2 and are illustrated on Plate 2.

*T. californiensis* appears to affect a wider variety of definitive hosts than have any of the other known

TABLE 2

Counties in California from which *T. californiensis* has been reported.

| | | | |
|---|---|---|---|
| Alameda | Lassen | Orange | Sierra |
| Butte | Los Angeles | Plumas | Siskiyou |
| Contra Costa | Madera | Riverside | Solano |
| El Dorado | Marin | San Bernardino | Sonoma |
| Fresno | Mariposa | San Diego | Stanislaus |
| Glenn | Mendocino | San Francisco | Sutter |
| Humboldt | Modoc | San Joaquin | Tehama |
| Inyo | Mono | San Luis Obispo | Tuolumne |
| Kern | Monterey | Santa Barbara | Tulare |
| Kings | Napa | Santa Cruz | Ventura |
| Lake | Nevada | Shasta | |

species. By referring to Table 1 it may be seen that *T. rhodesi* has been found in the ox, horse, and buffalo; *T. callipaeda* in dog and man. The rest of the species indicated on the table have been limited to one animal each. *T. californiensis* has been reported in ten mammalian hosts, as listed in Table 3.

TABLE 3

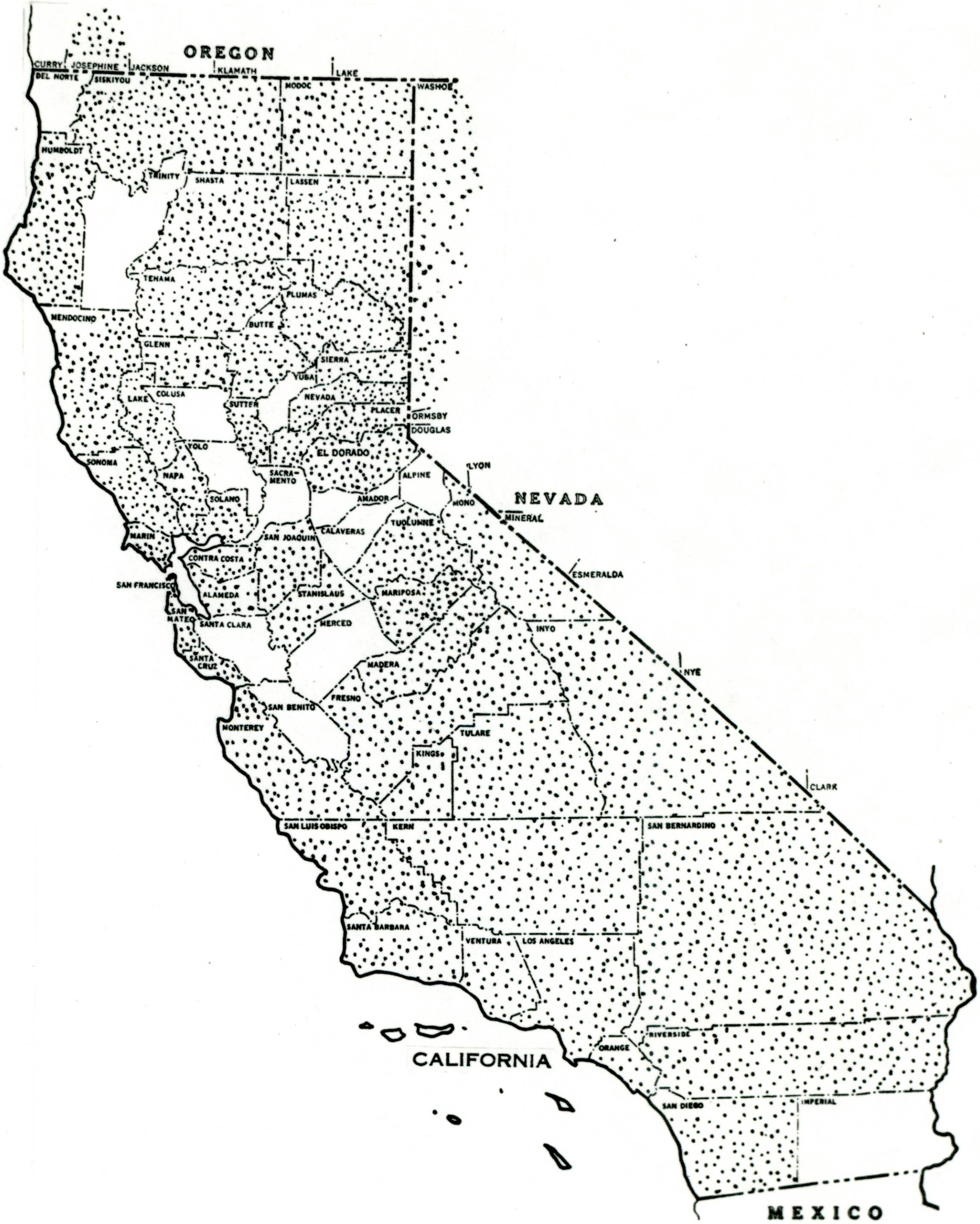
Definitive hosts of *T. californiensis*

| Host | Authority |
|---|---|
| Man | Kofoid and Williams (1935), etc. |
| Dog | Allerton (1929), etc. |
| Coyote | Herman (1949) |
| Cat (house cat) | Douglas (1939) |
| Fox (commercial silver fox) | Burnett and Wagner (1958) |
| Black bear | Hosford et al. (1942) |
| Sheep | Stewart (1940) |
| Deer (mule deer) | Oberhansley (1940) |
| (columbian black-tailed deer) | Herman (1944) |
| Horse | Parmelee et al. (1956) |
| Jack rabbit | Burnett and Wagnen (1958) |

PLATE 2

Counties in California, Nevada, and Oregon reporting T. californiensis.

PLATE 2


OREGON
CURRY
JOSEPHINE
JACKSON
KLAMATH
LAKE
DEL NORTE
SISKIYOU
MODOC
WASHOE
HUMBOLDT
TRINITY
SHASTA
LASSEN
TEHAMA
PLUMAS
MENDOCINO
BUTTE
GLENN
SIERRA
YUBA
LAKE
COLUSA
SUTTER
NEVADA
PLACER
ORMSBY
DOUGLAS
YOLO
EL DORADO
SONOMA
NAPA
SACRAMENTO
ALPINE
LYON
SOLANO
AMADOR
MONO
NEVADA
MINERAL
TUOLUMNE
MARIN
SAN JOAQUIN
CALAVERAS
CONTRA COSTA
SAN FRANCISCO
ESMERALDA
ALAMEDA
STANISLAUS
MARIPOSA
SAN MATEO
SANTA CLARA
MERCED
INYO
SANTA CRUZ
NYE
MADERA
FRESNO
SAN BENITO
MONTEREY
TULARE
KINGS
CLARK
SAN LUIS OBISPO
KERN
SAN BERNARDINO
SANTA BARBARA
VENTURA
LOS ANGELES
CALIFORNIA
ORANGE
RIVERSIDE
SAN DIEGO
IMPERIAL
MEXICO

## DESCRIPTION OF *THELAZIA CALIFORNIENSIS*

## PRICE, 1930

The following description of *T. californiensis* is based upon examination of 23 adult worms (10 females and 13 males) removed from the eyes of a jack rabbit near Banning, California. Body measurements are given in Table 4.

### Description

*Thelazia*. (Plate 3). The worm, *T. californiensis*, is creamy white, slender, and cylindrical, with an annulated cuticle. The caudal extremity of both sexes is bluntly attenuated, tapering from full body width to the tip in a distance of 0.5 to 0.66 mm. The anterior two-thirds of the worm tapers gradually to a hemispherical cephalic cap which protects the cephalic extremity. The cuticular annules begin at the posterior margin of the cephalic cap and continue to the caudal tip. Their posterio-lateral margins form acute angles, giving the sides of the worm a serrated appearance. The total number of annular rings counted on the 23 specimens ranges from 738 on a male 13.5 mm. long to 902 on a female 22 mm. long. The average number of annular rings per mm. per worm ranges from 41 to 51. The length of each annular ring is about 30 microns. At the extremities the rings diminish to 15 microns or less.

TABLE 4

Measurements of Thelazia californiensis*
(All measurements in millimeters)**

| | Male | Female |
|---|---|---|
| Length | 11.2 -- 14.5 | 19.0 -- 22.0 |
| Greatest diameter | 0.384 -- 0.53 | 0.397 -- 0.53 |
| Diameter at first striation | 0.081 -- 0.105 | 0.093 -- 0.105 |
| Buccal capsule--length | 0.024 -- 0.030 | 0.030 -- 0.036 |
| Buccal capsule--diameter | 0.030 -- 0.039 | 0.030 -- 0.042 |
| Esophagus--length | 0.50 -- 0.57 | 0.54 -- 0.646 |
| Esophagus--diameter | 0.051 -- 0.072 | 0.051 -- 0.075 |
| Anus from posterior end | 0.105 -- 0.15 | 0.111 -- 0.15 |
| Left spicule--length | 1.47 -- 2.45 | |
| Left spicule--diameter | 0.006 -- 0.009 | |
| Right spicule--length | 0.16 -- 0.204 | |
| Right spicule--diameter | 0.018 -- 0.036 | |
| Vulva from anterior end | | 0.875 -- 1.1 |

* Ten females and 13 males examined.
** These measurements were made with the worm intact. Measurements requiring dissection are given in the text.

Cephalic structures (Plate 3, fig. 1, 2). The cephalic cap which covers the head has two circles of papillae, an inner circle of six papillae that surrounds the buccal capsule, and an outer circle of eight. The papillae of the inner circle are situated as follows: two laterally, two subdorsally, and two subventrally. These structures arise in the anterior margin of the somatic wall beneath the cephalic cap and extend cephalad to the point where the latter structure joins the buccal capsule. Viewed en face, they appear as rays whose inner ends impinge slightly upon the outer margin of the buccal capsule, and whose outer ends fan out and merge with the surrounding structures which constitute the

## PLATE 3

### Anatomy of *Thelazia californiensis*

Figures 1, 3, and 6 were made with the aid of a camera lucida. Figure 2 is a composite.

1 . . Cephalic structures, lateral view.

2 . . Cephalic structures, en face view.

3 . . . . . . Esophago-intestinal valve.

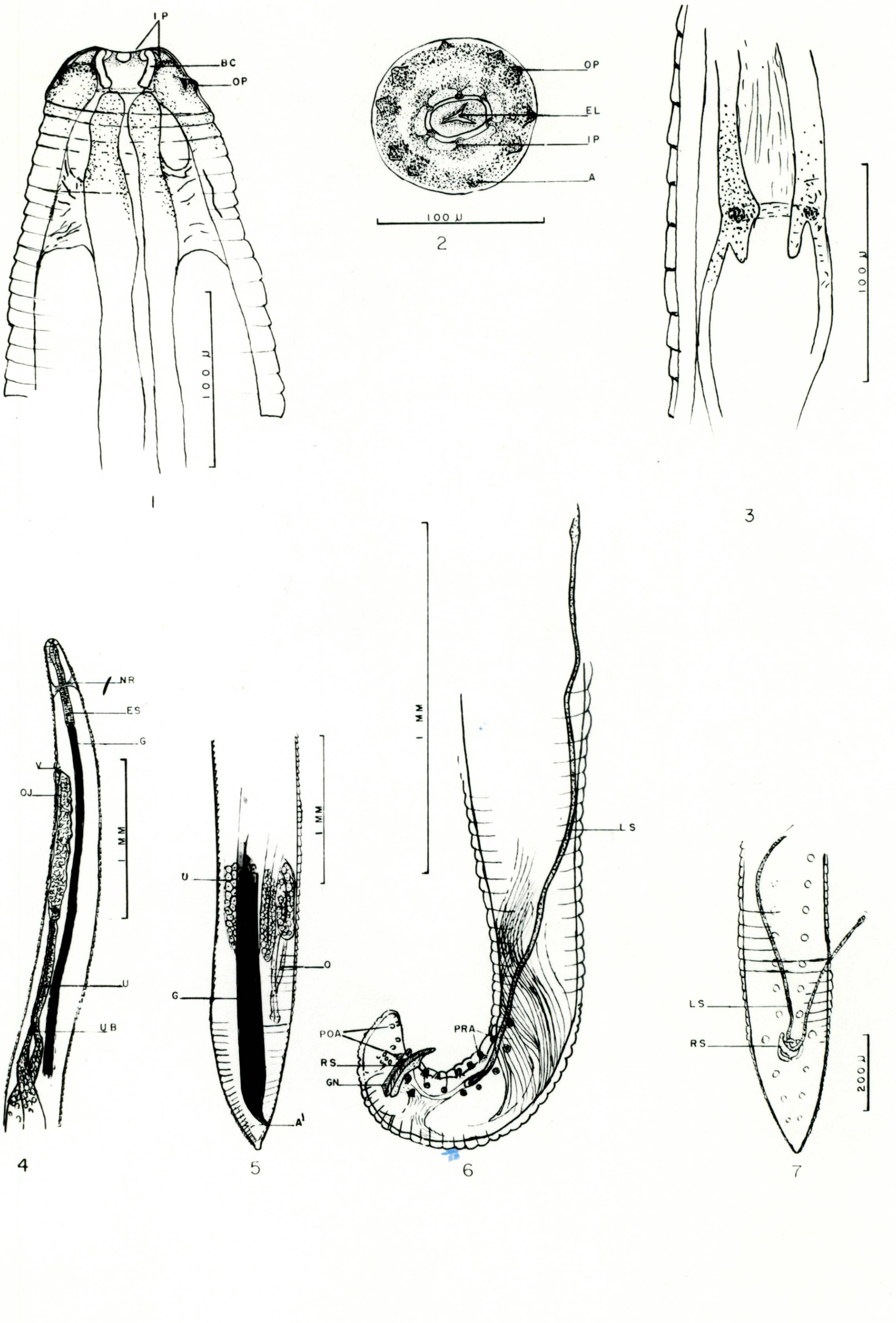
4 . . . . . . . . Anterior end of female.

5 . . . . . . . Posterior end of female.

6 and 7 . . . . . Posterior end of male.

A, amphid; $A^1$, anus; BC, buccal capsule; EL, esophageal lumen; ES, esophagus; G, gut; GN, gubernaculum; IP, internal papillae; LS, left spicule; NR, nerve ring; O, ovary; OJ, ovejector or larvejector; OP, outer papillae; POA, post anal papillae; PRA, pre anal papillae; RS, right spicule; U, uterus; UB, uterine bifurcation; V, vulva.

# PLATE 3


IP
BC
OP
100 µ
1
OP
EL
IP
A
100 µ
2
100 µ
3
NR
ES
G
V
OJ
1 MM
U
UB
4
1 MM
U
O
G
A1
5
1 MM
LS
POA
PRA
RS
GN
6
LS
RS
200 µ
7

most anterior portion of the body wall. The outer, or external circle of eight paired papillae lies even with the posterior end of the buccal capsule. Each pair lies caudad to one of the subdorsal or subventral members of the inner circle. Two amphids, one on either side of the cephalic cap, lie caudad to the lateral papillae of the internal ring approximately at the level of the external ring. This description of the cephalic structures is much the same as the description by Price (1931).

Alimentary canal (Plate 3, fig. 1--5). The buccal capsule is a cup-shaped cavity whose length is usually a few microns greater than its depth and whose base is narrower than its lipless oral aperture by about one third. It appears to be continuous with, though considerably thicker than, the cephalic cap, in which it is centrally located, and opens posteriorly into the triradiate lumen of the esophagus.

The esophagus is a thick-walled, muscular tube, having its terminus in the anterior end of the straight, tubular gut. The intestino-esophageal valve is simple, without bulb or diverticulum. The gut, whose anterior end compares in size to the diameter of the esophagus, gradually doubles in diameter as it progresses caudad. At the caudal tip it tapers abruptly to join the ventrally located anus.

Reproductive system, female (Plate 3, fig. 4, 5). T. californiensis is viviparous. The vulva, which is slightly prominent, is situated caudad to the posterior terminus of the esophagus and is followed by a cylindrical larvejector about 1 mm. in length and about 100 microns in diameter.

The uterus lies caudad to the larvejector as a single tube about 50 microns in diameter for a distance of about 1 mm., at which point it bifurcates into two limbs of identical construction and function. These limbs, about 50 microns in diameter at the point of bifurcation, expand in less than 1 mm. to 115 microns. Their total length is about 1.5 times the length of the worm. Their course is generally parallel to the long axis of the worm's body. Their extreme length is made possible by three reversals of direction and three convoluted folds at their most posterior position.

With the last convolution, about 0.6 mm. from the caudal tip, the limbs of the uterus turn cephalad for about 0.5 mm., abruptly lose caliber to 50 microns and join the seminal receptacles. These organs are from 1.75 to 2 mm. long and about 30 microns in diameter at their junction with the ovarian ducts with which they are continuous. The latter organs are about 5 mm. long and, at their distal ends, join the tubular ovaries which are 4 to 5 mm. long. These paired tubules taper gradually to about 20 microns, then expand slightly, forming terminal bulbs approximately 30 microns in diameter.

Reproductive system, male (Plate 3, fig. 6, 7). The male copulatory organ consists of two spicules of unequal size, the right being the broader of the two, but the left being 10 to 15 times longer than the right. A gubernaculum, as described in *T. californiensis* by Luckner (1950), is present. The single, tubular testis originates in the anterior quarter of the body and terminates about 2 mm. from the anus. Continuing caudad from the testis to the cloaca is the duct which makes up the ejaculatory system. In the unsectioned worm it cannot be differentiated into seminal vesicle and vas deferens.

For a distance of about 1 mm. the posterior end of the male is recurved ventrally to form a hook which is bent slightly to one side. On the ventral surface of the curved portion are eight to ten pairs of papillae. Three pairs are postanal and the remaining 5 to 7 pairs are preanal. In addition to the paired preanal papillae, 12 of the 13 males examined had one additional unpaired papilla on the left. The remaining male, which had but 5 paired preanal papillae, had 2 unpaired ones on the left. The left papillae appear to be more prominent than the ones on the right. Price (1931) states that there are 6 to 7 pairs of preanal papillae, whereas Kofoid et al. (1937) state that there are 5 to 7 pairs. They do not mention any unpaired ones.

To confirm the finding of the odd number of preanal papillae, two males were taken at random from each of five vials of preserved specimens received from veterinarians from various parts of the state, and the papillae examined. The papillae on three of the worms (one from each of three vials) could not be satisfactorily counted. The other worms from these vials, however, had one unpaired preanal papillae each. From the remaining two vials, only one worm was clearly without the unpaired member.

The embryo (Plates 4, 6, fig. 1-3). En utero, the embryos of this viviparous worm may be seen occuping the anterior half of the uterus and the larvejector. They lie in a coiled position, resembling three spirals of a coiled spring whose two end spirals lie against the middle spiral. Each embryo is within a transparent sheath which is coiled in such a manner that it assumes an ovoid shape. Upon ejection from the female, the embryo extends itself into a typical arched position, and the sheath adjusts itself into a banana-shaped envelop, slightly longer than its occupant and about 35 microns in diameter at its central point.

The embryos at the time of maximum development in the female are filiform, 130 to 170 microns in length and 8.8 to 9 microns in diameter. In their relaxed state they assume an arched position with the last ten microns of the caudal end reflexed sharply. The cephalic end is slightly bulged to form a somewhat spherical head which is crowned with a circle of highly refractile hook-like structures.

The ensheathed embryo is annulated but has no clearly defined internal structures.

# PLATE 4

Ensheathed embryos of *T. californiensis*.

From a motion picture by Dr. J. A. Howarth, 1934, showing embryos and eggs removed from a female *T. californiensis*.

PLATE 4

## METHOD OF EXAMINATION

For the general description of *T. californiensis*, male and female, the overall length was determined by the use of a dissecting microscope and a millimeter scale. Before examination, the male required special preparation in order to straighten the recurved portion of the posterior end. This procedure is best accomplished by using a freshly obtained, unfixed worm. The technique used is as follows. The worm is placed on a microslide in physiological saline under a 22 by 40 mm. cover glass (thickness 2), then a second cover glass of the same dimensions is pushed under the first cover glass until its inner edge lies close to, and parallel with, the dorsum of the worm. (Due to the hooked portion of the tail, the male invariably lies on one side.) By placing a ten gram balance weight on the top cover glass, on the side supported by the second glass, the arrangement is made fairly stable. A third cover glas is now slid under the opposite side of the first one, in such a manner that the worm is confined in the enclosure formed by the microslide and the three cover glasses. By carefully manipulating the second and third cover glasses, the worm may be forced into a perfectly straight position, between them.

The recurved portion of the worm is best straightened by sliding the third cover glass into position in such a manner that one corner is hooked by this portion of the worm as the glass slides diagonally into position. By placing an additional ten or twenty grams of weight on the top cover glass, the worm is not able to change its position, even though it be extremely active. By placing the entire assembly in a Petri dish with a filter paper saturated with 70% alcohol in the bottom, the worm dies in a few hours, the recurved portion remaining straight enough for careful examination.

Active worms of either sex can be confined for measurement and examination by this method, and by omitting the alcohol treatment, they can be kept alive.

Two methods were used in examining the cephalic structures. The first method consists of simple examination of the worm under the compound microscope. Structures are best observed in fresh unfixed material. By orienting the worm as straight as possible, parallel to the long axis of the slide, then wetting with 0.85% saline and covering with a cover glass, it is easily rolled for examination on all sides. Rolling is accomplished by sliding the cover slip carefully with the fingers or the eraser end of a pencil while viewing through the microscope.

By this method, the buccal capsule may be examined to advantage. By viewing the organ from every side without interruption, it may be seen to be a cup-shaped capsule whose sides are continuous and of unchanging shape and structure through 360 degrees. It may be seen that there are neither "plates," as described by Faust (1928) in *T. callipaeda*, nor (in harmony with Chitwood and Chitwood, 1950), any form of labia.

Although the method described above makes it possible to see the cephalic papillae, it is not possible to determine their anatomical relationship to each other or to other structures, except to see that they are approximately even with the posterior end of the buccal capsule.

In order to determine more completely the cephalic structures, the cephalic extremity may be carefully dissected off, oriented with the buccal capsule up, and magnified 100x. The method of dissection is crude but simple. The worm is placed on a microslide and dampened with saline. Then, under the dissecting microscope, using 40 magnifications, the worm is cut through with a small, sharp knife at the posterior margin of the cephalic cap. A myringotome (eardrum lancet) is excellent for this purpose. The free cephalic tip is then removed to a fresh slide on which has been placed a small drop of glycerine jelly, and viewed en face under the compound microscope.

The male structures may be examined without benefit of dissection. However, by extending the recurved posterior tip and rolling the worm as described above, the relative positions and comparative sizes of the organs described are not too difficult to determine.

Description of the vulva, larvejector, anterior part of the uterus, and the direction and course of the uterine limbs may be accomplished by examination of the undissected worm under the microscope. However, in order to examine and measure the limbs of the uterus and the more distal parts of the reproductive system, dissection is necessary. This is accomplished under the dissecting microscope.

A small lateral incision is made in the worm, anterior of the vulva, just large enough to free the larvejector from the vagina. Next, the posterior tip of the worm is completely severed just caudad of the most posterior portion of the ovaries. By holding the caphalid end of the worm against the slide with a teasing needle and, at the same time, exerting gentle pressure with the smooth back of the myringotome, starting at the point of the first incision and sliding the instrument caudad, the entire system of tubules may be expressed into the saline at the open posterior end of the worm. By careful manipulation, these filimentous organs may be extended, covered with a cover glass, and examined.

The nerve ring, esophagus, gut, and anus are identified without dissection.

These methods of examination are not adequate to identify either the excretory system or nervous system.

## FIELD STUDIES

Preliminary studies

In order to obtain a first-hand picture of thelaziasis and factors related to its transmission, it was necessary to locate a number of infested animals and to conduct several field studies and experiments.

Through the cooperation of several veterinarians, a number of dogs were located which were known to have been repeatedly infested. Some of the areas in which these dogs live lie near enough to this institution to be ideal for field study. One of the dogs, an English shepherd, living in Waterman Canyon, in the mountains near San Bernardino, was infested 14 times between July, 1955, and the end of 1957. One hundred forty four worms were removed from its eyes during that period. The largest number of worms removed from its eyes at any one time was 26 and the smallest number was 3. Another dog in the same area, a chow, was infested six times during the same period. The total number of worms removed from this dog is unknown, but between 62 and 70 were removed at one time. Another English shepherd living at Crestline, a few miles from Waterman Canyon and deeper into the mountains, was found to be infested three times between June, 1955, and June, 1956, with a total of 93 worms. The greatest number removed from this dog at one time was 65 and the smallest number was

10. A mongrel dog living in San Dimas Canyon, about 30 miles west of San Bernardino, was found to have 51 worms in its eyes. This dog was examined but once.

Except for transient opacities observed in one eye of the dog at Crestline, a varying degrees of conjunctivitis in all four of the dogs, no detectable pathological changes occurred as a result of these infestations. At the time that 65 worms were removed from the dog at Crestline, narrow, cloudy, bluish lines were visible across the sclera and cornea of the affected eye. These lines were scroll-like, forming a net-like pattern across the eye ball. Thirty days after the removal of the worms, the eye appeared to have recovered its normal sparkle and luster, and every visible trace of the opacities had disappeared.

No such marking of the eye ball has been noticed in the eye of any other animal during this study. It is common, however, to find the infestation associated with mild to moderate conjunctivitis. Infested dogs indicate annoyance in various ways. One dog owner learned that signs of photophobia displayed by the animal (lying with the head in a dark closet) indicated infestation. Another owner reported that when infested, his dog would rub its eyes against the cushions of the davenport. Other infested dogs were seen to rub their eyes with their paws.

By questioning the owners, it was learned that the four dogs mentioned above lived continually in the vicinity of their homes, without traveling to other areas. The nonitinerant habits of the animals suggest that within the areas in which they lived is a source of infestation. Intermediate host studies were conducted in these areas.

Intermediate host studies

Experiments (to be discussed later) in which the ensheathed embryos expressed from gravid female eye worms failed to develop in the eyes of rabbits in which they were placed, led to the conclusion that these fresh larval forms are non-infective to the definitive host. This conclusion was not based upon the negative results of a few experiments alone, however. It will be recalled that Kofoid et al. (1937) failed in similar experiments, and that Krastin (1949, 1950, and 1952) and Klessov (1949 and 1951) found that thelazias in the U.S.S.R. require intermediate hosts (muscid flies). In the light of these facts, attention was directed toward the study of flies in the three endemic areas already discussed.

Large numbers of *Fannia canicularis*, the common house fly, were found in the Waterman Canyon area during the summer months. It was noted that these flies displayed a marked avidity for the eyes of dogs. The dog with the highest reinfestation record displayed a remarkable tolerance for them, allowing them to move about on the margin of the eye lids and even enter the eyes. Many of the flies appeared to be ingesting lacrymal secretions.

During the months of September and October, swarms of Fannia benjamini, flies often found in mountainous regions, appeared in the San Dimas Canyon area. These flies, like the F. canicularis, display an affinity for the eyes of mammals.

Flies of both species were caught and taken to the laboratory for culture and special study. An effort was made to determine if Fannia species were present in every area studied.

Reservoir host studies

In order to determine which wild animals might act as reservoir hosts, two special studies were conducted. In November, 1955, with the cooperation of the California Department of Fish and Game, the research team assigned to the study of Thelazia examined 129 mule deer, Odocoileus hemionus sub spp., in Mono County. The deer were killed by deer hunters during a special hunting season and examined at checkout stations established by the Fish and Game Department. Seven of the 129 deer examined were infested.

The following year, again through the cooperation of the Fish and Game Department, a number of wild animals were examined. These animals were: 154 jack rabbits, Lepus californicus; 53 bob cats, Lynx rufus; 52 coyotes, Canis latrans; 12 gray foxes, Urocyon cinereoargenteus; 10 cottontail rabbits, Silvilagus audoboni; 6 ground squirrels, *Citellus beecheyi*; 5 raccoons, *Procyon lotor*; 3 skunks, *Mephitus mephitus*; 2 badgers, *Taxidea taxus*; and 1 oppossum, *Didelphus virginiana*. Of the above listed animals, eight coyotes and seven jack rabbits were infested. Table 5 is a list of areas in which *T. californiensis* was found in wild animals, with a list of animals examined in each area and the number of each found infested. For purposes of comparison, in areas where more than one animal species was found infested, the ratio of infestations in these animals is shown. Since jack rabbits and coyotes are the only combination of infested animals found in any of the areas, these are the only animals which can be compared. Infested jack rabbits from two areas where no coyotes were examined obviously cannot enter into the comparison.

Although the number of bob cats and foxes examined in the Banning Canyon area constitutes a fair sampling, noe one of these animals was found to be infested. The raccoon also apparently escaped the infestation in the same area. The ground squirrel, skunk, and oppossum, although free of the parasite, were too few in number to be significant.

The data summarized in Table 5 appear to indicate a marked susceptibility to thelaziasis by the jack rabbit and the coyote. The comparative data regarding these two animals, though meager, coupled with the fact that the jack rabbits were more heavily infested than the coyotes,

TABLE 5

Areas in which wild animals were found infested with T. californiensis

| Area | Animal | Number Examined | Number Infested | Per Cent Infested | Ratios* |
|---|---|---|---|---|---|
| Colville | Mule deer | 39 | 2 | 5.1 | |
| Fales Hot Springs | Mule deer | 70 | 3 | 4.3 | |
| Tom's Place | Mule deer | 20 | 2 | 10.0 | |
| Cajon Canyon | Jack rabbit | 24 | 2 | 8.7 | |
| Lone Pine | Jack rabbit | 14 | 1 | 7.1 | |
| Banning Canyon | Jack rabbit | 4 | 2 | 50.0 | 1.5/1* |
| | Coyote | 6 | 2 | 33.3 | |
| | Bob cat | 22 | 0 | 0 | |
| | Gray fox | 10 | 0 | 0 | |
| | Raccoon | 5 | 0 | 0 | |
| | Skunk | 2 | 0 | 0 | |
| | Opossum | 1 | 0 | 0 | |
| | Squirrel** | 3 | 0 | 0 | |
| Anza | Jack rabbit | 6 | 2 | 33.3 | 2.6/1* |
| | Coyote | 39 | 5 | 12.8 | |
| | Bob cat | 15 | 0 | 0 | |
| | Fox | 2 | 0 | 0 | |
| | Skunk | 1 | 0 | 0 | |
| | Squirrel** | 1 | 0 | 0 | |
| Barton Flats | Jack rabbit | 1 | 0 | 0 | |
| | Coyote | 2 | 1 | 50.0 | |
| | Squirrel** | 1 | 0 | 0 | |

* Ratio of infested jack rabbits to infested coyotes.
** Gound squirrel.

suggest that the former is the more susceptible of the two. A total of 57 worms was found in the four infested rabbits, an average of 14.2 worms per animal, whereas in the eight infested coyotes, a total of only 17 worms was found, an average of 2.1. One of the rabbits had 30 worms and another had 28. In contrast to these figures, the largest number found in any of the coyotes was five. The worms found in one of the rabbits were larger than any previously reported, some of the females measuring 22 mm. in length. The longest reported by Price (1931) in his description of the species was 17 mm. Herman (1944), however, found females 18.8 mm. long.

In three areas where a comparison of animals can be made, 4 (36.3%) of 11 jack rabbits and 8 (17.0%) of 47 coyotes were positive, an aggregate ratio of 2.1/1.

The data summarized in Table 5 do not account for all of the wild animals examined. Animals from areas where the eye worm was not found are not included.

Data tabulated during these studies support the suggestion made by Hosford et al. (1942) that infestations are associated with "hilly or mountainous areas which are more or less covered with brush." Table 6 is a tabulation of the physical characteristics of areas studied. It also indicates whether or not F. benjamini and infested animals were found, indicating their presence or absence by use of the plus (+) or minus (-) signs, respectively. Presence or absence of streams and potential fly breeding places are indicated by the same signs. The nature of the terrain and the amount of shade offered by trees or large bushes are indicated by use of a numeral scale of 0 to 4. The scale applied to the terrain as follows: 0=plateau or flat valley floor; 1=rolling hills; 2=hilly country with valleys and plateaus; 3=mountainous country with canyons and plateaus; 4=mountainous country with steep ridges and deep canyons. It applies to the amount of shade as follows: 0=no shade or only that amount offered by sage brush or small shrubs; 1=25% or less of the ground shaded by trees or large bushes; 2=over 25% but not over 50% of the ground shaded by trees or large bushes; 3=over 50% but not over 75% of the ground shaded by trees or large bushes. Elevation is given in feet above sea-level. The term "breeding places" applies to any deposit of debris or rotting vegetation large enough to provide a possible breeding place for flies. These areas are shown on the map, Plate 5.

TABLE 6

Physical characteristics of areas studied. Endemicity of the areas for _T. californiensis_ and _Fannia_ species.

| Area | Elevation | Nature of Terrain | Amount of Shade | Streams | Eye worms | _Fannia benjamini_ | Breeding Places |
|---|---|---|---|---|---|---|---|
| Anza | 4000-4500 | 1-2 | 1-2 | + | + | + | + |
| Banning Canyon | 3500-4200 | 1-3 | 1-4 | + | + | + | + |
| Barton Flats | 5000-7200 | 2-4 | 1-3 | + | + | + | + |
| Big Bear Lake | 6700 | 1-3 | 1-2 | + | + | * | + |
| Big Pine | 4000 | 0 | 0 | + | - | * | * |
| Cajon Canyon | 4000 | 0-2 | 1-2 | * | + | * | * |
| Cedar Springs | 3400 | 0-3 | 0-3 | + | + | + | + |
| Colville | over 5000 | 1-3 | 1-3 | + | + | * | + |

TABLE 6 (Con't.)

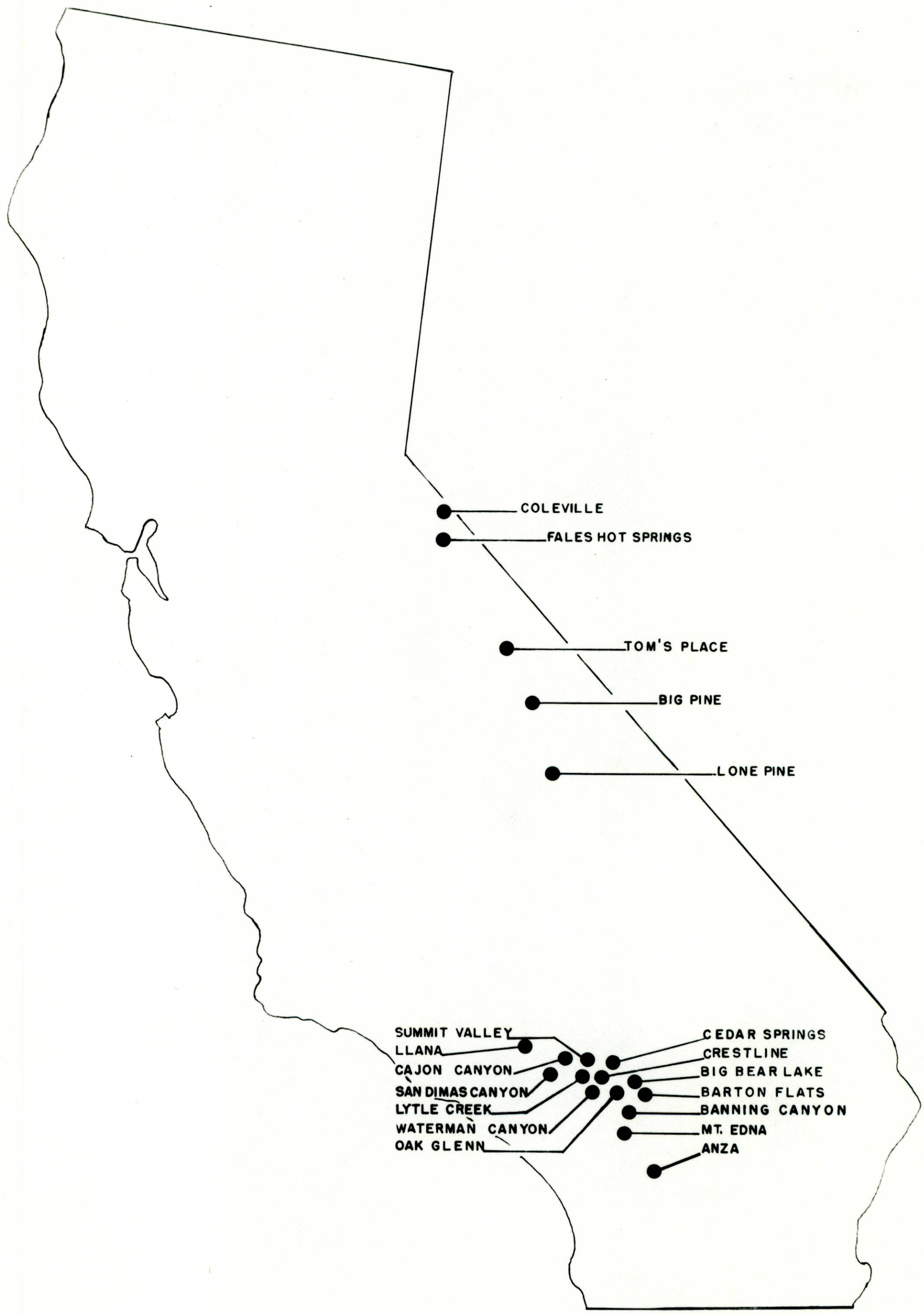
| Area | Elevation | Nature of Terrain | Amount of Shade | Streams | Eye worms | *Fannia benjamini* | Breeding Places |
|---|---|---|---|---|---|---|---|
| Crestline | 4800 | 3 | 2-4 | + | + | + | + |
| Fales Hot Springs | over 7000 | * | * | + | + | * | * |
| Llano | 3500 | 0-1 | 1-2 | - | - | - | - |
| Lone Pine | 3700 | 0-1 | 0-2 | + | + | ** | + |
| Lytle Creek | 3000 | 2-4 | 2-4 | + | + | + | + |
| Mt. Edna | 3500-4000 | 1-4 | 1-3 | + | - | * | + |
| Oak Glenn | 4200-4700 | 2-4 | 1-3 | + | + | + | + |
| San Dimas Canyon | 1450 | 3-4 | 2-4 | + | + | + | + |
| Summit Valley | 3300-3800 | 0-3 | 0-3 | + | - | + | + |
| Tom's Place | over 6000 | 1-3 | 0-2 | + | + | + | + |
| Waterman Canyon | 3000 | 1-3 | 2-4 | + | + | + | + |

* Not investigated.
** Tentatively identified.

PLATE 5

Outline map of California, showing areas in
which animals were examined during this study.

PLATE 5


COLEVILLE
FALES HOT SPRINGS
TOM'S PLACE
BIG PINE
LONE PINE
SUMMIT VALLEY
LLANA
CAJON CANYON
SAN DIMAS CANYON
LYTLE CREEK
WATERMAN CANYON
OAK GLENN
CEDAR SPRINGS
CRESTLINE
BIG BEAR LAKE
BARTON FLATS
BANNING CANYON
MT. EDNA
ANZA

## EXPERIMENTAL

### Definitive host experiments

During the early part of the work on the life history of *T. californiensis* several attempts were made to infest laboratory rabbits with ensheathed embryos of the parasite, but in no case was the experiment successful. Adult worms placed in the eyes of rabbits survived for about a year, but diminished in number in spite of the fact that freshly deposited embryos could be found in the lacrymal secretions as long as gravid females were present. Larval forms instilled into the conjunctival sacs of rabbits which were proven to be susceptible to the parasite failed to survive. Kofoid et al. (1937) reported negative results in similar experiments on dogs.

These indications that the larval forms extruded from the female are non-infective, supplemented with the knowledge that muscid flies have been found to be intermediate hosts of related thelazias in the U.S.S.R., indicated a need for intermediate host experiments.

### Intermediate host experiments

*Experiment 1.* Before suspicion had fallen on any particular fly as the intermediate host, an effort was made through a number of experiments to determine if *Musca domestica* might be involved in this capacity. A stock colony of this fly was obtained from the Department of

Entomology at the University of California Citrus Experiment Station, at Riverside, California, and maintained for these experiments.

An effort was made to infest these laboratory reared flies with ensheathed embryos of the eye worm by the method of Rentdorff (1953) as follows: ten flies were suspended by their necks in small v-shaped notches cut in the edge of a 3 x 5 file card. Larvae of the worm, freshly procured by rupturing a gravid female, were placed in physiological saline in sufficient numbers to average 20 larvae per drop. One drop of the larva-saline mixture was placed on a round cover glass of 12 mm. diameter which was placed on the file card in such a position that the preparation was in contact with the fly's proboscis. All ten of the flies were fed in this manner. All ate avidly.

One fly was sacrificed five minutes after feeding, the body carefully dissected and examined under the compound microscope, using 100x magnification. Eleven larvae were found in the head which was not dissected but crushed under a cover glass. Three flies were examined after 24 hours but no larvae were found in any part of the alimentary canal or body cavities. Three more flies were examined after 48 hours and the remainder after 72 hours. All of these flies were examined in the same manner as the first but no larvae were found in any of them.

Experiment 2. Twelve *M. domestica* were placed in a small cage and allowed to eat from a 5 cc. pH beaker containing 1 cc. of human blood mixed with T. californiensis larvae. The larvae numbered 22 per drop. The flies were fed canned milk and a sucrose syrup solution daily until death occurred. As soon as possible after they died, they were carefully dissected and examined. The first fly died on the 13th day and the last one on the 30th day. All were negative.

Experiment 3. Twenty four M. domestica were placed under individual inverted Petri dishes. Each was fed 1 drop of the above larva-blood suspension as described above. The feeding was accomplished by placing one drop of suspended larvae on a cover glass and sliding it under the edge of the inverted Petri dish. The flies survived from 24 hours to 17 days. Examination after careful dissection revealed no larvae in any of them.

Experiment 4. In this experiment a special fly-feeding cage was used. It was made from a round card-board food container of one gallon capacity, one end of which was replaced by nylon netting with about 25 meshes per inch. A sleeve of muslin was attached to the other end as a flyproof means of access to the inside.

Twenty five flies were placed in the feeding cage. They were the progeny of a wild strain of Fannia canicularis caught in one of the endemic areas and successfully reared in the laboratory by a co-investigator. The flies were 48 hours old when used in this experiment. They had been fed on dextrose solution for 36 hours and then starved for 12 hours. Small drops of a heavy suspension of _Thelazia_ larvae in a physiological-dextrose solution were placed on the upturned mesh cover of the feeding carton containing the flies. Feeding participation by the flies was good. The flies were fed dextrose solution for 24 hours and again starved for another 24 hours after which they were again fed larvae in the same manner as before. Again feeding participation was good.

Five days after the second feeding, three flies died. All were males and were found to be negative. Ten days after the second feeding, all of the remaining flies were examined. Examination was accomplished by crushing each fly under a cover glass in saline and examining microscopically. All were negative except one. One motile larva (Plate 6, fig. 4), apparently that of a nematode, was found among the extruded viscera of the fly. In general outline it compared favorably with larvae forced from the larvejector of _T. californiensis_ females. It was 331.5 microns long and 13.25 microns in diameter; about twice as long and slightly under twice the diameter of the fresh larvae. It tapered abruptly to a point at the posterior end. Just behind the cephalic end it tapered quickly to about 7 microns. The extreme cephalic end flared slightly, giving the head a "riveted over" appearance. Unlike the fresh larvae, its motions were active and vigorous. About one third of the anterior end of the worm flexed constantly in all directions and the mouth parts adhered occasionally to the cover glass, at which times a strong expansion and contraction of the buccal cavity could be seen. Internal structures were poorly defined.

Experiment 5. Twenty four F. canicularis, again the laboratory strain, were fed T. californiensis larvae, this time using a modification of the technique used in Experiment 4. The flies were individually confined in 5 cc. pH beakers covered with the nylon mesh previously described. The larvae suspension was prepared in 0.85% saline sweetened with honey (one drop of honey to 5 cc. of saline), and placed on the nylon mesh, one drop per fly. The flies, attracted by the honey, ate avidly. By observing through a dissecting microscope, using 40 magnifications, the larvae could be seen as small refractile bodies passing into the meati of the flies' proboscises.

The flies were fed again the following day, again using the pH beakers and a honey-sweetened suspension of larvae. The method of feeding was different, however. The larva suspension was sucked into a dropped made of ¼-inch glass tubing about four inches long. The delivery end of the dropper had been drawn out to about the caliber of a 26 gauge hypodermic needle for a distance of one inch. The other end was equipped with a medicine-dropper bulb. By applying pressure to the bulb by means of a small screw clamp while holding the dropper with the large end up, the suspension was forced into the tip, just to the open end of the lumen. After several minutes

## PLATE 6

Larval forms of *T. californiensis*.

Figure

1 . . . . . . . . . . Ensheathed embryo en uteri.

2 . . . Ensheathed embryo, after larvaposition.

3 . . . . . . Anterior end of ensheathed embryo.

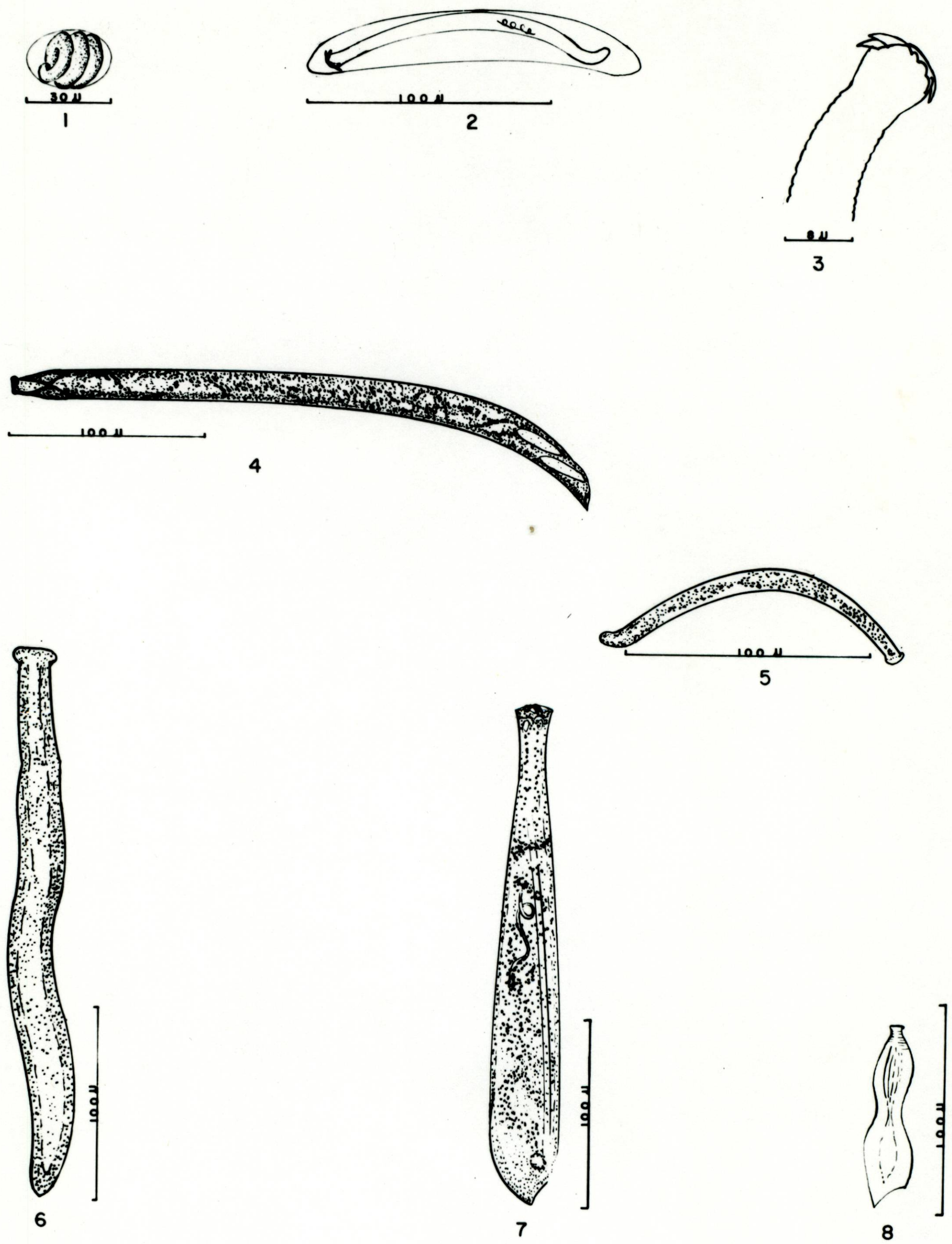
4 . . . . . . . . . . Larva found in Experiment 4.

5 . . . . . . . . . . Larva found in Experiment 5.

6 and 7 . . . . . . Larvae found in Experiment 6.

8 . . . . . . . . . . Larva found in Experiment 7.

PLATE 6



the larvae settled, causing a heavy suspension in the tapered part of the dropper and an almost continuous procession in the finely constricted portion.

While observing through a dissecting microscope, the dropper, held steady in a test tube clamp attached to a ring stand, was adjusted downward until it almost touched the nylon mesh cover of the pH beaker containing the fly. Attracted by the honey, the fly reached through the mesh with its proboscis and appeared to suck at the lumen of the dropper. Larvae could be seen progressing downward in the fine glass tip and disappearing into the fly's proboscis.

After feeding, the flies were transferred to one of the larger enclosures made of food cartons. Unfortunately, during the night all but seven of the flies died and became so badly dehydrated that they were unsuited for examination. The survivors were fed dextrose and milk daily for 14 days, then crushed and examined as in Experiment 4. One nematode larva was found (Plate 6, fig. 5), measuring 138 microns long and 9 microns in diameter, about the size of larvae freshly obtained from the female worm. Newly obtained larvae average 150 microns long and are about 9 microns in diameter. In the single larva found, neither motility nor evidence of internal organization was observed.

Experiment 6. The flies used in this experiment, as well as the next one, were wild *Fannia benjamini* caught in the San Dimas area. Thirty one of these flies, freshly caught, were crushed and examined as in Experiment 4. Two of them were found to have larvae in their abdominal contents. These larvae resembled nematode juveniles and in many respects resembled the larvae found in the laboratory in Experiment 4, but were less active. Two larvae were found in one of the flies. These appeared to be identical in every respect (Plate 6, fig. 6), measuring 300 microns long and 25 microns wide for most of the body length. They tapered more abruptly posteriorly, and more gradually anteriorly than the one described in Experiment 4, but both displayed the same "riveted over" appearance at the anterior end. Again, no internal structure could be defined. The single larva found in the second fly in this experiment appeared somewhat different from those previously found (Plate 6, fig. 7). It was 275 microns long and 38 microns in diameter at the greatest width near the posterior end. From the point of greatest width, 30 microns from the posterior terminus, the body tapered gradually to a diameter of 15 microns at a point 15 microns caudad to the typically flared cephalic end, which was 20 microns in diameter. Internal structures were more definable than in any larva previously seen. A segment of a long, straight tubular structure about 3 microns in diameter was seen extending lengthwise through the posterior two thirds of the body. A second tubular structure, coiled and following an indefinite course was also seen. The larva periodically contracted energetically to the dimensions described and then, during a period of slow side-to-side activity of the anterior quarter, it gradually relaxed to somewhat longer and narrower dimensions.

Experiment 7. Thirty of the wild F. benjamini from the San Dimas area were crushed and examined under cover glasses, then transferred to 0.85% saline in a 5 cc. pH beaker, where they were finely diced with a small scissors. The mass was then filtered through one thickness of surgical gauze into a 15 mm. centrifuge tube, after which enough saline was poured through the gauze to wash it well. After centrifuging, the sediment was examined. One larval nematode was found (Plate 6, fig. 8). This larva did not resemble any of those found in previous experiments except for the flared cephalic end. Its length was 93 microns. The width at the first quarter was 21 microns, 18 microns at the middle, and 27 microns at the third quarter. No definable structures were observed except faint lateral striations at the anterior end.

The results of the above experiments were referred to in part by Burnett et al. (1957).

## DISCUSSION AND CONCLUSIONS

During this study about 30 infested animals have been examined, including the dogs and other animals discussed in this paper, as well as a few not mentioned. In none of these cases did permanent damage appear to be inflicted upon the eye or its adnexa. However, it will be recalled that other workers have reported more serious findings. Faust (1928) and Kofoid et al. (1937) consider the worm capable of causing reduced vision and even blindness. More important are the findings by Douglas (1938) and Wagner (1958) of blindness in dogs suffering with thelaziasis, and the report by Lee and Parmelee (1958) of serious damage to the eye of a human.

Although thelaziasis associated with marked or permanent damage to the eye is not common, the cases mentioned above establish the worm _T. californiensis_ as an important parasite.

At last report, ten animal species have been known to harbor the infestation. No information is available to establish the area of distribution. Except for the isolated reports of one infestation in Oregon and another in Nevada (both in counties adjacent to California), the worm does not appear to range to the north or east of this state.

There are no known published reports of the worms' occurrence to the south of California in Mexico (Lower

California). However, it was reported by Friedmann (1951) in a human in San Diego County, near the Mexican border.

Ryckman (1958) has reported that the topography, fauna, and flora typical of the southern part of California extend southward into Lower California between 150 and 200 miles, and that there is an abundance of flies including Fannia spp. in the north central portion of the peninsula. Residents of that part of Mexico have stated that the wild sections of the region mentioned by Ryckman are well populated with deer, coyotes, and jack rabbits. The multiplicity of dogs in the populated areas is an observed fact. In short, the northern part of Lower California is well populated with the mammals found most often infested with T. californiensis, flies are present which are suspected of being the intermediate host of the parasite, and the terrain and flora compare to those in endemic areas in this state. There is a great likelihood that thelaziasis is present in animals in Lower California.

The relative position of various animals as reservoir hosts of T. californiensis is questionable. The full value of the data furnished by Wagner (1958) in regard to the incidence of thelaziasis in deer is not known. If the deer examined in various counties in California were selected at random, the indication is that the incidence of infestation in that animal is extremely high. It is routine, however, for members of the Department of Fish and Game, from whom Wagner obtained his data, to collect animals with obvious or suspected defects for examination. A report by Oberhansley (1940) indicates that deer with "sore eyes" were being studied. Dixon and Herman (1944) state that a deer examined by them "was collected because observation showed the right eye to be partially shut." Examination revealed the presence of eye worms in both eyes.

The few cases of thelaziasis found among mule deer in Mono County during this study furnish no dependable information concerning incidence of infestation in that area, but do supply valuable information concerning distribution. Most of the deer examined had been dead at least 12 hours, with the probability that the worms in some cases had been lost. The loss of worms is made more probable by the fact that most of the deer had been dragged from the places where they were killed to the nearest road. As a result of dragging the deer through the dirt, the eyes were often mutilated and filled with dust and gravel. It is of interest to note that one of the two infested deer found at Colville was examined at the spot where it had just fallen after being shot.

The position of coyotes as natural reservoir hosts of the parasite in certain areas is fairly well indicated by the fact that 17% of a fair sampling of these animals were infested. The position of the jack rabbit, however, is more controversial. While the ratio of infested jack rabbits over infested coyotes is 2.1/1, the number of rabbits examined was unfortunately small. However, the comparative data cannot be wholly discounted; the ratio suggests that the jack rabbit plays a relatively important role as the natural reservoir. Furthermore, the jack rabbit is undoubtedly more numerous than the coyote, and possibly more so than the deer, especially in the southern part of the state. The large number and great size of the worms found in one of the rabbits suggest that this animal is a more optimal host than the other animals studied. The idea that the worms attain their greatest size in the optimal host is subscribed to by Kofoid and Williams (1935), and Hosford et al. (1942).

The role played by the jack rabbit may be still further emphasized. In the last three areas listed in Table 5, more than four times as many coyotes were examined than jack rabbits. Yet the number of infested coyotes was only twice the number of infested rabbits. Tracks and signs left by the two animals indicated that jack rabbits were present in the three areas in much larger numbers than were the coyotes. Furthermore, farmers and other local residents in all of the areas studied stated that the rabbit population at the time was radically depleted. In some areas where but one or two jack rabbits were found in a day, residents stated that from 30 to 60 might be killed in a day during an average year. These figures, if dependable, add further evidence that the jack rabbit stand high on the list of reservoir hosts.

The bob cat and the gray fox appear to have escaped the parasite, even though they have been found living in endemic areas. The fact that no infested bob cats were found among the 37 examined (in areas where other infested animals were found) suggests that these animals either have a relatively high immunity to the parasite or that their habits render them less vulnerable to contact with the intermediate host. This suggestion is less applicable to the gray fox since it was examined in the same areas in considerably smaller numbers. The infested silver fox reported for the first time by Burnett and Wagner (1958) cannot be considered in evaluating the status of the wild fox, since the habit of the former is influenced by its man-made environment. However, this can serve as a lead to experimental methods.

The other animals reported are not represented in large enough numbers to be significant.

The dog, though often found infested with thelaziasis, occupies a questionable position on the list of susceptible animals. Hosford et al. (1942) believe the dog to be an accidental host. They base this opinion upon their observations that the worms reported in dogs are smaller than those secured from sheep and deer. They state that it is a well known fact that resistance on the part of the host to certain nematode parasites results in dwarfing of the worm. The relationship of worm size to host species has been noted during this study, especially in regard to the jack rabbit, as previously mentioned.

Thelaziasis has been previously reported in man and other mammals not included in the study (Table 3). The position of man, undoubtedly, is minor, in terms of importance as a natural reservoir host. This may also be said of sheep and horses, since, if commonly infested, it seems logical to conclude that they would be reported more often.

The black bear, reported only once (Hosford, et al. 1942), is so rarely disturbed in this state by man, that its relationship to T. californiensis cannot be even speculated upon.

Information concerning the intermediate host of the parasite is meager. That certain muscid flies have been found to fulfill that function in the life cycles of related species of Thelazia in the U.S.S.R. indicates strongly that flies of the same family may function similarly in the life cycle of T. californiensis. The experiments conducted with flies during this work, though not conclusive, implicate certain Fannia species.

Although ensheathed embryos of the worm were fed to F. canicularis with the resultant growth of the larva within the fly, this fly appears to be exonerated as a natural intermediate host in that it flourishes equally well in both endemic and non-endemic areas, with a longer seasonal occurrence in many of the latter. It is found in both high and low regions.

*Fannia benjamini*, on the other hand, has been observed during this study only in hilly and mountainous regions which are moist and well shaded. It is probably coincidence that *T. californiensis* was also found only in areas of the same description. It was observed that if one of the three characteristics, high elevation, shade, or moisture, was lacking, *F. benjamini* was not found, and that such regions, though well populated with animals known to be susceptible to thelaziasis, appeared to be free of the parasite. These observations, plus the affinity of the fly for the eyes of animals, together with the finding of nematode larvae in several flies of this species caught in endemic areas, suggest that *F. benjamini* is the natural intermediate host for *T. californiensis*.

## SUMMARY


Nine species of *Thelazia*, known as eye worms, are parasitic in the eyes of mammals. *Thelazia* spp. have been reported on every continent but Australia.

*T. californiensis*, first reported in this country in the eyes of dogs in California, has previously been reported in eight mammalian species including man, dog, horse, sheep, deer, coyote, and black bear.

The effect of thelaziasis (infestation by *Thelazia* spp.) may lead to serious pathological changes in the eye. Blindness has been known to follow in cows and dogs.

Fifteen cases of thelaziasis in humans have been reported, six of which are in California. Two cases (one in China, the other in California) were associated with marked pathological changes in the adnexa of the affected eye.

Herd infestations have been reported in cattle in Russia and England, and in sheep in the United States.

Reports indicate that the infestation is unknown in the United States outside of California except for one case each in Nevada and Oregon.

A detailed description of the viviparous parasite and its ensheathed embryo is given.

During observations of infested dogs in endemic areas, it was noted that the flies, *F. canicularis* and

*F. benjamini* revealed an avidity for lacrymal secretions. Experiments conducted on these flies indicate the probability that the latter is the intermediate host of the parasite.

The experiments conducted on flies were of two types. In the first experiments, ensheathed embryos of the viviparous female eye worms were fed to laboratory reared *F. canicularis*. Subsequent examination of the flies revealed *Thelazia* in a few of them. The second type of experiment consisted of examining wild flies caught in an endemic area. During these examinations, nematode larvae resembling those found in the laboratory infested flies were found within the bodies of *F. benjamini*.

During an extensive reservoir host survey, infestations were found in dogs, silver fox, deer, coyotes, and jack rabbits. Evidence indicates that the incidence of infestation in the latter three is high. Further evidence indicates that the jack rabbit may be the optimal host.

During the reservoir host survey, attention was given to the physical characteristics of the areas investigated. It was noted that endemic areas invariably were in mountainous or hilly regions which were well supplied with shade and moisture. In most of these areas, *Fannia benjamini* was found.

The jack rabbits and silver fox were first reported by Burnett and Wagner (1958).

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COLLEGE OF MEDICAL EVANGELISTS

School of Graduate Studies

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NATURAL AND EXPERIMENTAL INFECTIONS

OF <u>THELAZIA CALIFORNIENSIS</u> PRICE (1930,

(NEMATODA: THELAZIIDAE)

WITH DESCRIPTIONS OF THE ADULT

AND LARVAL PARASITES.

by

Howard S. Burnett

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An Abstract of

A Thesis in Partial Fulfillment

of the Requirements for the Degree

Master of Science in the Field of

Microbiology

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June, 1958

ABSTRACT

The purpose of this work was to determine the extent and effects of infestation of the eye worm of mammals, Thelazia californiensis, and to elucidate through experimentation or observation the mode of transmission.

A survey of literature revealed that thelaziasis (infestation by Thelazia species) may cause serious pathological changes in the eye and its adnexa, sometimes resulting in blindness. It was found that reports of thelaziasis in the United States were (with two exceptions) of cases in California. It was further found that T. californiensis has been reported in a wider variety of hosts than has any of the other thelazias. The hosts were found to include many wild animals, most of the domestic animals, and man. From Russian literature it was learned that muscid flies were the intermediate hosts of the thelazias found in that country.

A detailed description of the viviparous worm (male and female) and the larva is given.

One phase of the work is concerned with observing the conduct of muscid flies in the vicinity of infested dogs in areas endemic for the eye worm. The flies, Fannia canicularis and F. benjamini, were observed to manifest a marked avidity for the eyes of dogs and other mammals. Information was gained through laboratory experiments and

field observations strongly suggests that the latter is the intermediate host of the parasite.

A second phase of the work is concerned with reservoir host studies. Animals found involved were dogs, domestic silver fox, deer, coyotes, and jack rabbits. Information gained during this phase of the work suggests that the jack rabbit may be the optimal host, and that during years when these animals are plentiful, they may constitute a large portion of the reservoir. During this part of the study it was learned that the endemic areas are in mountainous or hilly regions which are amply shaded and supplied with moisture.

On the basis of these studies it was concluded that thelaziasis is a serious infestation, endangering the sight of the host, that it has infested a wider variety of animals than has any other member of the genus, and that transmission is probably through the fly, _F._ _benjamini_.