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## Echinococcus in Dogs from Pretoria and vicinity.

### By R. J. ORTLEPP, M.A., Ph.D., Empire Marketing Board Research Officer, Onderstepoort, Transvaal.

THIS Institute is from time to time supplied by the Pretoria Municipal authorities with stray dogs which are caught in its boundaries. In this way the writer was able to examine and post-mortem 25 dogs, and the results of this survey is embodied in the following. It is difficult, if not impossible, to trace the exact origin of the dogs, but it may be taken, with little ground for doubt, that they have for the most part migrated into the municipal boundaries from the native locations situated in the vicinity of Pretoria. Except for three, all the dogs were infected with one or more helminths; eleven were infected with Toxacara canis, seven with Toxascaris leonina, five with Ancylostoma caninum, nineteen with Dipylidium cananum and five with *Echinococcus granulosus*. One dog was infected with all five. Of the five dogs which carried an Echinococcus infection, two harboured only a few worms, one had a fair infection and two had a heavy infection, the worms being located all the way down from the middle of the duodenum to near the caecum. Although these two dogs carried such a gross infection the intestinal mucosa appeared quite normal and it would thus appear that they suffered little, if any, inconvenience from their presence.

An examination of this material under the binocular microscope revealed the fact that the majority of worms had only three segments, the last of which was gravid. None were observed to have more, and a few had only two segments. This observation led the author to examine the material more carefully, especially as the European material from the English fox which he had examined while in London, had four or five segments, and material from the Hunting dog, Lycaon pictus, in the collection of this Institute, normally showed the presence of five segments, although some specimens with as many as seven segments were also present. This examination convinced the author that in South Africa at least two, and perhaps three, species of Echinococcus are present, all of which are different from the fox material which Cameron (1926) described as E. granulosus. The author identifies his material from the dog as E. granulosus, that from Lycaon pictus as a new species to which the specific name lycaontis is given, and he renames the material from the English fox giving it the specific name cameroni. The third South African species is probably E. longimanubrium which Cameron (1926) described from the Cape Hunting Dog, Lycaon capensis.

### ECHINOCOCCUS CRANULOSUS (Batsch, 1786), Rud., 1805.

The material available for examination consisted of numerous specimens collected from the domestic dog. The specimens were collected alive and were killed and fixed in various ways; the most satisfactory method was to open out and place a portion of the intestine with the attached worms in slightly warmed tap water, and to leave them here until the worms were dead; this happened in

about two hours. The worms thus died beautifully extended and most of them detached themselves from the intestine; by carefully picking them up and dropping them into cold 10 per cent. formalin, they were found to undergo no shrinkage whatsoever. If, however, they were fixed in 70 per cent. alcohol they underwent considerable shrinkage, and the same also happened if the live worms were dropped into alcohol or formalin, hot or cold. The above method of killing and fixing, may not be satisfactory for histological studies, but for preserving the specimens in their natural condition it gave very satisfactory results; besides it was now possible to make out all the details of the genitalia and excretory systems by direct examination of the worms without any preliminary staining, and as the worms had died and been fixed in an extended position no flattening and consequent distortion of the specimens was necessary.

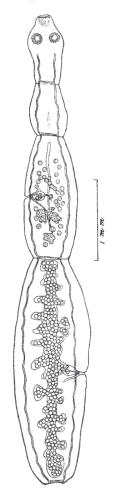


Fig. 1.—*Echinococcus Granulosus.* Entire specimen killed in lukewarm water and fixed in 10 per cent. formalin.

Mature worms, carrying gravid segments, vary in length from 5 to 8.5 mm. with a maximum width across the gravid segment of 0.9 to 1.1 mm. Every worm carrying a gravid segment is built up of three segments plus a head and neck (Fig. 1), the first segment being immature and just showing the beginnings of the genital organs, the second segment contains the fully developed genital organs, and the last carries only the uterus with the contained eggs. Specimens with two segments have evidently lost their gravid segment as the second in these cases only contains the genitalia, or are still immature.

The head carries a prominent rostellum which is broader than it is long, varying in breadth from 0.16 to 0.25 mm. and from 0.1 to 0.2 mm. in length. At its apex it carries a crown of from 30 to 36 hooks arranged in two rows of 15 to 18 each. The hooks are fairly large, those of the anterior row being from 0.042 to 0.049 mm. in length, while those of the second row are 0.032 to 0.042 mm. long. The larger hooks have a stout blade placed at an angle to the axis of the handle and is strongly curved (Fig. 2). The guard is massive

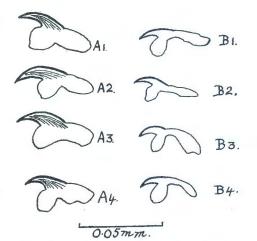


Fig. 2.—*Echinococcus granulosus* hocks. A1—A4 hocks from anterior row. B1—B4 hocks from posterior row.

and often has a cordate shape; the handle is also massive and generally thickens out towards its middle to taper towards its distal extremity; the guard and handle generally give the appearance of a bi-lobed base to the shaft, but a tri-lobed ventral outline is also seen in some of the hooks. Their edges are smooth. The smaller hooks are markedly different from those described above; the blade is smaller and lighter and is also less curved. The guard is relatively very massive and is somewhat oval in shape and its axis generally makes a more acute angle with the axis of the blade than is the case in the larger hooks. The handle is relatively very long and lies almost in a direct line with the axis of the blade. It may broaden out slightly towards its distal extremity, but in the majority of cases it maintains an even width and its edges may be slightly wavy; very

often its distal extremity is turned downwards. The suckers are round and prominent and vary in diameter from 0.16 to 0.2 mm. The head narrows immediately behind them to form the neck which is from 0.4 to 0.54 mm. long and 0.33 to 0.4 mm. in thickness.

The first segment is somewhat barrel-shaped and is slightly longer than broad; it varies in length from 0.38 to 0.75 mm. with a maximum thickness of 0.38 to 0.55 mm., it contains no definite organs, and the only indications of the genital organs is a median patch of darker cells in the posterior half of the segment.

The second segment varies in length from  $1 \cdot 1$  to  $1 \cdot 8$  mm. with a maximum breadth of 0.44 to 0.8 mm. It contains the mature genital organs; the number of testes varies from 30 to 53 and they are rounded with a diameter of 0.05 to 0.06 mm. They are situated in the middle portion of the segment, leaving its proximal and distal areas free. The majority are located on the aboral side anterior to the female genitalia. The vasa efferentia unite to eventually form a coiled vas deferens which enters the cirrus pouch at its distal extremity. The cirrus pouch is pyriform in shape and about 0.2 mm. long; it passes between the excretory canals and extends obliquely inwards and forwards, but does not reach the centres of the segment. The cirrus is retractile into the cirrus pouch and carries numerous rows of small spines. The genital pore lies at the centre of the segment and alternates irregularly. The vagina opens posterior of the cirrus pouch, the two forming a short common genital canal passing to the exterior. The ovary is roughly horse-shoe shaped, the limbs being however slightly lobed. In the isthmus connecting the two halves there is a distinct and spindle-shaped receptaculum seminis; from its anterior end the vagina passes obliquely forwards and outwards to the genital pore, while a thin duct passes backwards from it to terminate in the shell gland. This gland is roughly spherical in shape and is located slightly posterior of the ovaries. An irregularly-shaped but somewhat oval vitelline gland is present towards the posterior margin of the segment and its duct passes forwards to the shell gland where it meets the other ducts. The uterus also originates from the shell gland and this passes forwards in the centre of the segment as a slightly dilated tube.

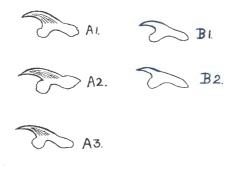
The last or gravid segment only contains the uterus filled with eggs; it is relatively very large, being in most cases longer than the rest of the worm. It varies in length from 3 to  $5 \cdot 2$  mm., with a maximum thickness of 0.8 to 1.1 mm. The uterus occupies most of its central area and is provided with about 12 to 15 lateral pouches. Most of its cavity is occupied by the numerous eggs. The embryophores are slightly oval with thick smooth and radially striated shells; they vary in size from  $0.031 \times 0.037$  to  $0.030 \times 0.038$  mm. and the hexacanth embryo varies in size from  $0.021 \times 0.026$  to  $0.022 \times 0.028$  mm., with hooks 0.01 mm. long. The wall of the empryophore is about 0.006 mm. thick. The excretory system is represented by two pairs of longitudinal ducts passing down each side of the worm; in the head region they become confluent with each other, and at the posterior end of each segment transverse ducts unite the corresponding ducts from each side.

#### DISCUSSION.

The most satisfactory description of this species available to the writer is that of Leuckart (1881). His description is based on material from dogs and differs in a few minor respects from the writer's material. He gives the maximum length of his specimens as 5 mm. whereas in the writer's material some of the specimens attained a maximum size of 8.5 mm. This difference in size may possibly be due to different methods of fixation and too great an importance can therefore not be assigned to this difference. Further, he says there may be three or four segments; in the writer's material no specimen was seen with more than three segments, but as Leuckart explains, it is quite possible that before the gravid segment is cast off an additional segment may in some cases have been formed, so that even if no four-segmented specimens were seen by the writer, the possibility of this number being present is not thereby excluded.

Leuckart states that the number of Rostellar hooks may vary from 28 to 50. The writer, however, has not seen such a wide variation in his material where the number varied only from 30 to 36. As to the sizes and shapes of the hooks in the two series, the writer's and Leuckart's findings agree very closely, the latter stating that the larger vary from 0.04 to 0.045 mm. and the smaller from 0.03to 0.038 mm. as against the writer's 0.042 to 0.049 and 0.032 to 0.042 mm. respectively. Krabbe (1865), who also examined material from the dog, gives the number of hooks as from 38 to 40, and the variations in sizes as from 0.029 to 0.046 mm. for the larger and 0.021 to 0.033 for the smaller hooks; however, it is quite possible that the smallest hooks in both series may have belonged to immature individuals where the hooks had not yet grown to their full size. The writer also observed that the heads which carried the least number of hooks, tended to have their hooks larger than in those specimens with a larger number. Hall (1910) gives the sizes of the larger hooks as 0.022 to 0.03 mm. and those of the smaller as from 0.018 to 0.022 mm. Unfortunately he does not mention whether these measurements are based on his own observations, and if so, what the origin and nature of his material was, i.e. was it still immature or gravid? From Krabbe's, Leuckart's and Cameron's (1926) observations we know that the size of the hook (shaft and guard) increases in size with the development of the worm so that the size will depend to a great deal on the state of development of the parasite. Cameron, who based his observations on material from the English fox-Vulpes vulpes-gives the sizes of the larger and smaller hooks as 0.034 and 0.03 mm. respectively, but evidence will be brought forward later to show that Cameron did not have the true E. granulosus at his disposal, but a hitherto unidentified new species. Ross (1929) was able to examine numerous specimens of E. granulosus collected from the domestic dog in Australia. He found that the hooks varied considerably in size and shape, but he was not able to find any which corresponded closely with those figured by Cameron (1926). In his material the large hooks varied in length from 0.034to 0.038 mm. and the small hooks from 0.022 to 0.03 mm. Although these hooks are smaller than those seen by the writer, there does not appear to be any reasonable grounds to doubt the identity of these The most striking difference concerns the guard which materials. is elongate and set almost at right angles to the axis of the handle. in Ross' specimens, whereas in the writer's it is less elongate, stouter and rounded, and its axis forms an obtuse angle with that of the handle. Krabbe (1865), however, figures both types, his specimens originating from Danish and Icelandish dogs. The hooks, however, agree in having a relatively long handle in that the guard is large, and in that the blade of the large hooks is strongly curved and the dimorphism between the two rows of hooks is marked. These characters are also well brought out in both Krabbe's and Leuckart's figures.

As far as the genitalia are concerned there is little difference between the writer's and Leuckart's findings. The latter finds about 60 testes present as against the writer's 30 to 53 and their size is slightly larger (0.07 mm.). The cirrus sac is smaller in the writer's material and does in consequence not extend to the middle of the segment as observed by Leuckart. Despite the above-mentioned differences the writer is satisfied that the materials examined by Krabbe, Leuckart, Ross and the writer all belong to the same species. In all four cases the materials originated from the domestic dog, that of Krabbe from Iceland and Danish dogs, in the former of which place *E. granulosus* is known to be an endemic parasite of dogs.



0.05 mm

Fig. 3.—Echinococcus cameroni n. sp. hooks. A1—A3 hooks from anterior row. B1—B3 hooks from posterior row.

#### ECHINOCOCCUS CAMERONI n. sp.

SYN. E. granulosus of CAMERON, 1926, FROM Vulpes vulpes; NOT

#### E. granulosus (BATSCH) RUD., 1805.

In 1925 the writer collected adult *Echinococcus* from the common English fox—*Vulpes vulpes*—which had died in the Zoological Gardens in London. The writer identified this material as *E. granulosus*. Cameron (1926) reviewed the members of this genus and examined this same material and also concluded that it was *E. granulosus* and on this material he based his drawing and measurements of the hooks. Unfortunately, neither of us had an opportunity of carefully comparing this material with good material from the dog. The writer fortunately still has a few mounts of this fox material, and a comparison of these with the dog material described above has convinced the writer that the dog and fox materials are not co-specific. Cameron states that his fox material had two to three immature segments (i.e. four or five segments), whereas some specimens from the dog which he examined had only three segments. In the writer's mounts there are also four or five segments, but none with only three segments, and the hooks are also similar to those described and figured by Cameron except that they are slightly larger, the larger being from 0.035 to 0.038 mm. long and the smaller from 0.03 to 0.033 mm. (Fig. 3). As the material examined by Cameron and in the writer's possession possesses gravid segments with ripe eggs, it appears legitimate to assume that the specimens are fully adult and that in consequence the hooks have attained their final form and size. A comparison of these with those of *E. granulosus* from the dog reveals the following differences:—

(1) The hooks are appreciably smaller; (2) the size dimorphism between the larger and the smaller hooks is much less marked than in E. granulosus; (3) the axis of the blade of the larger hooks tends to lie in a direct line with that of the handle, which is not the case in those of the dog material; (4) the size of the guard and sheath is relatively much more slender than in E. granulosus, where these structures are robust and are collectively much larger than the blade; (5) the handle of the smaller hook is relatively much shorter than that from the dog material.

From the above considerations of the size of the parasite, the number of segments, the size and shape of the hooks the writer concludes that this fox material represents a hitherto unidentified species which he has pleasure in naming after his friend and former colleague, Dr. T. W. M. Cameron. This species may briefly be diagnosed as follows:—

Slender parasites provided with four or five segments and from 5 to 7 mm. long. The mature segment is always the third from the end, the last being gravid, and the preceding being intermediate in development between the mature and gravid. The rostellum carries two rows of 28-32 hooks; those of the first row vary in size from 0.035 to 0.038 mm. and those of the second row from 0.03 to 0.033 mm. Size dimorphism between the larger and smaller hooks is not marked. The axis of the blade tends to lie in that of the handle. Other characters as for E. granulosus.

Location: Small intestine. Locality: England. Host: Vulpes vulpes.

In addition to the above two species, the writer has been able to examine representative material collected from the wild dog— *Lycaon pictus*, which had died in the Johannesburg Zoological Gardens. The material had been preserved in alcohol so that it is quite possible that the size given hereunder is appreciably less than their natural size. This species differs from the known species in several respects and in consequence it is here described as a new species.

#### ECHINOCOCCUS LYCAONTIS sp. n.

In general anatomical characters this species is similar to the genotype except in the number and nature of its hooks, and in the number of its segments. In consequence only those wherein it differs will be described.

The preserved material, provided with gravid segments, is 4 to 7 mm. long and the average number of segments is five (Fig. 4); however, specimens with four to seven segments are also present. In all forms the mature segment is the third from the last and the gravid the last; in no case is there more than one mature and one gravid segment present. The most remarkable character of this parasite is the presence of four rows of hooks on the rostellum; the

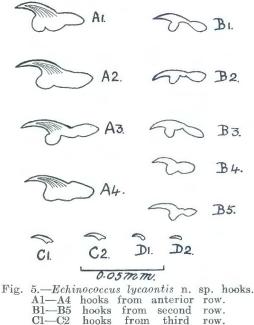


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Fig. 4.—*Echinococcus Lycaontis* n. sp. Entire specimen killed and fixed in alcohol.

first and second rows conform in general with those typical for the genus, but those of the third and fourth rows are very much smaller, and appear to drop off much more easily than those of the anterior two rows (Fig. 5). No scolex was seen on which the full number of these small hooks were still present, notwithstanding that all those

of the first and second rows were still present. Each of the anterior two rows carries 17 or 18 hooks, which are slightly smaller than those of E. granulosus. The larger hooks are from 0.04 to 0.045 mm. long and the smaller 0.028 to 0.033 mm. The former carry a stout blade which is not as strongly arched as that of the dog material, and the shaft (handle and guard) is also more slender. The axis of the blade meets that of the shaft at an angle, and there is, in most cases, a distinct notch on the dorsal surface where the blade meets the handle. The ventral margin of the shaft is seldom saddle-shaped, as is often the case in E. granulosus, but tends to have a tri-lobed outline. The guard is relatively small and is either rounded, oval or cordate in shape. The handle is plump and its distal extremity is either rounded or tapering. The hooks of the second row are much more slender than those of the first row. The blade is less arched,



D1-D2 hooks from fourth row.

the guard in general is oval in shape and the handle generally terminates in an oval swelling. This last character serves to distinguish these hooks easily from the corresponding hooks of E. granulosus or E. cameroni. The hooks of the third and fourth rows are very small, and are composed of only the blade, the absence of a shaft probably gives the reason why they are so easily lost. Those of the third row appear to be lodged opposite those of the first row, and those of the fourth row opposite those of the second row. It was not possible to determine their exact number as they were incomplete in all the specimens examined. The larger of these hooks are from 0.012 to 0.014 mm. long and the smaller from 0.005 to 0.006 mm. Their blades are only slightly arched. In the smaller hooks there is no indication whatever of a guard or handle, whereas those structures are represented in the larger hooks by two short processes at the

distal extremity of the blade. The neck is narrower than the rest of the head and leads to the first segment which is slightly longer than broad and shows the beginnings of the genitalia as a streak of darker staining cells in the middle of the posterior half of the segment. In specimens with five segments the second segment is about half as long as it is broad; in it the testes, ovaries and yolk gland and their ducts have become differentiated. The third segment in six and seven segmented forms is about half again as long as the second segments, and in it the genital organs are slightly better developed although not mature; in five-segmented forms the genital organs are fully mature in this segment; the details of this system are similar to those described by E. granulosus above, except that, on account of shrinkage (?), the individual organs are smaller. The fourth, or fifth, or sixth segments in five, six and seven segmented worms respectively show the disappearance of the genital organs and the enlargement of the uterus which becomes filled up with the maturing eggs. These segments are from two to three times as long as they are broad. The last segments are gravid and may form from a half to three-fifths of the entire length of the parasite, and is nearly five times as long as it is broad. The uterus, with about a dozen sacculations on each side, occupies the greater portion of its central area and is practically filled with embryophores indistinguishable from those of E, granulosus. The genital pores alternate irregularly and are situated just posterior of the middle of each segment.

IIost: Lycaon pictus. Location: Small intestine. Locality: Johannesburg Zoological Gardens.

#### DISCUSSION.

The presence of four rows of hooks on the rostellum places this species in a unique position among the remaining members of the genus *Echinococcus*. A careful search has been made for them in the specimens from the domestic dog and English fox, but in not a single case was there the faintest trace of them seen. It may thus be taken with confidence that this characteristic is specific for this parasite. The number of segments is also peculiar to this species, there being, as far as the writer is aware, no previous record of an *Echinococcus* possessing up to seven segments. The size and shape of the hooks on the first and second rows is also different from those known in described species. Taking these three characters together there does not appear to be any doubt that this material represents a hitherto unknown species.

#### Specific Diagnosis,

Slender parasites provided with four to seven segments and 4 to 7 mm. long in specimens preserved in alcohol. The mature segment always third from last. Rostellum carries four rows of hooks; the shapes and sizes of those of the first and second rows conform to those typical of genus and vary in number from 34 to 36 and in size from 0.04 to 0.045 mm. for the larger and 0.028 to 0.033 mm. for the smaller; hooks of third and fourth rows small, degenerate and easily lost, their handles and guards being entirely absent or only represented by short processes. Internal morphological characters as for E. granulosus. Cameron (1926) described from a nearly related host, the Cape hunting dog, Lycaon capensis, a new species which he named E. longimanubris. He was unfortunately not able to give a detailed description, because the material was not sufficiently well preserved. He, however, gives measurements and drawings of the hooks and further mentions that "in general appearance it closely resembles the ordinary species from the dog". As the dog specimens which Cameron saw had three segments, the writer concludes that this species also had only three segments. This character would thus serve to differentiate his species from the species described above. In addition there are considerable differences in the hooks, the larger hooks are smaller, 0.035 mm. as against 0.04 to 0.045 mm. the guard is remarkably large and situated at right angles to the axis of the blade and handle, and the handle of the smaller hooks are much larger and do not end in expanded extremities.

Principal Measurements in mm. of the Six Known Species of Echinococcus.

	E. gra- nulosus.	E. came- roni.	E. lyca- ontis.	E. oligar- thrus.	E. longi- manubris.	E. minus.
Sizemm.	5-8	5-7	4-7	$1 \cdot 7 - 2 \cdot 5$	?	?
No. of segments	3-4	4-5	5-7	3	?	?
No. of hooks	30-36	28-32	68-72 (34-36 large)	36-40	?	?
Size of large	040 040	095 090	040 045	0.45	0.95	09.0
hooksmm. Size of small	.042049	·035-·038	·040-·045	$\cdot 045$	$\cdot 035$	·032
hooksmm.	.032042	·030-·033	.028032	.032	·030	·020
No. of testes	33-53	50-60	40-50	20-33	2	?

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