SECTION A, BIOLOGICAL SCIENCES

SUBSECTION ZOOLOGY

Notes on the Helminths of *Pseudemys scripta elegans* (Wied, 1838) in Areas of Texas and Oklahoma

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A survey was made of the helminths of the Red-eared turtle, *Pseudemys scripta elegans* (Wied, 1838), in two diverse ecological areas. The turtles were obtained from two principal localities in Texas and from six in the vicinity of Stillwater, Oklahoma. The ecological aspects of the two localities in South Texas were quite different in spite of the fact that both had been subjected to a severe three-year drouth. The ponds in Oklahoma were also quite diverse ecologically. The drouth did not affect

the ponds and turtle populations as severely in Oklahoma as in the Texas localities. With few exceptions, however, the parasite fauna was similar for the turtles in Texas and Oklahoma, but the average number of parasites per host varied greatly in some cases, depending on the specific parasite involved. The data are based on parasites collected from a total of seventy-nine turtles: fifty-six from Texas and twenty-three from Oklahoma.

Methods and Materials

All of the specimens were collected and preserved by the usual parasitological techniques. The majority of the trematodes were stained and mounted for study using primarily cochineal, borax carmine and hematoxylin stains and the usual staining procedures.

Parasitologists have found it extremely difficult to stain nematodes for study except by elaborate and time-consuming methods. Consequently, most workers in the field have depended upon semi-permanent, unstained mounts using either lacto-phenol or glycerine jelly preparations for study. The lacto-phenol solution will destroy parasites if they are left in it too long and the glycerine jelly mounts are tedious to prepare. Both media, however, are serviceable. A mounting medium, Hoyer's, used by entomologists, was tried for mounting these worms. Excellent results were secured and later several stains were added to the medium which gave better definition of structures.

Hoyer's mounting medium (a modification of Berlese's gum-arabic mounting medium) is an aqueous solution of gum arabic, chloral hydrate and glycerine. According to Baker and Wharton (1952) the medium is prepared by mixing the following ingredients at room temperature in the order listed:

Distilled water	50 Gm.
Gum arabic (clear crystals)[1]	30 Gm.
Chloral hydrate	200 Gm.
Glycerine	20 Gm.

These writers recommended that the cover glasses of finished slides be ringed to prevent the absorption of moisture by the medium. Moisture absorption is especially apt to take place when slides are kept in humid areas.

The clear medium was used initially but it was observed that some of the smaller nematode specimens, such as *Spiroxys contortus*, overcleared. Goldberg (1954) reported that the addition of Giemsa or Acetocarmine stain to PVA (Polyvinyl alcohol) mounting medium enhanced its value for small nematodes. On the basis of this report these two stains were added separately to the Hoyer's solution and excellent staining results were obtained with nematodes and with acanthocephalans.

With Hoyer's medium, nematodes were mounted either directly from the living conditon or from the aqueous preserving solutions. Living specimens were mounted without dehydration since this medium relaxes, fixes, stains and produces excellent mounts of the parasites in one operation. Nematodes mounted in the Hoyer's medium containing Giemsa stain (one part stock stain to nineteen parts of the clear mounting medium) took up the eosin and the specimens were red in a blue field. This proved to be a very useful feature in that the blue field acted as a light filter and prevented a great deal of eye strain. The length of time required for stain-

[1] I found that powdered gum arabic also gave excellent results, but some entomologists have reported it to be useless.

ing and clearing specimens mounted in the Giemsa stain medium varied from a few minutes to several hours, and depended apparently on the size of the specimen.

In April, 1954, approximately twelve specimens of *Spironoura concinnae* were mounted in the clear medium. These nematodes were examined in October, 1954, for purposes of identification and overclearing had rendered them useless. The slides were placed in containers of water and left for 12 to 14 hours. The cover glasses loosened, the medium dissolved, the specimens absorbed water and regained their original opaque appearance. The worms were remounted in the Giemsa-stained Hoyer's medium and were very satisfactory for study.

Acanthocephalans, like the nematodes, were relaxed, fixed, stained, and permanently mounted in one operation. The Giemsa medium gave excellent results. Even the characteristics of the eggs, such as the three shell membranes in *Neoechinorhynchus emydis*, were easily observed within the mounted specimens and according to Hopp (1954)these membranes are difficult to see except in living eggs. If specimens were relaxed partially prior to mounting, the proboscis remained extruded and the hooks and proboscis could be measured. Some acanthocephalan worms were stained using borax carmine or various hematoxylin stains, destained, rinsed in a basic solution and mounted in the clear Hoyer's medium with results equally as good.

There are a few general observations that should be mentioned concerning the use of Hoyer's mounting medium and they apply to all groups of helminths. When specimens are mounted in any of these variations of Hoyer's medium they seem to collapse and appear worthless, but as soon as the medium is dispersed throughout the worm, its original shape returns and there is no apparent gross internal distortion. Very little shrinkage, if any, was observed in any of the specimens mounted. Specimens in the Giemsa medium tended to fade slowly unless stored in closed slide boxes.

MONOGENETIC TREMATODES

Polystomoides coronatum (Leidy, 1888) Price, 1939

A total of 68 specimens was collected from 20 turtles: the incidence in Texas turtles was 1.8 per cent as compared with 82.6 per cent in Oklahoma turtles. This was the greatest percentage differential in any of the species. The representative specimens that were examined agreed closely with the redescription of *Polystomoides coronatum* by Price (1939), except for one with only five suckers on the opisthaptor.

Neopolystoma orbiculare (Stunkard, 1916) Price, 1939

A total of 86 specimens was collected from 28 turtles. The incidence of infection was 20.0 per cent in Texas with an average of 2 worms while in Oklahoma it was 74.0 per cent with an average of 4 worms per host. The range in the number of hooks of the genital coronet was 31 to 36, which is quite narrow as compared with the range of 14 to 40 given by Price 1939). The bladder habitat of *N. orbiculare* is usually given as the "urinary bladder." The majority of the flukes in these turtles were in the accessory bladders instead of the urinary bladder "proper." Of the 28 turtles infected only 7 had infections in the bladder "proper" while the rest had the flukes in the accessory bladders only.

DIGENETIC TREMATODES

Telorchis corti Stunkard, 1915

A total of 521 specimens was taken from 14 turtles. The incidence of infection was 17.9 per cent in Texas with an average of 29 worms

while it was 17.4 per cent in Oklahoma, with an average of 59 worms per host.

Telorchis singularis (Bennett, 1935) Wharton, 1940

A total of 281 specimens was recovered from 10 turtles. The incidence of infection was 12.5 per cent in Texas, with an average of 21 worms while it was 13.2 per cent in Oklahoma, with an average of 46 worms per host.

Spirorchis artericola Ward, 1921

A total of 28 specimens was removed from 5 turtles. The incidence of infection was 5.4 per cent in Texas with an average of 6 worms while it was 8.3 per cent with an average of 5 worms per host in Oklahoma.

The size of the specimens appeared to be influenced by the location in the host. Those from the liver were the smallest, those from the lungs and heart were intermediate in size and the one from the spleen was the largest.

NEMATODES

Camallanus microcephalus (Dujardin, 1845) Railliet and Henry, 1915

A total of 1,486 specimens was collected from 65 turtles. The incidence in Texas was 85.7 per cent with an average of 18 worms per host, while in Oklahoma 74.0 per cent harbored this parasite with an average of 38 worms per host. This was the most common parasite in the turtles from Texas while in Oklahoma it was surpassed by the mouth trematode, *Polystomoides coronatum*.

Spironoura concinnae Mackin, 1936

Only 24 specimens were collected. All were from Texas turtles; 14 per cent of 56 hosts were infected, with an average of 3 worms per host. This is the first report of *Spironoura concinnae* in turtles from Texas and it is a new host record. It was reported from Oklahoma by Mackin (1936) from *Pseudemys concinna*.

Spiroxys contortus (Rudolphi, 1819) Schneider, 1866

These agreed anatomically with the description of *Spiroxys contortus* by Hedrick (1935); however, some of the minute details in Hedrick's description could not be discerned in preserved specimens.

A total of 12 mature specimens was recovered from 4 of 56 turtles examined from South Texas, and no mature specimens were observed in any of the 23 turtles examined from Oklahoma. All of the turtles collected in Oklahoma contained only encysted larvae of *Spiroxys* sp. and 39 of 56 turtles collected in South Texas harbored encysted larvae in addition to the mature specimens. The fact that both immature (encysted) and mature specimens have been reported from several hosts is interesting and, apparently, the cause is not well understood. More research on the life cycle should reveal the effects of certain biological conditions concerned with this cycle and explain the reason for the peculiar distribution of encysted larvae and mature worms.

Neoechinorhynchus emydis (Leidy, 1851) Hamann, Stiles and Hassall, 1905

These specimens were in agreement with descriptions given by Hopp (1954), and Cable and Hopp (1954) except for the length of the egg. According to Hopp, who adequately described and figured the egg for the first time, the eggs in a living condition measured 0.023 to 0.025 mm.

in length. These eggs measured 0.029 to 0.030 mm. in length and averaged 0.030 mm. when preserved.

A total of 377 specimens was collected from 11 turtles captured in Oklahoma. This species was not found in the turtles from Texas. Oklahoma or northern Texas probably represents its southern limit of distribution. Other hosts that have been reported for this species are: *Chelydra serpentina, Chrysemys emydis, Clemmys insculpta, C. guttata, Graptemys geographica, G. pseudogeographica, Pseudemys concinna, P. scripta, P. elegans, P. troosti.* The reports of Hopp (1954) and Cable and Hopp (1954) seem to indicate that this host list is based on a confusion of three or more acanthocephalan species and is therefore invalid. According to Hopp, *Graptemys geographica* was the only host of this species and on the basis of the present work, *Pseudemys scripta elegans,* is another host.

Cable and Hopp have started a new trend in acanthocephalan taxonomy, at least insofar as the species that inhabit North American turtles are concerned. In the past, the taxonomy of this group has been based primarily on one structure, namely the praesoma, with little emphasis on the rest of the organism. These workers are considering the differences in the organism as a whole as the criteria for speciation, which in my opinion is necessary.

A new species of Acanthocephala was found and it will be described in a later paper.

A Case of Extreme Parasitism

All of the turtles examined in this survey were infected with helminth parasites. One proved to be unusually interesting because of the extreme number and variety of parasites in a turtle of unusual size.

A small red-eared turtle, captured in the vicinity of Stillwater, Oklahoma, on September 15, 1954, was kept in captivity for 44 days until it died. It was kept in a small aquarium and fed on commercial turtle food. The turtle was dead approximately an hour before it was examined, hence it is quite unlikely that much postmortem migration of parasites occurred . The age of the turtle was estimated to be one year using the number of rings on the plastron as the criterion.

A total of 247 helminth parasites was recovered. There were 231 specimens of *Camallanus microcephalus,* the most abundant nematode. Fifteen monogenetic trematodes were collected; five from the mouth, five from the urinary bladder and five from the accessory bladders. Nine monogenetic flukes was the largest number taken from any other turtle. The largest specimen of *Spirorchis artericola,* a digenetic trematode, was recovered from the spleen.

CONCLUSIONS

The results of the survey show that the red-eared turtle, *Pseudemys scripta elegans,* is very heavily parasitized in parts of Oklahoma and Texas. The incidence of infection is shown to vary greatly with the different parasite species and with the geographical location of the host. For instance, the mouth trematode, *Polystomoides coronatum,* was found in only 1.8 per cent of the hosts obtained in Texas while 73.1 per cent of the turtles were infected in Oklahoma. Concentration and near starvation of hosts due indirectly to drouth conditions did not appear to affect the parasite burden of the turtles as one might expect.

SUMMARY

1. Seventy-nine specimens of the red-eared turtle, *Pseudemys scripta elegans,* were examined for helminth parasites; fifty-six were collected

from two localities in Texas and twenty-three were taken from ponds in the vicinity of Stillwater, Oklahoma.

2. *Pseudemys scripta elegans* is reported as a new host for *Spironoura concinnae* and *Neoechinorhynchus emydis*.

3. New distribution records are reported for *Spirorchis artericola* (Texas and Oklahoma) and *Spironoura concinnae* (Texas).

4. The other helminths reported are *Telorchis corti; Telorchis singularis; Polystomoides coronatum; Neopolystoma orbiculare; Spiroxys contortus* and *Camallanus microcephalus*. A new species of acanthocephala will be described elsewhere.

5. A new technique is described for staining and mounting nematodes and acanthocephalans.

6. A case of extreme parasitism is reported.

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