# OBSERVATIONS ON THE BIOLOGY AND MORPHOLOGY OF ECHINOCOCCUS GRANULOSUS IN LEBANON AND SYRIA

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ECHINOCOCCUS GRANULOSUS

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### ABSTRACT

This study gives some insight into the taxonomic position of the hydatid parasites of local and imported sheep and cattle, the Syrian camel and the donkey from Lebanon. By using biological, morphological and physiological criteria, these infections, with the exception of those of donkey origin, proved to be conspecific with Echinococcus granulosus granulosus. The worms from dogs fed cysts from donkeys along with those from a naturally infected dog from Hermel, Lebanon, show a definite affinity toward Echinococcus granulosus equinus, a distinct taxonomic form involving an equine intermediate host. A single infection in a wolf proved to be  $\underline{E}$ . g. granulosus. Foxes (Vulpes vulpes), badgers (Meles meles), feral cats (Felis domesticus) and 2 species of wild rodents (Meriones tristrami, Microtus geuntheri) were not infected experimentally with hydatid material which originated from either cow, sheep or donkey. Nor were these as well as other species of wild mammals from Lebanon, Syria and Jordan found infected naturally. A sylvatic cycle is therefore unlikely.

Observations on the morphology of hydatid material from the jackal and the Sambur deer from Ceylon as well as cysts from rhesus monkeys and worms from an infection from India show many features in common with each other but differ in some details from the types of hydatid in Lebanon and Syria.

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### INTRODUCTION

A. Hydatid Infection With Particular Reference to

The Middle East

Hydatid disease, caused by <u>Echinococcus granulosus</u>, was reported in man as early as 460 to 397 B.C. when Hippocrates described a patient with an infected liver (<u>in</u> Dew, 1928). Redi, who lived from 1626 to 1694, was the first to recognize that the hydatid cyst was of animal origin. This was followed by other early workers such as Tyson, Goeze, Van Siebold and Küchenmeister, all of whom contributed to our knowledge of the parasite (<u>in</u> Dew, 1928). It was not until 1867, however, that a complete account of the pig-dog life history was observed by Leukart.

In Lebanon and other parts of the Middle East, the incidence of human infection with hydatid disease is considered high. Schwabe and Abou Daoud (1961) mention I the reported occurrence of 385 human cases from Lebanon, Syria, Palestine and Iraq during the 25 year period from 1923 to 1949. They compared the rate of 3.82 per 100,000 people per year in Lebanon from 1949 to 1959 with Uruguay, Chile, Argentina, Cyprus and New Zealand which are known to have high levels of infections and noted Lebanon's rate was second only to Cyprus and New Zealand. The

incidence of hydatid cysts in domestic animals has been reported from the Middle East by various workers. Studies by Luttermoser and Koussa (1941) showed the incidence in 32 sheep and goats at the Beirut abattoir to be 23.1%. A survey by Babero et. al. (1963) in Iraq showed similar results with a 29.5% in sheep and 26.6% in goats. In Syria, Hörchner (1964) found 21% of 5000 sheep to be hosts of the parasite. The incidence of pig and cow infections were also noted by Luttermoser and Koussa in Lebanon to be 33.3% of 300 examined and 37.8 of 164 examined respectively, while Babero et. al. found the incidence in Iraqi cattle to be only 13.9%. Camels (Camelus dromidarius) have been reported with hydatid infections from Lebanon, Syria and Iraq by Pipkin, Rizk and Balikian (1951) who also gave a summary which includes the work of Turner, Dennis and Kassis (1936) and Senekjie and Beattie (1940). They stated the incidence in camels ranged from 67.4% to 100%. Other camel infections have been reported from West Kuzakastan (Shumilina, 1955), Saudi Arabia (Salah, 1961) and Egypt (El-Garhy and Selim, 1957). The latter authors noted the incidence of echinococcosis to be as low as 7.3% and stated that most of the camels were imported from the Sudan.

Hydatid infection in the horse had been reported throughout Europe to as far east as Hungary (in Williams and Sweatman, 1963) with occasional and perhaps introduced infections in America and

Australia. It has also been seen in horses in Ceylon by Dissinaike (1962). No horse infections apparently have been reported from the Middle East in spite of the prevalence of horses in most Arab countries. Williams and Sweatman (1963) demonstrated that the English horse hydatid was a distinct subspecies of Echinococcus granulosus which they named E. g. equinus. Another equid common in the Middle East is the donkey (Equus asinus). Donkey infections have been noted in Uzbekistan S.S.R. (Zhdnova, 1961) and in Liverpool, England (Southwell, 1927) but no incidence studies appear to have been carried out. Sheep are known hosts of E. g. granulosus in both geographical regions, while horses are infected with E. g. equinus in England (Williams and Sweatman, 1963). The relationship of these to the donkey infections requires clarification.

The incidence of echinococcosis in the horse and donkey has not been reported from the Middle East and no biological or morphological data on the donkey or camel material have been published. Observations on the biological relationship of the donkey and camel infections with each other and with those seen in sheep, cattle, swine and horses, designated as <u>E</u>. g. granulosus (Sweatman and Williams, 1963) and <u>E</u>. g. equinus (Williams and Sweatman, 1963) form the basis of this dissertation.

#### B. Taxonomy

The cestode Echinococcus granulosus was first described in accordance with the binomial system of nomenclature by Batsch in 1786 under the name of Hydatigena granulosa. Gmelin, changed it to Taenia granulosa in 1790, and Rudolphi in 1801, placed it in the newly established genus, Echinococcus with the type species granulosus. Currently, taxonomic features include the morphology of the parasite, hosts, georgraphical location, comparative susceptability to primary cystic infection, secondary cyst development and development of the adult worm in carnivores not known to be natural hosts.

Table I shows that there have been 12 species and 4 subspecies described in the genus Echinococcus. Only 2 species (E. granulosus and E. multilocularis) and 4 subspecies (E. g. granulosus, E. g. canadensis, E. g. borealis and E. g. equinus) have been described following experimental confirmation. Rausch (1958) and Vogel (1955) made careful descriptions of E. multilocularis, while a thorough morphological and biological description of the 4 subspecies in E. granulosus was made by Sweatman and Williams (1963) and Williams and Sweatman (1963). When these observations are compared with the recorded descriptions of E. intermedius, E. patagonicus, E. longimanubrius and E. minimus, these fall

into synonomy with E. granulosus granulosus, while certain features established in the original descriptions of E. oligarthrus, E. felidis, E. cameroni and E. cruzi maintain these as being currently valid. Rausch and Nelson (1963) suggested the synonomy of six species, E. intermedius, E. cameroni, E. longimanubrius, E. lycaontis, E. minimus and E. ortleppi with E. granulosus, but the criteria available for some of these cannot be considered adequate. They also list 3 species that are considered valid (E. granulosus, E. multilocularis, E. oligarthrus) and 2 (E. felidis, E. patagonicus) as uncertain and possibly conspecific with E. granulosus.

Three subspecies besides the nominate subspecies <u>E</u>. g. granulosus now exist. These are <u>E</u>. granulosus canadensis,

Webster and Cameron, 1961, <u>E</u>. granulosus borealis, Sweatman and Williams, 1963 and <u>E</u>. granulosus equinus Williams and Sweatman, 1963. Subspecific rank, as indicated in Table I, was: created using biological and morphological criteria noted in the first paragraph of this section. Similar criteria were used in this thesis to assess the taxonomic status of the type of hydatid infections seen in donkeys and camels.

SPECIES	DEFINITIVE INT	TERMEDIATE HOST		No OF HOCKS
E.g. granulosus	dog	bovids & domestic pig	New Zealand	
E.g. borealis	( <u>Canis lupus</u> ) ( <u>Canis latrans</u> ) dog	Primarily cervids par- ticularly <u>Alces alces</u>	Ontario, Canada	
E.g. canadensis	<u>dog</u>	( <u>Rangifer</u> <u>tarandus</u> )	Aklavik, Canada	
E.g. equinus	experimental dogs	horse	England	
E. multi- locularis	Vulpes vulpes Alopex. lagopus dog dog dog	microtine rodents	North America Europe, Russia	26-36 average 30
E. oligarthrus	Felis concolor Felis jaquarondi	Myocastor sp.	South America	36-40
E. lycaontis	Lycaon pictus		South Africa	34-35
E. felidis	Panthero leo		Transvaal South Africa	32-46

Table I

SPECIES AND SUBSPECIES DESCRIBED IN THE GENUS ECHINOCOCCUS

λ** Σ	Length Hooks Large	OF ADULT (#) SMALL	LENGTH OF STROBILA	No. OF PROGLOTTIDS	No. OF TESTES	DISTRIBUTION OF TESTES
	25-40 (34-2) (3 ro	19-35 (26) ws)	40° 600 600 au 600	3 - 5 usually 4	40-70	More than half anterior to genital pore.
The state of the s	36-46 (41.8) (3 rows	25-37 (3 <b>2</b> .6) , rarely 4)		3 - 5 usually 3	35-55	More than half anterior to genit pore. Genital por posterior to mid point.
	32-43 (38.1) (3 rou	27-37 (31.5) vs)		2 - 3 usually 2	21-40	Majority posterio to genital pore.
	36-52 40.4) (3 ro	22-44 (34.2) ows)		3 - 4 occasionally	35	Generally 2 to 3 rows posterior to vitelline gland. Testes persist in gravid proglottid
	<b>27</b>	19-26	1.2-3.7 mm.	usually 2-3 may be 4-5 in old worms	30-53	Posterior half of segment to genital pore level.
4	6-50 49)	36-39 (38)	1.9-2.3 mm.	usually 2 may be 3	23-29	Posterior half of segment.
3	6-42 (4 row	28-30 s)	4 - 6 mm.	usually 5 more may exist	40	Extending well anterior.
<b>?</b>  .	7-42 41)	28-35 (33)	4-5.5 mm.	3 - 4	28-46	Equally anteriorly & posteriorly, distributed to genita pore level.

Pear-shaped 140x100 m Horizontal or tilted slightly anterior.	Cyst development rare in deer. Strobilate form aborted in foxes. Forms secondary cysts in mice.	Sweatman & Williams, 1963
Round shaped 100x80 m Generally a slight tilt anteriorly	Cystic development rare in domestic sheep. Strobilate slow but complete in some foxes. Secondary cysts rare and slow in immature white mice.	Sweatman & Williams, 1963
Pear-shape 90x75 µ Horizontal or tilted slightly anterior.	Nome or only aborted development in domestic sheep and pigs. Secondary cysts development insignificant in mouse.	Webster & Camero 1961; Sweatman & Williams, 1963
Round and some tilted acutely anterior beyond midline 88%.	Cyst rare in domestic sheep. Secondary in mouse and rat, but not rabbit.	Williams & Sweatman, 1963
		Vogel, 1955; Rausch, 1958
		Cameron, 1926
***		Ortlepp, 1934
		Ortlepp, 1937

SPECIES D	EFINITIVE IN HOST	NTERMEDIATE HOST	TYPE LOCALITY	No. OF HOOKS
E. ortleppi	dog		South Africa	30-36
E. intermedius	dog	<b>~~~</b>	<b>S</b> pain	38
E. cameroni	Vulpes vulpes		England	28032
E. patagonicus	( <u>Dusicyon</u> <u>culpaeus</u> )		Argentina	33 <b>-</b> 36
- A				
E. longitana	Lycaon capensis	<b>9</b> 1 1 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2	South Africa	
		Tarian in India		
E. minimus	Canis lupus	645 april 1640 1640 \$	Macedonia	
		•		
E. cruzi		· ·		
		(Dasyprocta aguti)	South Americ	•
			· · · · · · · · · · · · · · · · · · ·	
Length (µ) of c	ystic hooks: 1- I	E.g. granulosus Large hooks: 22- Small hooks: 17-	29 (25.9) 27 (22.6)	

2- E.g. canadensis Large hooks: 27-33 (30.4) Small hooks: 23-29 (26.2)

j.

Table I

# Continued

LENGTH HOOKS LARGE	OF ADULT (µ) SMALL	LENGTH OF STROBILA	No. OF PROGLOTTIDS	No. OF TESTES	DISTRIBUTION OF TESTES
<b>(\$2-4</b> 9	32-42	5-8.5mm.	3	<b>5</b> 0-53	Concentrated in middle portion of segment.
<b>35-38</b>	24-30	3 • 3mm •	4	5 <b>2-</b> 60	Extending from posterior to anterior segment
35-38	30-33	5-7mm.	4 or 5	50-60	Extending well anterior to genital pore
	25		3 - 4	28-38	5-to 8 anterior to cirrus on one side only.
35	30	gage with mind drop date	THE MAY AND	gate. 1950 Arts	
32	20	. *** **** **** **** ****	400 Min Nan		and were their size suppliers and were some way.
					å <b>√</b>

E.g. borealis
Large hooks: 26-33 (30.2)
Small hooks: 22-28 (25.3)

<sup>4.</sup> E.g. equinus
Large hooks: 26-35 (30.4)
Small hooks: 21-31 (27.8)

SHAPE & SIZE OF CIRRUS	HOST SUSCEPTIBILITY	SOURCE
200 #	## ### ### ##########################	Ortlepp, 1934; Lopez-Neyra & Soler, 1943.
100x38 μ		Ortlepp, 1934.
		<b>Szi</b> dat, 1960
		Brumpt & Joyeux, 1924.
	***************************************	Cameron, 1926.
		Cameron, 1926

Cameron, 1926

# C. Sylvatid Cycles and Their Possible Existence In Lebanon and Syria

Sylvatic infections have been investigated in other parts of the world. Echinococcus multilocularis, the cause of alveolar hydatid disease in man, is maintained by a fox-rodent cycle and is found in arctic North America, central Europe, Russia and Japan (Rausch, 1958; Vogel, 1960; Choquette et. al., 1962; Fay and Williamson, 1952). E. granulosus borealis and E. granulosus canadensis occur in Canada, possibly Alaska and Norway and primarily involve a moose-wolf and reinder-dog cycle respectively (Sweatman and Williams, 1963). Domestic carnivores, especially dogs, become infected from a wild animal source and appear to be the main reservoir of infection for mak, but not farm animals. Dissanaike and Paramananthan (1960) in Ceylon, found gravid worms of Echinococcus in a jackal (Canis aureus) while hydatid cysts were seen in one of 4 Sambur deer (Cervis unicolor) a toque monkey (Macaca sinica), local cattle, buffaloes, goats and horses (Dissanaike, 1958; Dissanaike and Paramanantha, 1962; Dissinaike, 1962). Whether or not the deer and monkey infections indicate the presence of a sylvatic cycle or simply transfer of a domestic animal cycle has yet to be clarified. Two rhesus monkeys (Macaca mulatta) purchased in India were reported with hydatid cysts by

Healy and Hayes (1963) who also noted two other reports in the same host.

Alvarez (1961) carried on a 5 year survey in Chile Which included 12,274 animals of 28 species. There was, however, only a single hydatid infection. This occurred in the rodent Octodon degus. Other natural infections of Echinococcus in rodents have been seen in a cavy (Microcavia australis) in Argentina (De la Barrera, 1958), in a Cape molerat (Georhynchus capensis) in South Africa (Verster, 1962), and in a nutria (Myocastor coypus) in Europe (Wolfhugel, 1951). Echinococcus cruzi, a species of doubtful validity, was reported in its cystic form from an agouti (Dasyprocta aguti) in South America (Brumpt and Joyeux, 1924). Experimental primary infections of E. granulosus borealis were established by Sweatman and Williams (1963) in the squirrel (Scuirus carolinesis) and chipmunk (Tamias striatus), while infections of E. granulosus granulosus have been produced in cotton rats (Sigmodon hispidus) and laboratory white mice (Webster and Cameron, 1961; Sweatman and Williams, 1962).

Since camels and donkeys are used in the Middle East for transportation over long distances in country inhabited by Wild dogs (Canis familiaris), wolves (Canis lupus), foxes (Vulpes vulpes), badgers (Meles meles), marten (Martes faina syriaca) and possibly hyaenas (Hyaena hyaena) and jackals (Canis aureus), their cystic

infections could conceivably be related to worms from these wild carnivores rather than the domestic animals. The material used by Webster and Cameron to produce primary infections in white mice originated from cattle slaughtered in Lebanon. The fact that various rodents are abundant throughout Lebanon and Syria (See Table II) in areas where wild carnivores live, raises the question of possible sylvatic cycles. The fox, one species, (Vulpes vulpes) of which is common in Lebanon and Syria (Table II) has been seen naturally and experimentally infected in other parts of the world. Natural infections have been reported in Vulpes vulpes (both wild and farm), Vulpes corsac, and Dusicvon gymnocercus in a wide geographical range including Wales, Bavaria, Australia, Poland, Eastern Russia and Argentina (Mendheim, 1955; Sinclair, 1956; Gemmell, 1959; Melczewski, 1961; Shumakavich and Nikitin, 1962; Blood Lelijveld, and Lord, 1963). Szidat described a new species of Echinococcus in 1960 from the South American red fox (Dusicyon culpaesus). All the worms were immature and the morphological features fell within the range of E. g. granulosus (Sweatman and Williams, 1963). Whether or not this species is valid requires further clarification. E. granulosus, when fed to foxes usually fails to become established or remains immature but some positive results have been seen. Sweatman and Williams (1963) observed that E. granulosus borealis became gravid in one of the 5 red foxes (Vulpes fulva) as seen at autopsy at 89 days. This indicated a

prolonged prepatent period. Gerina (1959) observed gravid worms (but no excreted eggs) at the autopsy of 4 immature foxes (<u>Vulpes</u> <u>vulpes</u>) at 102 days after they had ingested hydatid cysts from pigs in Russia. Further observations are obviously required on the significance of foxes in the spread of <u>E. granulosus</u>.

The wolf (Canis lupus) is the important natural definitive host of E. g. borealis in Canada (Sweatman and Williams, 1963).

This same species of wolf occurs in Lebanon and Syria but as indicated in Table II is seen only rarely. The jackal (Canis aureus) as indicated previously, was found naturally infected in Ceylon. The jackal may be more common in Lebanon and Syria than the wolf. The badger (Meles meles) and possibly the marten (Martes foina) (Table II) occur not infrequently in Lebanon.

Their significance in a possible sylvatic cycle of hydatid disease requires assessment, although it is known that mustelids are refractory to E. granulosus granulosus and E. granulosus borealis (Sweatman and Williams, 1962; Sweatman and Williams, 1963).

Domestic and stray dogs are commonly found infected with E. granulosus. In the Middle East, Babero and Al.Dabagh (1963) observed the parasite in 38.4% of 169 dogs from 7 liwas of Iraq and referred to two previous publications by Senekji and Beattie (1940) and Kelly and Izzi (1959) of an incidence of 18% of 123 dogs and 85% of 27 dogs from the same country. In Istanbul, Turkey,

5 of 22 dogs were found infected by Merdivenci (1963) while early reports by Turner, Berberian and Dennis (1936) and Pipkin, Rizk and Balikian (1949) from Lebanon showed the incidence to be 20 to 25% of 500 dogs and 11.75% to 32.9% of 467 dogs respectively. The possibility of sylvatic infections being present in Lebanon and Syria has been assessed in the present study with particular reference to those infections seen in camels and donkeys.

# Table II TERRESTRIAL MAMMALS KNOWN TO OCCUR IN SYRIA AND LEBANON

			Habitat				
Species .	Prevalence	Distribution	Coastal Cities	High & Rocky		Uncul- Desert tivated Land	
Rodentia		arian kanning-an kan kannin darika darika kanning menganin kannin dan menandi dalam					
Myomorpha Muridae Murinae							
Rattus norvegicus Rattus rattus Mus musculus Apodemus sylvaticus	common common common uncommon	general general general Faraya & Dog Rive	x Synantropic Synantropic er	x			
Apodemus mystacinus	common	Lebanon Faraya, Lebanon	•				
Cricetidae Cricetinae Cricetulus migratorius	common	general			x		
Mesocricetus auratus	common	Aleppo & Palmyra, Syria			x		
Gerbillinae Gerbillus dasyurus	common	Aleppo & Palmyra, Syria				x	
<u>Meriones tristrami</u> <u>Meriones libycus</u> Jaculus jaculus	common uncommon common	general P <sub>almyra</sub> Syria			x	x	
Microtinae <u>Microtus guentheri</u> <u>Microtus nivalis</u> <u>Microtus socialis</u>	common common uncommon	general Faraya, Lebanon Faraya, Lebanon	4	x x	x	x	
		55.5			endoni.	The state of the s	
Spalax ehrenbergi	common	general			х	X	
Hystricomorpha Hystricidae Hystricinae Hystrix indica	common	Kartaba, Lebanon	-	×		**************************************	
Hyracoidea Procaviidae Procavia capensis	common	Kartaba, Lebanon				x	
Lagamorpha Leporidae Lepus europaeus	rare	South Lebanon			x	x	
Insectivora Erinaceidae Erinacus europaeus	common	general					
Hemiechinus auritus	common	Aleppo, Syria					
Carnivora Canidae Vulpes vulpes Canis lupus Hyaena hyaena Canis aureus	common uncommon rare uncommon	general Schmistar, Lebano Ainab, Lebanon Palmyra, Syria	on	x	<b>x</b>	x x	
Felidae Feral Cat <u>Felis</u> <u>domesticus</u>	common	general					
Mustelidae <u>Meles meles</u> <u>Vormela peregusna</u> <u>Martes foina</u>	common rare uncommon	Kartaba, Lebanon Byblosk, Lebanon Kartaba, Lebanon		x x	x	x	
Lutra lutra	rare	Amik, Lebanon (seen once)		x		•	

Data primarly from Lewis (1962), Bodenheimer (1958).

### MATERIALS AND METHODS

Experimental Technique

#### A. Primary Infections

Brood capsules were collected from hydatid cysts found in naturally infected donkeys, camels, sheep, cattle and man. A few scoleces were retained for taxonomic study while the remainder were examined microscopically for viability, using flame cell motility and muscular contraction as criteria. These were then used experimentally. It was possible to feed scoleces and cyst membranes to some immature dogs. Other untractable animals, both immature and adult were force-fed with gelatin capsules filled with scoleces, whereas wild carnivores were anaesthetized and the scoleces administered by stomach tube.

Worms were removed from infected dogs by scraping the small intestine with the edge of a spatula blade. Some worms were retained for taxonomic study, while eggs were collected from the remainder by grinding. Ova were divided into appropriate portions and force-fed to donkeys, camels and lambs. The monkeys and rodents were exposed by mixing the eggs with food before feeding. At necropsy, the carcasses were examined

grossly, while the liver, lungs, kidneys, heart and spleen were usually cut into strips for an accurate count of the total number of cysts.

### B. Secondary Infections

Scoleces from donkey, camel and cattle cysts were examined for viability and an estimation of their number was made from 3 replicate samples. A suspension with a known number of scoleces was then injected intraperitoneally into immature 40 to 45-day-old rabbits, mice and rats. The animals were necropsied at various times and the susceptibility and rates of infections were noted and compared.

### Taxonomic Technique

Hook counts and measurements were made from both fresh and preserved material under oil immersion. The cystic scoleces and rostella of adults were placed on a slide and pressed by a glass coverslip.

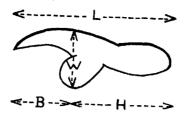


Fig. 1. Hook measurements. Total length, L; Blade length B; Handle length H; and Width, W.

Adults to be stained were allowed to relax in distilled water and then fixed with 10% formalin. Several stains were used but the best results were obtained with a combination of Erlich's and Delafield haematoxylin (Van Cleave, 1953). The worms were dehydrated in an ethyl alcohol series, cleared in xylol and mounted in Canada Balsam.

### NATURAL INFECTIONS

### A. Camel, Donkey and Horse

Camels and donkeys were examined to ascertain the rate and incidence of hydatid infections. Although the camels were examined at the Damascus abattoir, during their lives they would have ranged over a wide region of Syria. All but one was at least one year old. The donkeys ranged from 4 to about 6 years old and were purchased from inhabitants and Bedouins around the villages of Khider, Shmistar and Labwi, located in the Beka valley of Lebanon.

Table III shows that both the 100% incidence and infection rate of about 8 cysts per positive animal in camels were approximately twice that seen in donkeys. The cysts were mostly fertile with some undergoing degeneration. Many camel cysts had unusually thick, calcified adventitia which were not seen in the cattle, donkey or sheep cysts. Also no infertile, non-infected cysts, as is common in cattle, were seen in either camels or donkeys. Seventy percent of the infections in the camel were pulmonary while this high percentage occurred in hepatic infections in donkeys. One cyst found in the

mesentary, a rare site of infection, measured 5x5 cm.

A preserved horse lung containing a very heavy pulmonary infection with some cysts as large as 10 cm. was observed at the veterinary experimental station operated by the Free University of Berlin, Damascus, Syria. According to the owner it had never been out of Syria. This infection differed from that in English horses reported by Williams and Sweatman (1963) where 91 positive cases revealed only liver cysts but no pulmonary infections.

### B. Stray Dogs and Wild Animals

As shown in Table IV, 31% of 71 Beirut dogs were infected with E. granulosus. Although the samples from the 3 Lebanese villages were smaller than from Beirut, some dogs were infected, suggesting that the cestode may be widespread in Lebanon.

Certain wild animals which could be considered possible hosts for the adult or metacestode stage of <u>E. granulosus</u> exist in Lebanon and Syria. A survey of these animals (See Table V) was made to determine what role they played,

if any, in the transmission of the disease in these countries.

Table III

COMPARISON OF HYDATID INFECTIONS IN CAMELS AND DONKEYS

EXAMINED IN SYRIA AND LEBANON

HOST	MATURITY	No. EXAMINED	No. POSITIVE	TOTAL No. OF CYSTS	LOCATION LUNGS LIVER OTHER
CAMEL	Adult Calf	31 1	31 (100%)	254	205 48 1 (Spleen) (71%) (28.7%) (0.3%)
DONKEY	Adult	42	24 (60%)	93	28 64 1 (Mesen- (30.1%) (69.8%) tary) (0.1%)

Table IV

STRAY DOGS EXAMINED FOR E. granulosus IN LEBANON

LOCALITY	TOTAL No. EXAMINED	No. POSITIVE	PERCENT
Beirut	71	22	30.9%
Hermel	15	2	13.3%
Halba	4	2	50.0%
Nabatieh	10	2	20.0%
	·		
Total	100	28	

Of the 9 carnivores and 21 other mammals listed in Table II, there are 3 of the former (<u>Vulpes vulpes</u>, <u>Meles meles</u> and <u>Felis domesticus</u>) and 15 of the latter which are seen commonly. Opportunity was taken to examine the 3 common carnivores for adult worms and 9 of the other species of mammals for hydatid cysts (Table V). Only one infection was seen. This occurred in a wolf (<u>Canis lupus</u>) taken at Schmistar, Lebanon.

Table V ANIMALS EXAMINED FOR ECHINOCOCCUS

	SPECIES	LOCALITY	No. EXAMINED	No. WITH E. granulosus		
Wolf	Canis lupus	Schmistar, Lebanon	1	1		
Fox	Vulpes vulpes	Kartaba & Balbeck,	65	0		
Badger	Meles meles	Lebanon Kartaba, Lebanon	51	0		
Stone Marten	Martes foina syriaca	Kartaba, Lebanon	10	0		
Feral Cat	Felis domesticus	Throughout Lebanon	22	0		
Hedgehog	Erinaceus europeaus	Throughout Lebanon	30	O		
Coney	Procavia capensis syriaca	Kartaba, Lebanon	27	0		
Porcupine	Hystrix indica	Kartaba, Lebanon	14	0		
Mole Rat	Spalax ehrenbergi	Schmistar, Lebanon	17	0		
Tristrams Jird	Meriones tristrami	Throughout Lebanon,	54	0		
Levant Vole	Microtus guentheri	Palmyra, Syria Beka valley, Lebanon	36	0		
Black Rat	Rattus rattus alexandrinus	Beirut, Lebanon	12	0		
Brown Rat	Rattus norvegicus	Beirut, Lebanon	95	0		
Wagner's Gerbil	Gerbillus dasyurus dasyurus		21	0		
Levantine Field Mouse	Apodemus mystacinus	Palmyra, Syria Faraya Lebanon	11	0		
Common Field Mouse	Apodemus sylvaticus	Nahr el Kelb, Lebanon	1	0		
		No. Exam	ined N	o. Infected		

### EXPERIMENTAL INFECTIONS

### A. Biological Comparison

a. Secondary infection experiments using hydatid scoleces of cattle, camel and donkey origin.

Secondary infections have previously been used as biological criteria for taxonomic separation at the subspecific level (Sweatman and Williams, 1963; Williams and Sweatman, 1963). Using this criterion, it is possible to separate E. g. equinus from E. g. granulosus. Secondary cysts of the former subspecies develop in white mice and white rats but not rabbits, while E. g. granulosus develops secondarily in white mice and rabbits but not rats. Two other subspecies E. g. borealis and E. g. canadensis have also been separated by secondary growth in white mice. In E. g. borealis development of cysts is rare and slow while in E. g. canadensis there is only abortive or no growth.

As shown in Table VI, secondary cysts developed in immature white mice following the injection of 3,000 to 12,000 scoleces from primary cysts taken from the cow, camel and donkey. Autopsies were performed from 108 to 119 days and cysts ranged from 1 mm. to 10 mm. at 115 and

117 days with material from the 3 sources. Many cysts were fertile. Those cysts from a donkey source differed from the others in that they possessed hard, opaque walls (Plate 1) around vesicles containing brood capsules and scoleces. The cysts from the other sources had a translucent vesicular wall (Plates 2 and 3). Two fertile cysts measuring 9x10 mm. and 5x7 mm. at 108 days were found in one rabbit injected with scoleces of camel origin, while those exposed to cow and donkey material were negative. White rats were injected only with scoleces from camels and were found negative at autopsy. The rabbit and mouse infections suggest that the type of hydatid in camels may be related to E. g. granulosus, while the negative results in the rats injected with scoleces from camels also indicates an affinity to E. g. granulosus.

## b. Primary infections with scoleces

Hydatid cysts collected from cattle, sheep, camels, donkeys and man from Lebanon and Syria were fed to a total of 26 dogs, 8 foxes (<u>Vulpes vulpes</u>), 9 badgers (<u>Meles meles</u>) and 8 domestic cats.

Table VI

SECONDARY INFECTION EXPERIMENTS IN THE ,IMMATURE WHITE MOUSE, RAT AND RABBIT

SOURCE OF	WHITE MOUS	3E	RAT	r	RABBIT		
SCOLECES 1	No. EXPOSED	No. POSITIVE	No. EXPOSED	No. POSITIVE	No. EXPOSED	No. POSITIVE	
Camel	7	5	4	0	3	1	
Cow	4	3	-	-	2	0	
Donkey	4	3	••	-	3	0	

Table VII

INFECTION EXPERIMENTS IN CARNIVORES USING HYDATID MATERIAL FROM DOMESTIC ANIMALS AND MAN FROM LEBANON AND SYRIA

SOURCE OF CYSTS	EXPERIMENTAL ANIMAL	No. EXPOSED	DAYS INFECTED	No. POSITIVE	No. GRAVID
CATTLE	dog fox badger cat	6 2 2 2	90-100 2,10 2,10 2,10	6 0 0	4 0 0 0
SHEEP	dog fox badger cat	3 2 2 2	90-100 2,10 2,10 2,10	3 0 0 0	1 0 0 0
CAMEL	dog fox badger cat	9 2 3 2	79-100 2,10 2,3,10 2,10	9 0 0	6 0 0
DONKEY	dog fox badger cat	7 2 2 2	90-180 2,10 2,10 2,10	6 0 0	0 0 0
MAN	dog	1	102	O	0

As shown in Table VII foxes, badgers and cats were refractive to material from all sources. The pre-autopsy

period ranged from 79 to 102 days in dogs fed on material from cattle, sheep, camel and man and up to 180 days in those fed on donkey cysts. Over were seen in some stools at 7 weeks in the case of one camel-dog infection, but not all dogs became infected following ingestion of scoleces from this source of from cattle, sheep, or human sources even after 11 to 14 weeks. But in no case did any dogs fed hydatid cysts from donkeys have gravid worms, some of which were held as long as 25 weeks. The failure to produce gravid worms in dogs fed donkey material is not understood.

c. Primary infections using eggs of cattle-dog, camel-dog origin

Eggs from cattle-dog and camel-dog sources were fed to sheep, camels and donkeys, toque monkeys, and 2 species of rodents to determine differences, if any, which may occur in their susceptibility to ova of these sources.

As seen in Table VIII the exposed sheep, camels and monkeys were infected while the donkeys and the rodents were refractive to ova from both sources. Faeces from the rodents were collected for a week following exposure and examined by the zinc sulfate floatation technique. Since

no eggs were seen, it seems likely that the eggs hatched in the rodents but failed to infect. Cyst size ranged from 1 to 3 mm. in the 31 and 48 day-old sheep infections (Plate 4) and up to 3 mm. to 5 mm. in the older camel and monkey infections (Plate 5). Fulmonary cysts were more numerous in the camel infections while the majority were hepatic in sheep and monkeys.

These experiments suggest that the <u>Echinococcus</u> from the cattle and camel sources may be biologically identical. The fact that the donkeys were refractive is suggestive of <u>E. g. equinus</u> of horse-dog origin seen in Great Britain.

The refractive nature of the rodents, together with the absence of hydatid cyst in wild animals noted previously, suggests that any sylvatic cycle involving these is unlikely.

Table VIII

EXPERIMENTAL PRIMARY INFECTIONS USING HYDATID EGGS
FROM CATTLE-DOG AND CAMEL-DOG
ORIGIN

SOURCE OF EGGS	EXPERIMENTAL AN IMAL	APPROXIMATE No. OF EGGS	PERIOD OF INFECTION (DAYS)	No. OF CYSTS RECOVERED
Cow-dog	sheep	5,000	38	158
	sheep	2,000	31	86
	camel	5,000	85	43
	donkey	6,000	85	0
	monkey	2,000	84	Numerous
	Meriones tristrami	2,000	82	0
	<u>Meriones</u> tristrami	2,000	82	0
	Microtus guentheri	2,000	82	0
	Microtus guentheri	2,000	82	0
Camel-dog	sheep	100,000	49	267
	sheep	100,000	49	342
	camel	100,000	49	Numerous
	donkey	100,000	49	0
	monkey	100,000	41	Numerous
	monkey	50,000	41	<i>L</i> <sub>+</sub>
	Meriones tristrami	100,000	39	0
	<u>Meriones</u> tristrami	50,000	39	0

### B. Morphological Comparison

Tables IX, X, Figures 2 through 26, and Plates 6 to 11 show observations on the morphological features of Echinococcus from cattle, camel and donkey in Lebanon and Syria with additional comparative information on material from India and Ceylon. Adult worms were recovered from experimental cattle-dog, camel-dog and donkey-dog infections after 3 months of development.

### Sheep, Cattle Source

Rostellar Hooks. Cystic hooks from the cow and sheep are virtually identical (Plates 6, 8). The maximum number of hooks from both sources, reaches 39 (Table IX) and the range, mean and standard deviation of total length are similar in both hosts for large and small hooks (Fig. 2). No incomplete third row of tiny hooks or any distinguishing characteristics of the hook shapes occur in either source (Figs. 4, 6).

Strobila. The total length of the strobila is 4 to 5 mm., usually the latter. The usual number of proglottids is 3, with 4 seen commonly and 5 only occasionally. When 3 proglottids are present they consist of an immature, mature and a terminal gravid segment. In these worms with 4 segments, a second immature

Table IX

THE NUMBER OF ROSTELLAR HOOKS FROM HYDATID CYST SCORECES

OF DIFFERENT HOSTS

HOST	SOURCE	No. OF CYSTS	No. OF SCOLICES EXAMINED	RANGE AND MEAN OF LARGE HOOKS (µ)	RANGE AND MEAN OF SMALL HOOKS (µ)	RANGE AND MEAN OF TOTAL No. OF HOOKS	MAXIMUM No. OF THIRD ROW TINY HOOKS
Cow	Lebanon	3	20	15-20 (16.6)	12-20 (15)	27-39 (30.5)	O
Sheep		3	25	16-23 (19)	10-19 (15)	31-39 (34)	0
Camel	Syria	3	20	17-24 (19)	15-21 (17)	36-52 (40)	9
Donkey	Lebanon	3	45	13-22 (16)	10-19 (13.5)	23-37 (30)	6
Donkey	Syria	_	20	15-22 (20)	15-19 (17)	35 <b>-4</b> 0 (36)	0
Horse	Syria	4.	20	12-19 (16.4)	13-18 (16.1)	29-35 (32.7)	10
Man	Lebanon	2	20	15-25 (19)	14-22 (17)	29-47 (37.1)	4.
Rh <b>es</b> us Monkey	India	2	20	17-22 (20)	17-24 (21)	36-48 (41.2)	10
Cow	Ceylon	?	21	1 <b>6-</b> 22 (18)	13-18 (15)	29-40 (33)	4
Sambor Deer	Ceylon	?	20	16-19 (17.5)	16-19 (17)	32-37 (34.5)	o

Table X

# COMPARISON OF MORPHOLOGICAL CHARACTERISTICS OF ADULT E. GRANULOSUS FROM EXPERIMENTAL AND NATURAL INFECTIONS

SOURCE N	lo.		o. Of Segments	TE No.	STES DISTRIBUTION	CII AVERAGE SIZE IN (	RRUS SHAPE µ	LOCATION OF GENITAL PORE
SYRIAN CAMEL- DOG	20	2.5-4mm. (3)	3-4 (3)	21-33 (27)	Primarly posterior to genital pore	ll0x70μ	Pyriform horizontal or tipped slightly anterior	Anterior to middle segment
LEBANESE DONKEY- DOG	20	2-3mm. (2.5)	3	34-50 (39)	Anterior third of segment to posterior to vitelline gland	100 <b>x87</b> μ	Round to pyriform, tipped anterior	Middle of segment to slightly posterior
LEBANES COW-DOG	SE 20	4-5.5mm. (5)	3-5 (3)	35-52 (40)	About equally distributed from anterior margin to posterior to vitelline gland	1 <b>71</b> x9 <b>6</b>	Pyriform and horizontal	Posterior to middle of segment
HERMEL DOG	20	4.5-7.5 (5.75)	3 <b>-4</b> (3)	33-45 (42)	Equally dis- tributed anters to genital pore 2 rows common below vitelline gland	93x74	Round and tipped acutely anterior	Middle or slightly posterior of segment
WOLF	1	2.75mm.	3	43	Equally dis- tributed anteri to posterior	ior 140x10	Pyriform 00 and horizontal	Middle of segment

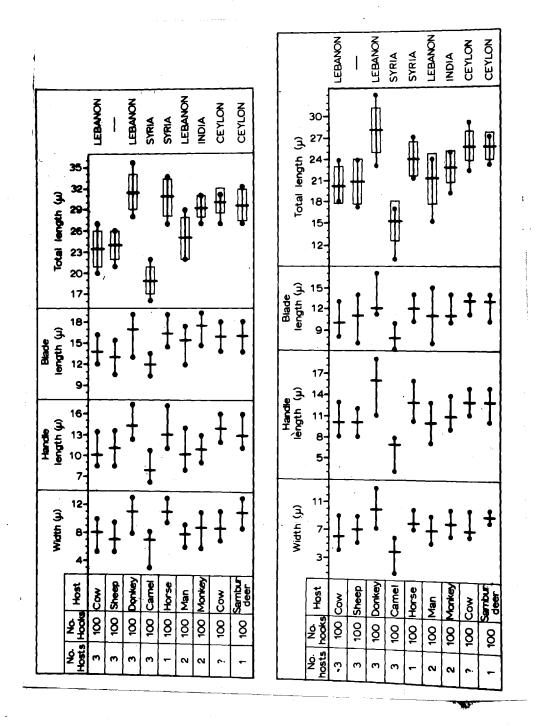


Fig. 2.- The total length, blade length, handle length and width of large (left) and small (right) cystic hooks of Echinococcus from Lebanon, Syria, India and Ceylon. The box around the mean denotes the distance of two standard deviations on either side of the mean.

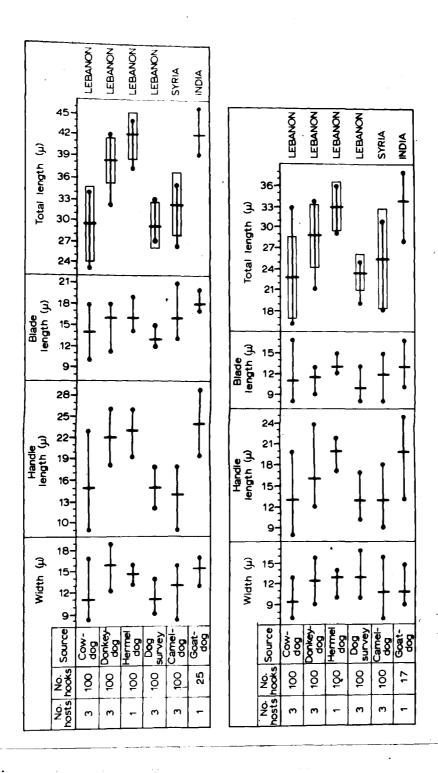
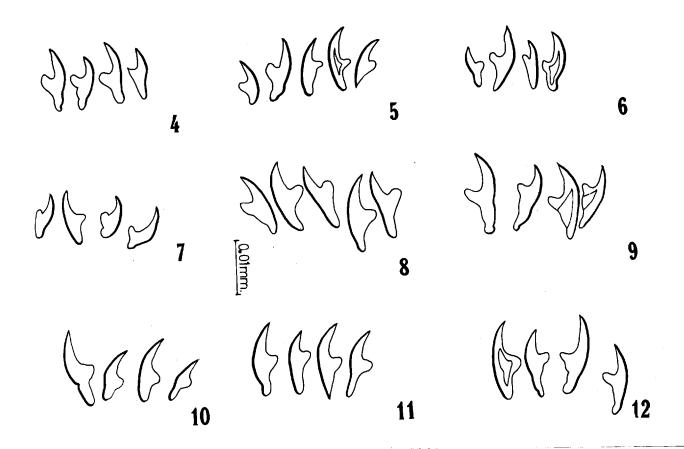
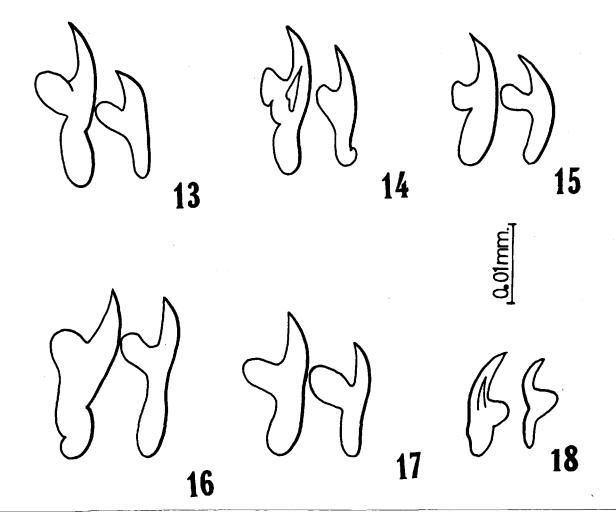


Fig. 3.- The total length, blade length, handle length and width of large (left) and small (right) adult hooks of Echinococcus in experimental and natural infected hosts from Lebanon, Syria and India. The box around the mean denotes the distance of two standard deviations on either side of the mean.



Representative shapes of some large and small cystic hooks from various hosts. Fig. 4. Cattle from Lebanon. Fig. 5. Camel from Syria. Fig. 6. Locally slaughtered sheep. Fig. 7. Man from Lebanon. Fig. 8. Donkey from Lebanon. Fig. 9. Horse from Syria. Fig. 10. Rhesus monkey from India. Fig. 11. Cattle from Ceylon. Fig. 12. Sambur deer from Ceylon.



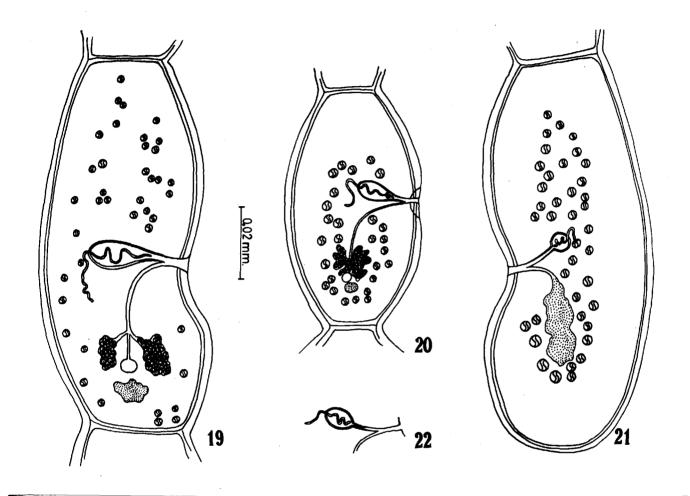
Figs. 13-18.- Some common morphological features seen in adult large and small hooks from experimental and natural infections.

Fig. 13. Naturally infected dog from Hermel, Lebanon.

Fig. 14. Donkey-dog origin. Fig. 15. Camel-dog origin.

Fig. 16. Indian goat-dog origin. Fig. 17. Cattle-dog origin.

Fig. 18. Sample of hooks from naturally infected dogs collected during survey.

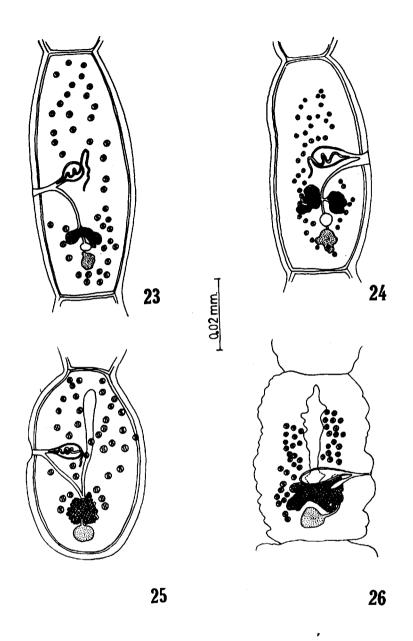


Figs. 19-22.- Fig. 19.- Mature proglottid of E. granulosus of cattle-dog origin.

Fig. 20.- Mature proglottid of  $\overline{E}$ . granulosus of Syrian camel-dog origin.

Fig. 21.- Mature proglottid of a non-gravid  $\underline{E}$ . granulosus of donkey-dog origin.

Fig. 22.- Pear-shaped cirrus also seen commonly in the donkey-dog mature proglottid.



Figs. 23-26.- Fig. 22.- Mature proglottid of E. granulosus from a naturally infected dog from Hermel, Lebanon. Note the round anteriorly tilted cirrus sac together with the occurrence of two rows beneath the vitelline gland.

Fig. 24.- Mature proglottid of  $\underline{E}$ .  $\underline{granulosus}$  from a naturally infected wolf from Lebanon.

Fig. 25.- Mature proglottid of Echinococcus from a 39 day old experimental infection of Indian goat-dog origin.

Fig. 26.- Mature proglottid of Echinococcus from a naturally infected jackal from Ceylon.

proglottid is seen. Where 5 proglottids exist, the first 2 segments are immature followed by 2 mature proglottids in different stages of development, usually with the fourth proglottid showing incipient atrophy of the genitalia, and with number 5 being gravid.

The number of testes ranges from 35 to 52 and are distributed from the anterior margin to below the vitelline gland posteriorly, with the majority commonly being anterior to the genital pore. The pear-shaped cirrus sac extends horizontally to the middle of the segment and has an average size of  $171 \times 96 \times 10^{-3}$ . The vagina lopes posteriorly between the ovarian branches to enter the seminal receptacle below the anterior margin. The seminal receptacle is round while the vitelline gland is large and bizzare shaped with an irregular margin.

# Syrian Camel Source

Rostellar Hooks. As shown in Table IX the maximum number of cystic hooks observed in this study occurs in the camel. The incomplete third row of tiny hooks is also most common in this host, occurring in 75% of those examined. These tiny hooks measure 6 to 12 long, with most of the growth in the blade.

Another feature of the cystic camel material is the total length measurements of the large and small hooks which are considerably

smaller than those from other sources (Figs. 2, 5). Morphologically typical cystic hooks appear in Fig. 5. Plate 8. The large and small hooks from adult camel-dog worms show mean lengths of 22µ and 27µ respectively which is slightly higher than that shown for the cow-dog source (Fig. 3). The morphological appearance of the adult hooks is slightly different from the cow and other local sources in that the handle of the small forms have a bent appearance and the gnard is less pronounced than in the cattle-dog material (Fig. 15. Plate 9).

### Strobila.

The total length of the strobila is 2.5 to 4 mm. with 3 mm. being the most common (Table X). Usually 3 proglottids are seen, the immature, mature and gravid. Occasionally 4 segments are present in which case the first and second are immature followed by the mature and gravid proglottid. In the mature proglottid the testes are of a large size and are comparatively fewer than those from cattle-dog and donkey-dog worms, ranging from 21 to 33 of which about two-thirds occur posteriorly to the genital pore. These lie in a lateral line along the female genitalia and form a single row posterior to the vitelline gland. The remaining anterior testes are relatively few in number (3 to 10) and rarely reach the anterior third of the proglottid (Fig. 20). The cirrus sac is pear-shaped and lies horizontally

or in some cases it is tipped slightly anteriorly. The average cirrus size is  $110x70\mu$  and it commonly extends to the middle of the segment or slightly less. The vagina slopes gradually posterior after leaving the genital atrium and passes through the istmus of the irregularly branced ovary to connect dorsally with a round seminal receptable. The vitelline gland stains as an irregular ovoid mass just posterior to the seminal receptable.

The adult morphology of the cattle-dog and camel-dog worms is similar in hook size, shape of cirrus, location of genital pore and both commonly have 3 segments, but differ primarily in cystic hook measurements and the number and distribution of testes.

### Lebanese Donkey Source

Rostellar Hooks. Cystic hooks from the donkey and horse of Lebanese and Syrian origin were compared as to the total number, length and morphology. There is virtually no difference between the two donkey sources while the Syrian horse material shows two distinct types (Figs. 8, 9. Plate 10). One of these is an up-turned handle on the posterior end and another where it appears to be just an extension of the blade with no up-turned process present. When cystic and adult hooks from these equine sources are compared with those previously described for E. g. equinus Williams and Sweatman (1963) we find little similarity. Although the size

is consistently larger than those from cow-dog and camel-dog infections as well as the figures given for E. g. granulosus by Sweatman and Williams in 1963, but these fall short of E. g. equinus in maximum length by 8 in the donkey-dog large hook measurements. This may be explained by the lack of maturity in the worms from this source. No previous drawings of equine cystic hooks are available for comparison but several differences in the morphology can be seen in the hooks from adult worms. In the donkey the small hooks have a very distinct up-turning (Fig. 14. Plate 11) at the posterior end of the handle while none is shown for E. g. equinus. In the large hooks of E. g. equinus a characteristic notching and up-turning of the handle is described as a consistent feature. These were not observed in the donkey-dog material.

## Strobila

No gravid worms were recovered from dogs infected with viable scoleces of Lebanese donkey origin even after a 5 month period. Immature worms measured from 2 to 4 mm. with an average of 2.5 mm. The number of segments was always 3 when a terminal mature proglottid was present.

In the mature proglottid the total number of testes ranged from 34 to 50 of which more than half are found anterior to the

genital pore. The distribution extends from near the anterior margin to just posterior to the vitelline gland (Fig. 21). The cirrus sac has an average measurement of  $100x87\mu$  and can be either round or pyriform (Fig. 22). These are commonly tfited anteriorly and rarely extend beyond the middle of the segment. The vagina has a slightly more angular bend posteriorly than those seen in the cow-dog or camel-dog worms. Due to immaturity, the ovary, seminal receptacle and vitelline gland have not sufficiently differentiated and are seen as an irregular confluent structure that extends to the posterior third of the segment.

# MORPHOLOGY OF ADULT WORMS FROM NATURAL INFECTIONS IN LEBANON

### Hermel Dog Source

In the survey of Lebanese dogs (Table IV) one out of the 100 dogs showed unique worms. All the others, including worms from Iraq, from which the hooks had become detached and were not available for study, although examined only supperficially appeared identical with the classical <u>E. granulosus</u>. This one dog was taken from the village of Hermel in Eastern Lebanon. It revealed a number of comparatively large <u>Echinococcus</u> worms

reaching a total length of 7.5 mm. These also had long hook measurements reaching 44  $\mu$ . Aside from their large size the hooks have no distinguishing morphological features that would set it apart from other material (Fig. 13).

### Strobila

Although the total length of 20 worms reached a large maximum size the average is 5.75 mm. and only 3 or 4 segments are present. The most common number is 3, represented by the typical immature, mature and gravid proglottids. When 4 segments exist, 2 are immature. The number of testes is similar to those previously mentioned in the donkey-dog and cow-dog sources. The occurrence of several rows posterior to the vitelline gland (Fig. 23) and their persistence in the gravid proglottid is reminiscent of <u>E. g. equinus</u> as is the large hook size and does not coincide with our other observations on material of Lebanese and Syrian origin. This, along with the fact that the cirrus sac is more acutely tipped anteriorly and is commonly round instead of pyriform, indicates that these could possibly be gravid worms of a dog naturally infected from an equine source.

Some similarities exist between the immature donkey-dog Echinococcus and the worms from this natural infection. Both

have long hooks and a similar number of testes, location of the genital pore and an anteriorly tipped, round cirrus sac. This plus the absence of infections in donkeys fed ova of cowdog and camel-dog sources could indicate this to be the grawid form of the immature worm of donkey origin.

### Wolf Source

Two whole worms and 3 segments were recovered from a wolf taken in the Beka valley of Lebanon (See Table V). No hooks are available for study and only one intact worm could be used for morphological comparison. It consists of 3 segments measuring a total of 2.75 mm. Although no gravid segments are found, one of the free segments is seen to contain developing ova. Close examination of the intact worm showes jagged edges at the posterior periphery of the terminal proglottid indicating the possible loss of segment during preparation.

The testes number 43 and range from the anterior third of the proglottid to behind the vitelline gland and are approximately evenly distributed anteriorly and posteriorly to the genital pore. The cirrus sac measures  $122x61\mu$ , lies horizontal to the long axis and extends to the middle of the mature proglottid.

The visible female genitalia consists of the vagina passing between the two irregular lobes of the ovary and enters the round seminal receptacle at a sub-anterior level. The irregularly shaped vifelline gland is just posterior to the seminal receptacle (Fig. 24).

The characteristics in this specimen are indistinguishable from the experimental cow-dog or camel-dog infections.

### INFECTIONS FROM CEYLON AND INDIA

Preserved and mounted cystic and adult Echinococcus from a naturally infected cow, sambur deer and jackal from Ceylon also 2 rhesus monkeys (Macaca mulata) along with some 39-day-old adults from an experimental Indian goat-dog infection were examined morphologically for a comparative study.

# Rostellar Hooks

Cystic hooks were available from the monkey, cow and deer material and adult hooks from a 39 day old Indian goat-dog source but not from the adult jackal worms (Figs. 10,11,12,16).

The high total number of hooks in cysts from the monkeys (36 to 48) were second only to the camel material with the maximum number of incomplete third row tiny hooks being one of the highest. Material from cattle and sambur deer of Ceylon origin were very similar in total number of hooks to the local cow, sheep and donkey scoleces (Table IX). The morphology and measurements of hooks from the Ceylon and Indian sources showed some variation but for the most part are larger than the local material falling between the donkey and the sheep and cow figures (Fig. 2). Seven immature Echinococcus from a 39 day infection were examined. The fixation process used in these immature worms and the jackal specimens was not the same as the method used in this study. Hooks from the immature worms of Indian goat-dog origin were the largest hooks examined arranging up to 45.5 in the large hooks to 38 in the small (Fig. 3). The gross morphology of these hooks show a different pattern than do those from the other sources. The small hooks have a very long handle while the larger hooks have a turning under of the distal posterior end forming a small ventral notch (Fig. 16).

All worms are 2 to 3 mm. long and consist of 3 segments of which the first 2 are immature. In the terminal mature proglottid the testes range from 36 to 41, with the majority being found

anterior to the genital pore and never extending past the ovary posteriorly. The cirrus sac is horizontal, pyriform and measures  $110x66\mu$  in size. In the female genitalia the seminal receptable is not seen but it is assumed it is located behind the ovary instead of in a more posterior location as those previously described (Fig. 25).

### Jackal Source

From the jackal material only one intact worm was able to be used for morphological study. This worm was 3.5 mm. in length and had 4 segments, 2 immature, a mature and terminal segment which was presumably gravid. Due to the thick and highly contracted musculature the internal structures of this segment prevented the observation of eggs in this segment. However, in other single gravid segments fully developed ova were visible. The mature segment contains 44 testes arranged in rows parallel to the developing uterus. More than half are anterior to the genital pore and like the above worms extend posteriorly only as far as the posterior portion of the ovarian lobes. The pear shaped cirrus is unusually long and extends more than half way across the segment (Fig. 26).

The female genitalia is similar to the goat-dog material

in that the seminal receptable is apparently located behind the ovary and could not be seen.

The similarities between these experimentally produced worms from a domestic animal source and those taken from the jackal as well as monkey infections may indicate that this is not a sylvatic situation involving both wild definitive and intermediate hosts. However further investigation is needed in these areas to clarify these relationships.

These morphological observations on hooks and strobilate worms demonstrate that the donkey-dog worms can be separated from both the cow-dog and camel-dog material. The cow-dog and camel-dog worms are essentially the same morphologically. Sheep, camels and toque monkeys were more or less equally susceptible to ova from both sources, suggesting their biological similarity. These data demonstrate that it is <a href="Echinococcus granulosus granulosus">Echinococcus granulosus</a> granulosus that infects both cattle and camels. The presence of an incomplete third row of tiny hooks and the small size of the cystic large and small hooks in the camel material may indicate that it is an abnormal host. This same situation was shown to occur in known abnormal hosts by Sweatman and Williams (1963). It follows that camels may have become hosts of E. g. granulosus following their association with sheep brought about by the

activities of man during historic times.

The donkey material differs from the above in morphology, host susceptibility and secondary cyst development. It appears similar to Echinococcus granulosus equinus even though it did not successfully infect dogs experimentally. Not all dogs, however, were infected with material from cattle or camels either. The difference appears to be primarily related to factors associated with the dogs rather than the parasite. Nonetheless, it is very unlikely that any other carnivore in Lebanon could be the definitive host of the donkey cysts.

The significance of the material from Ceylon and India is difficult to assess. The cystic hooks from the cow, rhesus monkey and sambur deer are almost as large as hooks from the Lebanese donkey material which are distinctly larger than those from local cattle and sheep. Like the cow infection unusually large hooks were noted previously (Williams and Sweatman, 1963) in material from British cattle. Also noteworthy in the Indian material is the large size of the hooks on the goat-dog worms which are more consistent with the donkey-dog than the bovid-dog or camel-dog worms. The mature proglottids of the Ceylon and Indian forms show similarities to each other which are not seen in local worms. The taxonomic significance of these morphological differences, however, must await critical biological comparisons.

### EPIDEMIOLOGY

Sheep and goats are the most common ungulates in Lebanon and Syria while cattle occur less abundantly. The prevalence of hydatid cysts in Lebanon and Syria as reported by Pipkin, Rizk, and Balikian in 1951 ranged from 6.6% to 44.1% of 7.019 sheep, 45% of 715 cattle and 67.4% to 100% of 49 camels. In Beirut, the adult worm was found in 11.75% to 33% of 967 dogs examined by the same authors. The figures for intermediate hosts have probably not changed appreciably during the intervening years, and our 31% incidence in dogs is similar to theirs. Recently Luttermoser and Moussa (1961) reported 30% of 300 pigs infected in Lebanon. Pigs are raised in Lebanon (being a Christian as well as a Moslem country) but are not found commonly in Syria or any of the other predominately Moslem countries of the Middle East. The swine infections demonstrate an indigenous hydatid cycle in Lebanon. By contrast, many of the locally slaughtered bovids, particularly sheep and goats, are raised in Syria and driven overland into Lebanon. Also large numbers of sheep and cattle as well as buffaloes are imported by sea from Turkey, Greece and Yugoslavia. Reliable infection records in bovids raised in Lebanon are therefore virtually impossible to obtain.

Conditions of slaughter in Lebanon and Syria, however, presuppose a dog-sheep cycle. In abattoirs the butchering practices undergo only rudimentary meat inspection procedures. Trimmings are generally flushed out with the wash-water into an adjoining stream, directly into the sea, or sometimes into a large pit behind the abattoir. Many hydatid cysts are disposed of in this way. These effluents attract scavenging animals, particularly dogs, as shown in Plate 12. Outside some abattoirs, uncut lungs and livers of sheep are sold from carts (Plate 13) while other parts (head, omentum, etc.) are sold from the cemented ground (Plate 14). Some hydatid infections are most probably carried to the household by this means.

Besides abattoirs, individual shops (Plate 15) butcher a few sheep more or less daily. This is particularly true in smaller villages which do not have ready access to city abattoirs. Trimmings are disposed of in various ways, often being fed directly to dogs waiting nearby. Stray dogs which feed promiscuously are common in Lebanon and Syria, particularly in villages and at the periphery of cities. The high incidence of 28% reported for Lebanon in this study, like that of 38% noted by Babero and Al-Dabagh (1963) in Iraq, is undoubtedly related to the habits of the dogs and rudimentary methods of slaughtering food-animals. However, other sheep and goats

killed by the Bedouins at their tent sites, and animals that die in the fields no doubt also serve as a source of infection for scavenging dogs, jackals and wolves.

Camel meat is sold as an inferior type in Syria and less commonly in Lebanon. In Syria, where the demand is high, many animals are butchered in abattoirs and the infected trimmings handled in the same manner as noted previously for sheep.

In spring and summer, camels range over wide areas in search of adequate pasture, reaching as far as Iraq and Jordan. During the winter, camels are more sedentary, often feeding on land used for hay and other crops in summer. These migratory habits are facililated by almost complete absence of fences. The very high incidence (97%) and rate of infection of hydatid cysts in Syrian camels indicates that animals which die in agricultural or semi-desert regions could be an important source of infection for scavenging animals. The infection of E.g. granulosus in a wolf, together with the fact, that the subspecies E.g. granulosus in camels is the same as that in bovids, further enhances the chances of survival and dissemenation of the parasite.

The donkey is the most abundant beast of burden in Lebanon

and Syria but occurs less commonly than sheep and goats. Donkeys can be seen in virtually every Bedouin campsite, small village and large city. Donkey and horse meat is not sold for human consumption in these countries, and except for the hide, appears to have no commercial value. Consequently, these animals are not slaughtered in abattoirs, instead when they become of no further value, are left at the side of the road or driven outside the village to die. No apparent attempt is made to burn or bury the carcasses. Dogs have been seen feeding on a donkey carcass in southern Syria. It is apparent from the 60% incidence and high rate of infection in donkeys noted in this paper, together with their longevity and method of disposal, that infective material is accessable to scavenging carnivores. In the worms of donkey-dog origin a morphological relationship can be seen between them and E. g. gquinus. This fact plus the dog infected with worms similar to, if not identical to, E. g. equinus suggests that dogs may be the natural hosts.

Since the wolf and jackal are uncommon in Lebanon (Table II) the actual amount of hydatid disease spread by these is probably minimal. Experimental infections as well as a wild animal survey failed to reveal any wild rodents or other for-

aging animal with the metacestode stage. This along with our inability to infect foxes (<u>Vulpes vulpes</u>) or badgers (<u>Meles meles</u>) with hydatid material from cattle, camel and donkey sources, demonstrates that the dog is the significant definitive host and that domestic herbivores are the primary intermediate hosts of the parasite in Lebanon and Syria and play the major role in the epidemiology of hydatid disease in these countries.

### CONCLUSIONS

- 1. The comparison of hydatid infections in 32 camels and 42 donkeys examined from Lebanon and Syria showed an incidence of 100% in adult camels and 50% in adult donkeys. Of these 71% were pulmonary and 28.7% were hepatic in camels while in donkeys 30.1% were in the lungs and 69.8% were found in the liver.
- 2. Natural infections were found in 28 of 100 dogs examined from Beirut, Hermel, Halba and Nabatieh, Lebanon.
- 3. One wolf from Schmistar, Lebanon, was found with worms that appeared identical with E. g. granulosus. None of 466 other wild animals were positive for either the strobilate or metacestode stage.
- 4. Dogs fed hydatid material from the cow, sheep or camel produced gravid worms in some but not all individuals. A single dog was not infected following the ingestion of cystic material from man.
- 5. Dogs fed scoleces of donkey origin developed only non-gravid worms even after 6 months.
- 6. Foxes, badgers and cats were refractive to material of cattle, camel and donkey origin.
- 7. Attempts to infect immature white mice, rabbits and rats by intraperitoneal injection of scoleces of cattle, camel and donkey origin produced secondary infections in all white mice. Rabbits were only susceptable to the camel material, and negative to that of cattle and donkey origin. White rats were infected only with material of camel origin and all were negative.
- 8. Infections using eggs of cattle-dog and camel-dog origin showed comparable infection rates in sheep, camels and monkeys but failed to infect donkeys and 2 species of wild rodents.

- 9. Cystic large and small hooks from 6 host species from Lebanon and Syria can be grouped to include (a) camel with very short hooks (b) cow, sheep and man with distinctly longer hooks and (c) donkey and horse with still longer hooks.
- 10. An enlarged, turned-up end to the handle of the cystic hooks from the horse and adult hooks of donkey-dog origin is suggestive of E.g. equinus, but the notches in these ends so characteristic of E.g. equinus in the original description do not occur. Since the donkey infections produced only immature worms, it is not known whether the characteristic ends had yet to develop or whether the subspecies will require re-definition.
- 11. The strobila of the cow-dog and camel-dog worms are essentially similar to each other. Both the biological and morphological data suggest that the camel and cattle infections are referable to  $\underline{E}$ .  $\underline{g}$ .  $\underline{g}$   $\underline{r}$   $\underline{a}$   $\underline{n}$   $\underline{g}$   $\underline{g}$   $\underline{r}$   $\underline{a}$   $\underline{n}$   $\underline{g}$   $\underline{g}$
- 12. The strobila of the donkey-dog worms with (a) the testes distributed below the vifellarium (b) the tilting up of the cirrus sac, and (c) the round shape of the cirrus sac are characteristic of E. g. equinus. The refractory nature of exposed donkeys to eggs of camel-dog and cow-dog sources is also indicative of E. g. equinus.
- 13. One dog naturally infected with worms morphologically like <u>E</u>. <u>g</u>. <u>equinus</u> was collected at Hermel, Lebanon, while 27 other dogs were infected with <u>E</u>. <u>g</u>. <u>granulosus</u>.
- 14. The dog and domestic herbivores appear to play the major role in the epidemiology of hydatid disease in Lebanon and Syria.
- 15. Observations on Echinococcus from a jackal in Ceylon and an experimental goat-dog infection from India show common features with each other but differ in some details from the type of hydatid in Lebanon and Syria.

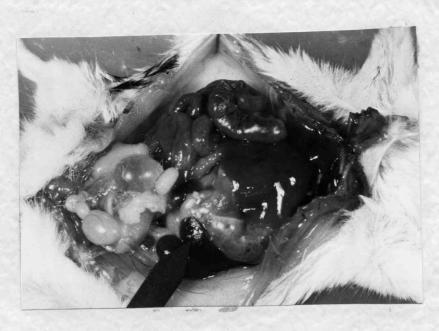


Plate 1. Secondary hydatid infection in a white mouse injected with scoleces of donkey origin. Note the non-translucent cyst walls.

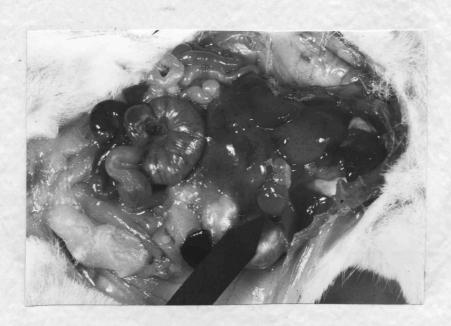


Plate 2. Secondary hydatid infection in a white mouse injected with scoleces of camel origin. Compare with Plate 1.



Plate 3. Secondary hydatid infection in a white mouse injected with scoleces of cattle origin.



Plate 4. Liver of sheep with a 48-day-old infection from ova of camel-dog origin.



Plate 5. Liver of monkey with a 84-day-old infection from ova of cattle-dog origin.

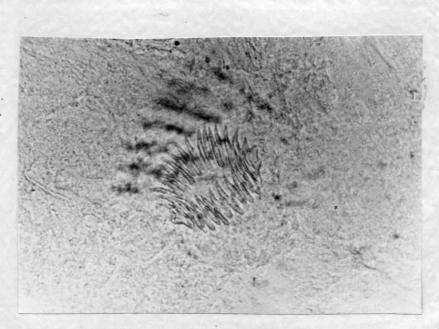


Plate 6. Large and small cystic hooks of cow origin.



Plate 7. Large and small hooks from a gravid E. granulosus of cow-dog origin.

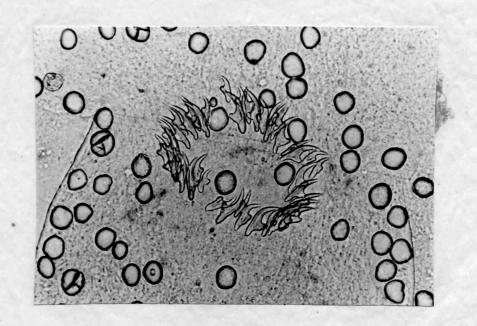


Plate 8. Large and small cystic hooks of camel origin.

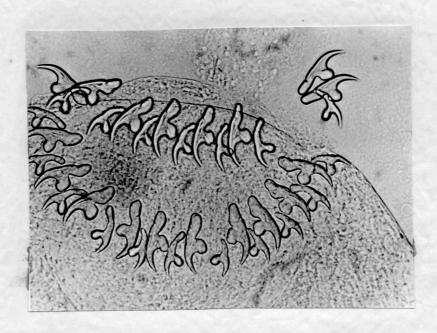


Plate 9. Large and small hooks from a gravid E. granulosus of camel-dog origin.

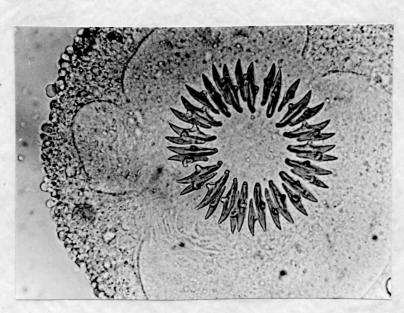


Plate 10. Large and small cystic hooks of donkey origin.



Plate 11. Large and small hooks from a non-gravid <u>E. granulosus</u> of donkey-dog origin.



Plate 12. Dogs scavenging for food in an effluent containing trimmings washed from the abattoir floor.



Plate 13. Lungs and livers of sheep being sold from a cart outside an abattoir.



Plate 14. Heads and omenta being sold from the cemented ground in front of an abattoir.



Plate 15. Butchering of a sheep outside a village shop.

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