Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 28 (1971)
Heft: 4

Artikel: Biology of "Echinococcus granulosus" as it occurs in South India
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DOI: https://doi.org/10.5169/seals-311732

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Biology of *Echinococcus granulosus* as it occurs in South India

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A high incidence of human hydatid disease was found in Kurnool, a city in the Deccan Plateau of South India. REDDY et al. (1968) conducted an epidemiological survey of the hydatid disease in Kurnool and found that (1) $33^1/_3\%$ of the street dogs had the adult worm; (2) 16.96% of goats, 21.75% of sheep, 60.94% of cattle, 7.7% of pigs slaughtered in the local abbattoir had hydatid cysts; (3) the cysts of the sheep were the most fertile; (4) in a nearby village a hydatid survey by Casoni test showed that 20–25% of the population in the various age groups gave a positive test. The above high incidence of the disease both in man and animals prompted us to investigate the type of the organism which is present locally. There was one other study from India of the biology and morphology of the *Echinococcus granulosus* of buffalo-dog origin from Lucknow, a city in Northern India (GILL & RAO, 1967).

Material and Methods

The local slaughter house was visited daily and cysts from various animals were brought and the following studies made:

(A) Fertility of the cyst was checked first and if found fertile the material was fed to dogs for the production of adult worms. The dogs were first kept in the laboratory for 10 days and motion was examined repeatedly to see that they were not previously infected. If no ova were found, the dogs were fed with the infective material. Two dogs were used for each type of infective material. The infective material was obtained from sheep, goat, cow and ox. The dogs were fed only once and the motion was examined on alternate days from 40 days after feeding and daily from about 50 days after feeding. The time taken for the appearance of the ova in the dog was noted. The dog was then killed and the adult worms in the intestine were collected and stained according to the method of NELSON & RAUSCH (1963). The morphology of the adult worm was studied with respect to length, number of segments, length and breadth of immature, mature and gravid segments; ratio of length of mature and gravid segments, cirrus sac shape and measurements in mature and gravid segments, number of testes and size, position of genital pore, measurements of scolex and rostellar pad, number and measurements of hooks and also of any odd shaped hook, shape of the female genitalia like the ovary, uterus, seminal receptacle and Mehlis's gland. In each dog more than 30 stained worms were studied.

Apart from dogs, 4 cats were also fed, with live scolices to look for any alternate definitive host.

(B) Live scolices from the fertile cysts from various sources were injected intraperitoneally into the animals and the development of cysts in them was studied. Live scolices from sheep hydatid cysts were injected into 2 white mice, 20 white

rats and 3 guinea pigs. Live scolices from ox were injected into 6 white mice, 10 white rats, 3 rabbits and 3 guinea pigs. Live scolices from cow were injected into 6 white mice and 2 rabbits. Similarly from goat into 2 white mice and 5 white rats and live scolices of buffalo origin into 5 rabbits and 5 guinea pigs. The number of animals which showed the development, with maximum size of the cysts with presence or absence of scolices inside, and the time taken for this, was also noted.

(C) Live scolices from cysts of sheep, goat, ox, cow, buffalo, pig and human origin were studied for the following. 10 cysts from 10 different animals of each type were chosen except in pigs and humans where only 2 cysts from pigs and 4 cysts from humans were studied. In each cyst 10 scolices were studied for the following: (i) number of rows of hooks; (ii) number of small hooks and large hooks; (iii) number in the third row; (iv) pairs and threes of hooks; (v) length and breadth of large and small hooks; (vi) length of the handle and length of blade of both large and small hooks. Altogether 200 large and 200 small hooks from 10 different fertile cysts from each source were measured in all.

(D) Fertility rate of the cysts of each animal and from each organ also were studied.

(E) Number of scolices in brood capsules were also noted.

(F) Three jackals obtained from the surrounding forest area were autopsied to see whether they had any *Echinococcus granulosus*.

Results

(A) The sheep-dog and the goat-dog showed ova in the motion 83 days after feeding whereas the ox-dog showed the ova 79 days after feeding and the cow-dog showed the ova 65 days after feeding live scolices. So the time taken for the development of a gravid worm and appearance of ova in motion in the definitive host ranged from 65 days to 83 days.

The main morphological characteristics of the adult worm as seen by us are given in Table 1 along with a comparison of the characteristics of the worms seen in New Zealand, South Africa and also Lucknow.

(a) Strobila: The total length of the worm varies from 5 to 7 mm (Fig. 1). The number of proglottids ranged from two to four. Usually the number were three, the first one immature, the second mature and the last one being gravid with uterus distended with eggs. The three segmented worms ranged from 86% in the sheep-dog worms to 96.6% in the cow-dog worms, the rest being 2 or 4 segmented ones. Further details are brought on Table 1 (length of segment, number of hooks, etc.).

(b) Male genitalia: The testes appeared early in the development of the proglottid as small dark staining masses, bigger in the posterior part showing that the posterior ones mature early (Fig. 2). The number in a mature proglottid ranged from 30 to 60 with an average of 48 in sheep-dog worm, 44 in goat-dog worm, 47 in cow-dog worm and 40 in ox-dog worm. Testes did not persist in the gravid segment.

The cirrus sac was pear-shaped and was horizontal or tilted posteriorly. The cirrus sac measurements are given in Table 1. In the

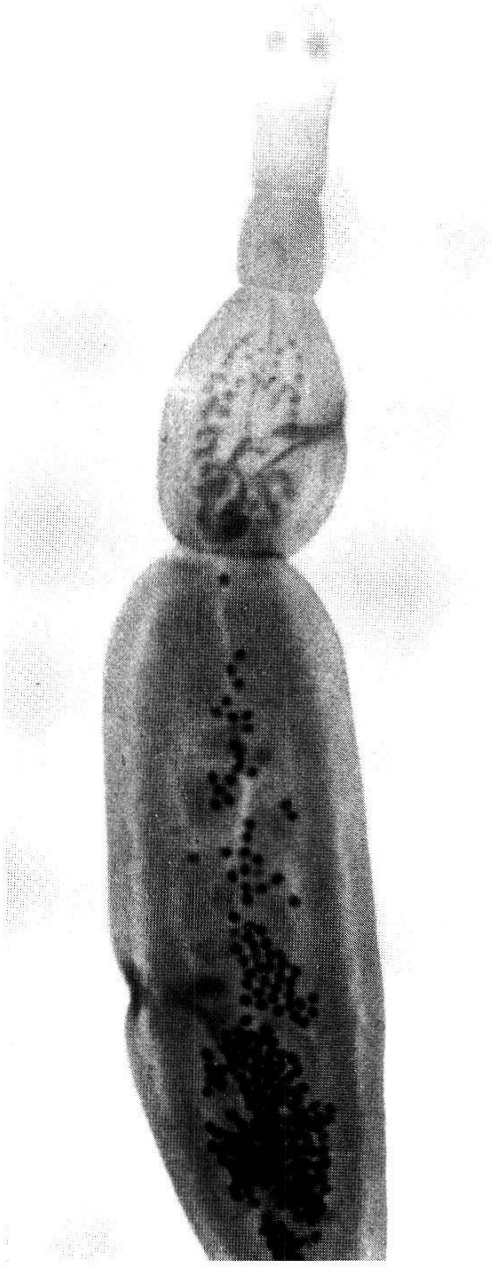

Fig. 1. Adult worm of sheep-dog origin, × 20.

mature proglottid it usually extended half way across the proglottid but not so in the gravid segment. The genital pore was more posterior than anterior.

(c) Female genitalia: The vagina leaves the genital pore and runs posteriorly up to the uterus and then opens into a round seminal receptacle. The ovary was bilobed joined by an isthmus. The lobes of the ovary were irregular and gave off irregular radiating lobules. The Mehlis's gland was dark staining and irregular. In the mature proglottid the uterus was a thin-walled blind sac, but in the gravid segment it got filled with eggs and had sacculations.

Cats: In none of the cats fed with live scolices from cow and ox source could we find ova on examination of the motion. At the end of

TABLE 1

Source of material:		New Zealand (Sweatman & Williams, 1963)	S. Africa (Verster, 1965)	Lucknow (Gill & Rao, 1964)	Present Material			
Definitive host:		dog	dog	dog	dog	dog	dog	dog
Total No. of cystic hooks		25–44 (34.9)	–	29–50	28–43	28–43	25–45	27–42
Total No. of adult hooks		30–42 (36.3)	–	27–32	26–38 (33)	28–40 (34)	28–38 (33)	28–36 (32)
Length of cystic hooks (in μ)	large	22–29 (25.9)	–	24–34	20–25 (22)	22–30 (24)	18–26 (33)	21–25 (23)
	small	17–27 (22.6)	–	18–30	15–23 (19)	17–23 (23)	17–22 (19)	15–22 (20)
Length of adult hooks (in μ)	large	25–40 (34.2)	30–46 (34)	24–43	32	32	32	32
	small	19–35 (26.0)	20–37 (30)	18–30	24	24	24	24
Max. length of the worm in mm		7 mm	4.84 mm	5.16 mm	7 mm	6 mm	6 mm	5 mm
No. of proglottids		3–5	2–4	2–3	2–4	2–3	3–4	2–3
Length of gravid segment in mm		0.936–2.160 mean: 1.728	1.105–2.934 mean: 1.794	0.790–3.260 –	1.5–3.15 mean: 2.416	1.925–3.150 mean: 2.620	1.750–3.500 mean: 2.688	1.400–2.800 mean: 1.876
Ratio of length of mature and gravid segment		1 : 2	1 : 2.7	1 : 2.7	1 : 2.5	1 : 2.6	1 : 2.5	1 : 2.2
No. of testes		about 48	about 40	about 26	about 48	about 44	about 47	about 40
Cirrus sac shape		pear shaped	pear shaped	pear shaped	pear shaped	pear shaped	pear shaped	pear shaped
Cirrus sac size in mature segment	length in μ	147.7±24.4	87.9±12.2	97	0.157±18	136±24	137±18	126±15
	breadth in μ	76.7±24.4	49.5±8.1	44.3	77±11	58±17	72±12	58±4
Cirrus sac size in gravid segment	length in μ	174.1±25.9	99.7±12.9	–	156±20	155±27	141±16	138±54
	breadth in μ	77.7±8.7	55.6±6.8	–	78±9	75±15	73±11	86±11
Position of genital pore		posterior	posterior	posterior	posterior	posterior	posterior	posterior

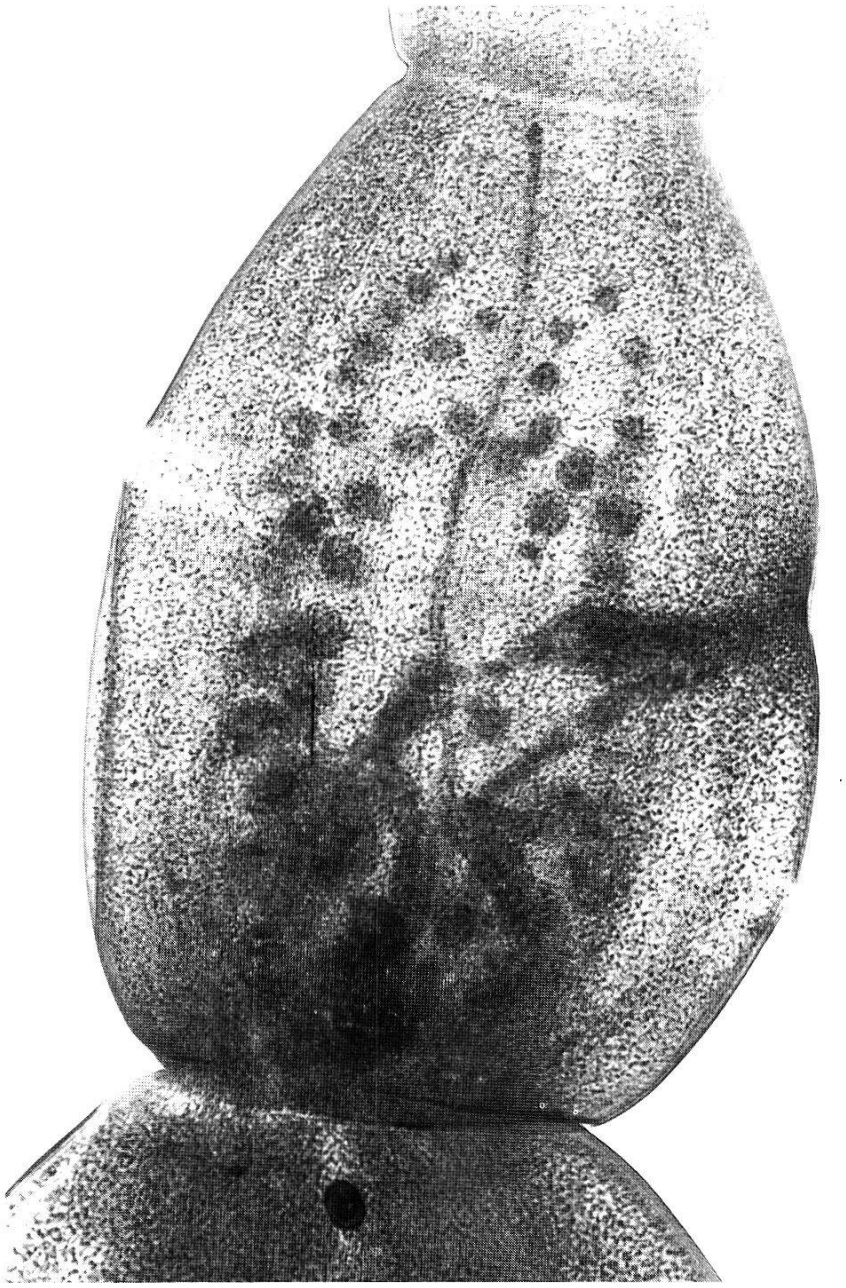

Fig. 2. Mature segment of *E. granulosus* of sheep-dog origin showing male and female genitalia, × 60.

3 months after feeding when the cats were autopsied no worm could be found in the intestine.

(B) Secondary cyst formation: In Table 2 the data on secondary cyst formation in various animals when injected with live scolices from various sources is given. No secondary cysts were formed in the guinea pig at all even after 4 months. In 25% of the white rats live scolices of sheep origin only could develop into secondary cysts whereas injection from any of the other sources did not give rise to any cysts. In rabbits only ox and buffalo source live scolices could develop and not those from cow. All the mice injected developed secondary cysts (Fig. 3) irrespective of the origin of the scolices. In the beginning we tried intra-

TABLE 2

Animal used	Source from	No. used	Number positive		Time when autopsied	Maximum size dia. in mm.	Minimum size dia. in mm.
I. Mice	sheep	2	2	100%	1–5 months	10	0.525
(amount in-	ox	6	6	100%	20 days–3 months	5.2	0.360
jected 0.25 ml)	cow	6	6	100%	2–6 months	10	0.380
		14					
II. Rats	sheep	20	5	25%	1 week–2 months	25	0.250
(amount in-	ox	10	–	0%	10 days–1½ mths.	–	–
jected 0.4 ml)	goat	5	–	0%	10 days–2 months	–	–
	human	2	–	0%	1 day–2 months	–	–
		37					
III. Rabbits	ox	3	3	100%	15 days–3 months	10	0.360
(amount in-	buffalo	5	4	80%	1–2 months	0.785	0.350
jected 0.6 ml)	cow	2	–	0%	2–3 months	–	–
		10					
IV. Guinea-pigs	sheep	3	–	0%	1–2 months	–	–
(amount in-	ox	3	–	0%	2–4 months	–	–
jected 0.6 ml)	buffalo	5	–	0%	1–2 months	–	–
		11					

peritoneal injection of the live scolices in older mice (70 days old) but failed to find any secondary cyst formation. But on trying the same in 30-day-old mice, all the mice developed cysts. In two of the mice even the diaphragm was infiltrated and the cysts entered into the thoracic cavity. The maximum size of the secondary cysts formed was in a white rat injected with sheep origin scolices and it was 2.5 cm in diameter. There were innumerable cysts in the mice and rats but in the rabbits there were very few. In none of the cysts was there any formation of scolices again. The origin of these secondary cysts from the live scolices injected was evidenced by the presence of hooks inside the cyst for quite some time after the formation of the cyst.

(C) Hooks. The rows of hooks ranged from 2–3 in all the cysts studied. In the 3rd row, the number of hooks varied from 1 to 5 and these were usually small and odd shaped and either present on the inner aspect of the regular rows or on the outer aspect. The hooks were also arranged either in pairs or even threes at times. These pairs or threes were present in both the large hooks and also the small hooks.

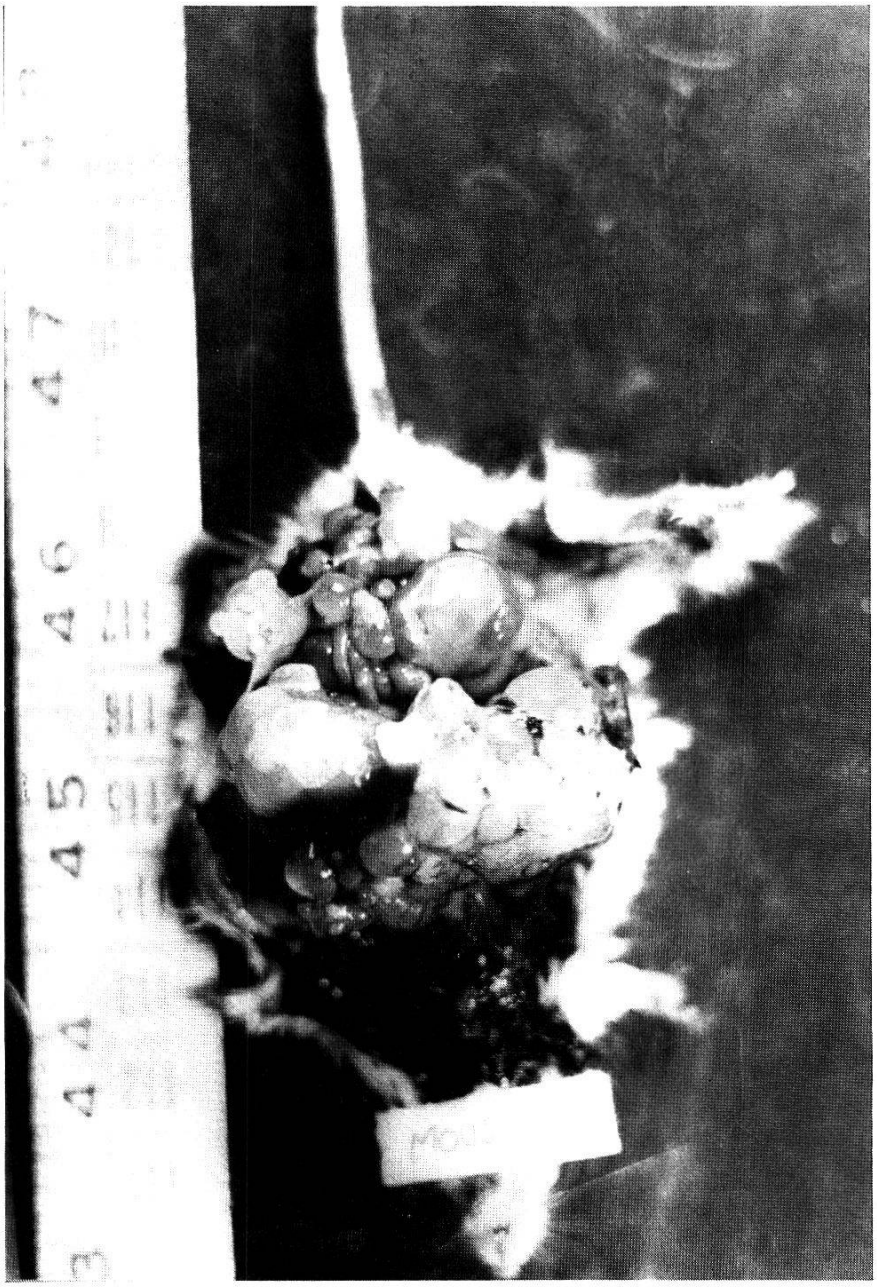

Fig. 3. White mouse showing large number of hydatid cysts.

(D) The fertility rate of hydatid cysts from various sources was studied by us (REDDY et al., 1970). Cysts from 247 cows, 165 oxen, 116 sheep, 33 goats, 18 buffaloes and 7 pigs were studied. 64.6% of sheep, 66.7% of buffaloes, 54.5% of goats, 41.7% of cows, 31.5% of oxen, 28.5% of pigs showed fertile cysts. But the number of buffaloes killed in the slaughter house are very small when compared to the number of sheep which are killed. The ratio being something like 150 sheep to 1 buffalo. Sheep having a large number of fertile hydatid cysts and also the large number of sheep slaughtered locally shows the epidemiological importance of sheep in the dissemination of hydatid disease locally.

(E) Brood capsules from the hydatid cysts of various animals were

studied for the number of live scolices seen in each (REDDY et al., 1969). 280 brood capsules from cow, 230 from sheep, 220 from ox, 30 from buffalo and 20 from goat were examined. The average number in each brood capsule was 8.6 for cow, 11.1 for sheep, 9.1 for ox, 8.9 for goat but only buffalo had a higher average with 31.9. Maximum number of 150 live scolices were seen in one buffalo hydatid cyst.

(F) To know whether there are any other natural intermediate hosts three jackals were obtained from the surrounding jungles and autopsied. None of the jackals showed any adult *Echinococcus granulosus* worm.

Comment

RAUSCH (1967) mentioned that *E. granulosus* probably has a wide distribution in Asia and northern Africa since ancient times, but subsequent introductions must have taken place repeatedly during the period of colonisation by Europeans. He also mentioned that *E. granulosus* has been reported from India, but does not mention the type of the parasite present here as evidently no work has been done regarding this. Only GILL & RAO (1967) studied the morphology and biology of *E. granulosus* of buffalo-dog origin at Lucknow.

HUTCHISON & BRYAN (1960) working with dog material found that the strobila matured in dog in 4 weeks but they took 8 weeks to become gravid. With our material, it took 9 to 12 weeks for the ova to be seen in the motion.

The length of the worm and the number of segments produced by us are similar to what has been described as occurring in *E. granulosus* described from New Zealand as seen from the Table 1. The value of the segments in taxonomy differs with different workers (RAUSCH, 1953; SWEATMAN & WILLIAMS, 1963). The number of testes seen in our material were more than what are described by GILL & RAO (1967) but similar to the number described in *E. granulosus* from New Zealand. The number of adult hooks, lengths of large and small hooks, ratio of the length of the mature and gravid segments, shape of the cirrus sac, number of testes, position of the genital pore, size of the cirrus sac in the gravid and mature segments of our material are almost similar to those described by SWEATMAN & WILLIAM (1963) as *Echinococcus granulosus granulosus* from New Zealand.

RAUSCH (1967) does not consider the number of rostellar hooks of any value for species identification. SWEATMAN & WILLIAMS (1963) and WILLIAMS & SWEATMAN (1963) relied upon characteristics of rostellar hooks of both larval and strobilar stages as one of the modes of species identification. VERSTER (1965) found that the size of the rostellar hooks depended upon the host and failed to give any taxonomic significance

to the size of the hooks. However, the measurements of the cystic hooks by us are similar to the measurements as reported by SWEATMAN & WILLIAMS (1963) for *E. granulosus granulosus* from New Zealand.

Our material slightly differs from what is described by VERSTER (1965) from South Africa as *Echinococcus granulosus africanus*. Our material differs from that of GILL & RAO (1967) in having more number of testes and a larger cirrus sac and being a longer worm.

We were unable to inject the cats with our material though successful development of immature worms in cats have been recorded. GILL & RAO (1967) and VERSTER (1965) reported to have obtained a mature *E. granulosus* with immature ova in it from a cat.

Echinococcus larvae can be maintained in the laboratory without passage through a definitive host by intraperitoneal inoculation with live scolices from a cyst into a suitable animal. Secondary hydatids of *E. granulosus* have been established in this way in white mice (SCHWABE, SCHINAZI & KILEJIAN, 1959) and rabbits (DEVE, 1949). SCHWABE et al. (1959) have demonstrated resistance to infection of older mice. In none of their mice was there any evidence of scolices in brood capsules, though SWEATMAN & WILLIAMS (1963) found scolices in their material.

In our studies all the mice developed secondary cysts but in none could we find scolices. In a few white rats secondary cyst formation occurred. In some rabbits also it occurred but in none of the guinea pigs did secondary cysts form. Similar to the findings of SCHWABE et al. (1959), we failed to infect the older mice. WLLIAMS & SWEATMAN (1963) reported development of secondary cysts in white rats, when injected with material from horse hydatid cysts. They also mentioned that white rats were refractory to secondary cyst formation from material of *Echinococcus granulosus* origin.

The fertility rate of the sheep, hydatid cysts seen locally is high. It is similar to what is described from New Zealand where sheep-dog cycle is important epidemiologically (LUTTERMOSER & KOUSSA, 1963). Though buffalo hydatid cysts show a slightly higher fertility, the number of buffaloes slaughtered are very few when compared to the number of sheep. So the sheep-dog cycle is important here epidemiologically.

The number of scolices in the brood capsules as studied by LEUCKART (1886) are not more than 22. CAMERON (1927) also was of the same opinion until he saw a large number of scolices in a brood capsule from a Canadian elk (DISSANAIKE, 1962). The number of scolices in the brood capsules as seen by us in goats, sheep, cow and ox are similar to the classical experience of LEUCKART (1886) and CAMERON (1927). The number seen in the buffalo are similar to what has been recorded by GILL & RAO (1967 a). But these are much less than what are recorded by DISSANAIKE (1962). The presence of large

numbers of scolices in each brood capsule as recorded in Ceylon where a sylvatic cycle is known to be present and also in hydatid cysts from areas known to have a sylvatic cycle is of interest.

Sylvatic cycle: The large number of cattle showing hydatid cysts (REDDY et al., 1968) makes one think of a sylvatic cycle here. But the small number of scolices in each brood capsule, the absence of adult worms in the few jackals autopsied show that probably the sylvatic cycle does not exist here.

SWEATMAN & WILLIAMS (1963) described two subspecies of *E. granulosus*. WILLIAMS & SWEATMAN (1963) described *Echinococcus equinus* as another variety. VERSTER (1965) has made a detailed study of *Echinococcus* species in South Africa and justified the separation of *E. granulosus* into nine different subspecies. RAUSCH (1967 b) is of different opinion and mentioned that it is not appropriate to describe the various types as subspecies as the differences observed though statistically significant are probably taxonomically insignificant and could be accounted for by host-species induced variations and physiological modifications. SMYTH & SMYTH (1964) concluded that *E. granulosus* and *E. multilocularis* may represent two extremes of a character gradient comprising an indefinite number of variants which may exhibit characteristics common to both. They also mentioned that since the organism is a hermaphrodite, with a larva reproduced by polyembryony, on theoretical grounds clones of new mutants consisting of a large number of identical individuals may be formed.

No single criterion is important or of significance for the separation of the species of *E. granulosus* into subspecies (RAUSCH, 1967 a, b; VERSTER, 1965). But when all the characteristics of adult worm, cysts, secondary cyst formation, natural intermediate hosts and definitive hosts, fertility rates of cysts and number of scolices in brood capsules are taken into consideration, one cannot escape from the fact that the adult worm as is seen by us at Kurnool is similar to *Echinococcus granulosus granulosus* of New Zealand. One has to remember also that India was colonized by the Europeans and live stock and dogs might have been brought by them here similar to what they did in Australia and New Zealand. Thus they might have brought or reintroduced the hydatid infection into this country. Positive proof that the infection probably existed here is difficult to obtain unless we can come across preserved specimens of hydatid cysts or parasites from dogs intestines. Medical museums in this country are not that old as to give such information as all the medical colleges were started after the Europeans colonized here.

Acknowledgments

Our thanks are due to Mr. G. Prahlada Rao and Mr. P. Haricharanapathi for helping us with the manuscript and photographs respectively.

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Zusammenfassung

Die Biologie von *Echinococcus granulosus* in Südindien wird untersucht.

a) Im Kot von Hunden konnten nach einer einmaligen Verfütterung von fertilen Larvenstadien nach 9 bis 12 Wochen Eier nachgewiesen werden.

b) Katzen konnten nach Verfütterung von Larvenstadien keine adulten Würmer entwickeln.

c) Die Autopsie von Schakalen, die in der Gegend vorkommen, ergab nie das Vorkommen von adulten Würmern.

d) Sekundäre Cysten konnten sich in jungen weißen Mäusen, weißen Ratten und Kaninchen, nicht aber in Meerschweinchen entwickeln.

e) Die Haken des Rostellum wurden in hydatidösen Blasen gemessen, die man vom Schaf, von der Ziege, vom Ochsen, von der Kuh, vom Büffel, vom Schwein und vom Menschen gewonnen hatte.

f) Die Fertilitätsrate hydatidöser Cysten von verschiedenen Tieren wurde untersucht. Cysten vom Schaf waren am fruchtbarsten.

g) Die Zahl der Scolices in den Kapseln der hydatidösen Cysten von den verschiedensten Tieren war sehr klein.

h) Es wird nachgewiesen, daß *E. granulosus* in Südindien identisch ist mit *E. granulosus* von Australien. Wahrscheinlich wurde er von Europäern während der Kolonisationszeit nach Indien wieder eingeschleppt.

Résumé

La biologie d'*Echinococcus granulosus* du Sud de l'Inde est étudiée.

a) 9 à 12 semaines après avoir nourri des chiens avec des hydatides fertiles, les œufs apparaissent dans les selles.

b) Les chats nourri d'hydatides ne montrent pas de vers adultes.

c) L'autopsie de chacals locaux ne montre pas de vers adultes.

d) Des kystes secondaires se développent dans de jeunes souris blanches et les rats blancs, mais pas dans les cobayes.

e) Les crochets du rostellum furent mesurés dans des hydatides de moutons, chèvres, bœufs, vaches, buffles, porcs et hommes.

f) La fertilité de kystes hydatiques d'animaux divers a été étudiée. Les plus fertiles sont ceux des moutons.

g) Le nombre de scolex dans les capsules des kystes hydatiques d'animaux variés reste très faible.

h) On montre que l'*Echinococcus granulosus* du Sud de l'Inde semble être identique à celui d'Australie. Il est vraisemblable que le vers ait été réintroduit dans le pays à la suite de la colonisation par les Européens.