OBSERVATIONS ON
THE MORPHOLOGY, BIOLOGY & PATHOGENICITY
OF
ECHINOCOCCUS GRANULOSUS (BATTISCHI, 1786)

Thesis

SUBMITTED TO
THE MAGADH UNIVERSITY
IN THE FACULTY OF VETERINARY SCIENCE
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF SCIENCE (Veterinary)

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B.V.Sc. (Agra)
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PATNA

1986

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1966

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Certified that the work described in this thesis entitled "OBSERVATIONS ON THE MORPHOLOGY, BIOLOGY AND PATHOGENICITY OF *Echinococcus granulosus* (Batsch, 1786)" is the bonafide work of Shri VIJAY SHANKER PANDEY, carried out under my guidance and supervision.

A K Varma
6.12.66

(A.K.VARMA)

A C K N O W L E D G E M E N T S

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(V.S.PANDEY)

A B S T R A C T

Title - Observations on the morphology, biology and pathogenicity of *Echinococcus granulosus* (Batsch, 1786).

This thesis incorporates the results of observations made on the incidence, morphology, biology and pathogenicity of *Echinococcus granulosus* of goat-dog origin.

1614 goats in different age-groups were examined at six slaughtering establishments at Patna and its suburbs for the hydatid cysts and 20 dogs for the adult stage of *E. granulosus*. The incidence of the hydatid cysts in found to increase with the age of the animals. It is as low as 0.33% in animals below one year and as high as 8.6% in animals above 3 years of age, the overall incidence being 6.5%. The organ-wise incidence with the intensity of infection and the variations in the size, shape and characters of the cysts, thus recovered, is recorded. The percentage of fertile cysts is found to be very high (77.7%). Two of the 20 dogs were found to be infected with the adult stage of the parasite.

Nine dogs and two cats were infected with scolices of hydatid cysts from goats. Twelve rabbits were exposed to 2000 mature eggs of *E. granulosus* orally and 12 guineapigs to 2000 scolices of hydatid cysts from goats I/p to study the development of primary and secondary hydatid cysts respectively

but they proved refractory.

The morphology of the worms, recovered from experimental dogs, was studied at different stages of development. Though some minor morphological and biological differences were observed, the worms are identified as Echinococcus granulosus.

The worms were found to be distributed upto the first four feet of the small intestine of experimental dogs, their maximum concentration being in the second feet.

The rostellar hooks, total body length and size of the different parts of the body have been observed to increase with the age of the worm, upto 117 days of infestation. The proportional growth at different ages of the worms has been studied. The prepatent period in 4 dogs has been found to be from 60 to 90 days. The cat does not seem to be a suitable host of E.granulosus,as only a few immature worms were recovered from experimental cats and that two without any visible sign of development of genital organs even at 42 days of infestation.

Observations have been made on the biochemistry of hydatid fluid and membrane. The specific gravity, pH, total solid (dry matter), inorganic substances (ash), organic substances, total, non-protein and protein nitrogen and the protein content of the fluid has been determined. The total solid and total nitrogen content of the hydatid membrane have

also been estimated.

The pathology of the larval stage of the parasite was observed in the liver, lungs and spleen from naturally infected goats. The gross pathological changes were dependant upon the number and size of the cysts. In heavy infections the external topography of the organs was greatly distorted. Microscopically the cyst wall has been found to be composed of two layers, around which there is a thick layer of dense fibrous connective tissue of host origin. The liver showed various degrees of cirrhosis, degenerative changes, disorganisation of hepatic cords and pressure atrophy. In lungs collapse and emphysema was a significant change. Changes in spleen were comparatively mild. Heavy infiltration with mononuclear cells, chiefly lymphocytes have been observed in all the organs especially around the pericystic region. Eosinophils, plasma cells and giant cells were rarely observed.

The clinical symptoms, observed in one of the experimental dogs were anorexia, emaciation and dysentery alternating with constipation.

C O N T E N T S

INTRODUCTION

INTRODUCTION

The genus Echinococcus is parasitic in adult stage in the carnivores. Its larval stage is also parasitic in herbivores, swine and man as hydatid cysts. Several species of this genus have been described but Echinococcus granulosus is universal in occurrence. As a result of its tremendous biotic potential and mode of propagation, it is a major source of economic losses and public health hazards.

Gemmell & Brydon (1960) has calculated the economic loss caused by the condemnation of carcases and offals from hydatid cysts alone around £ 600000 per annum in Australia. Although comparable statistics are not available for India, one can very well visualise the losses, as quite a fair number of food animals are found infected with hydatid cysts.

The joint W.H.O./F.A.O. Expert Committees on Zoonoses (1951,1959) and Meat Hygiene (1962) have recognised it as a world health problem and stressed the need for further research on this disease.

During the last decade morphology of this cestode has been studied in some detail but still there is much to be desired regarding its general biology and the effect of this parasite on the host.

Information on this disease in India is very scanty and there have been only occasional reports of its occurrence

in larval and adult stages here and there.

Keeping in view the importance of the goat as a meat animal in India, lack of precise knowledge on goat-dog material and complete absence of any report on experimental study in this country, it was considered worthwhile to undertake the present study notwithstanding the great human risk involved in maintaining the infected carnivores in the laboratory.

In the present study 1614 goats of different age groups have been examined in and around the city of Patna for the larval stage and 20 local dogs for the adult stage of the parasite. The gross and histopathology of organs infected with the hydatid cysts have been studied and some observations have also been made on the biochemistry of the hydatid fluid and its membrane. Pups and cats were infected with the goat material to study the biology and morphology of this parasite at different stages of development. Attempts were also made to produce primary and secondary echinococcosis in rabbits and guineapigs but they proved refractory.

A high incidence and fertility rate of hydatid cysts in goats and occurrence of adult stage of E. granulosus in local dogs is indicative of the possibility of the main cycle of transmission of echinococcosis between the goat and the dog in this locality.

The present study confines itself to the following items:-

(a) Morphology

(b) Biology and

(c) Pathogenicity.

Besides, it also incorporates incidental observations on the incidence of the parasite in the city of Patna in Bihar.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Historical

The cystic stage of this parasite was described by Goeze (1782) as Taenial socialis granulosa and by Batsch(1786) as Hydatigena granulosa (cited by Verster,1965). The genus Echinococcus was erected by Rudolphi in 1801. von Siebold (1852) proposed the name Taenia echinococcus for the adult worms from dogs. Since then 12 species have been described in the genus Echinococcus of which the two - Echinococcus granulosus (Batsch, 1786) and E.multilocularis Leuckart,1863; are well established ones on experimental evidences. Sweatman & Williams (1963), Williams & Sweatman (1963) and Verster (1965) have recently described six sub-species of E.granulosus viz; E. granulosus canadensis, E.g. borealis, E.g.equinus, E.g.newzealandensis, E.g.granulosus and E.g.africanus.

Incidence

The incidence of hydatid cysts in sheep and goats and adult E.granulosus in dogs in India, as reported by different workers from 1902 to 1963, is summarised in table no.22.

The organ distribution of hydatid cysts was found by Sharma & Chitkara (1963) as 33.3% in lungs only, 23% in liver only and 43.6% in lungs and liver both. None of the

workers has estimated so far the incidence in different age-groups and fertility-rate of the hydatids in goats in India.

Morphology

Recently this genus has been reviewed by Rausch (1953), Vogel (1957), Rausch & Nelson (1963) and Verster (1965). A summary of important morphological characters of E. granulosus, described by various workers, is presented in the table no.22.

Biology

(a) Distribution of worms in small intestine:

Yamashita et al. (1956b) found the worms distributed in the first 40-60 c.m. of the small intestine. Sweatman & Williams (loc.cit.) found them distributed in the first two-third of the small intestine. Babero et al. (1963) observed the worms distributed throughout the length of the small intestine.

(b) Growth and development:

Yamashita et al. (loc.cit.) using the sheep-dog material, recorded proportionate increase in dimensions during the development on the 16th, 35th, 135th and 375th day after infection, as follows:- Rostellum 1:1.4:1.5:2; Sucker 1:1.1:1.3:1.5; Large hooks 1:1.3:1.5:1.6; Small hooks 1:1.1:1.2:1.3; Length of strobila 1:3:4:8. Smyth (1962) described the sequence of maturation at 15th, 20th, 27th and 34th day of infection.

Smyth (1963) (cited by Smyth,1964) found the minimum development time for egg-production as 41 days.

(c) Prepatent period:

The prepatent period in dogs has been determined as 48-51 days by Ross (1936); 40-51 days by Chhoquette (1956); 64-97 days by Nosik (1954); 57 days by Drezancic & Wikerhauser (1956); 48-61 days by Yamashita et al.(loc.cit.) and 49-65 days by Sweatman & Williams (loc.cit.).

(d) Development in cat:

Cat was found completely refractory to experimental infection by Rosenbusch & Gelormini (1946), Hutchison & Bryan (1960) and Dailey & Sweatman (1965). However, Southwell(1927), Nosik (1954), Drezancic & Wikerhauser (loc.cit.), Gemmell (1959) and Verster (loc.cit.) recovered immature worms from experimental cats.

(e) Larval development in rabbits and guineapigs:

Sweatman & Williams (1962) have shown that the rabbits can be infected by ingestion of large number of E. granulosus eggs but Williams & Sweatman (loc.cit.) disagreed with this finding. Some workers have found the rabbits refractory to secondary echinococcosis of horse origin but susceptible to that of sheep origin (Bacigulapo, 1933; Deve, 1935,1938; de Waele & Pennoit-de Cooman,1938). Bacigulapo (loc.cit.) obtained similar results in guineapigs as well.

However, D

However, Dailey & Sweatman (loc.cit) could infect a rabbit by I/p injection of scolices of camel origin.

(f) Biochemical:

Carbone & Lorenzetti (1957) found 17.3-227 mg. protein per 100 ml. of hydatid fluid from man, sheep and cattle whereas Magath (1959) got only 7.5 mg. from man. Goodchild & Kagan (1961) recorded the nitrogen values in mg./100 ml. of hydatid fluid from bovine, pig, moose and man as 7, 34, 21 and 29 mg. Lapage (1962) has mentioned the sp.gr. of hydatid fluid as 1.017. Cmelik (1952) detected 4.5% nitrogen from the entire cyst wall which was later confirmed by Cmelik & Briski (1953).

Pathogenicity

(a) In larval stage:

The adventitious layer of hydatid cyst has two layers infiltrated with lymphocytes, few macrophages, eosinophils and plasma cells (Yamashita et al.,1956a,1957; Ahluwalia, 1960; Sweatman et al.,1962, 1963) and adjacent parenchymal tissue shows atrophy and degenerative changes. Dew (1925) observed a leading role of eosinophils instead of lymphocytes.

(b) In adult stage:

Chhoquette (loc.cit.) observed that three of his eight infected dogs developed diarrhoea, lost weight and died one month after the initial infection.

MATERIALS AND METHODS

MATERIALS AND METHODS

A total of 1614 goats (divided into 3 age-groups by an examination of teeth, viz. below one year, between one to three years and above three years) from six slaughter establishments in and around Patna, were examined during the period March to August,1966. These animals came from different districts of Bihar and eastern U.P. The dressed carcases were examined by visual inspection and palpation. Collections from each host were made separately and cysts were examined in detail in the laboratory.

2. Gross lesions were observed from freshly collected unpreserved organs. For histopathological studies 5-7/$^{\mu}$ thick sections were made and stained by Harris's alum haematoxylin and eosin (as a routine stain), van Gieson's and Gallego's iron fuchsin (as differential stains for connective tissue) stains.

3. The specific gravity of the hydatid fluid was determined by pycnometer method (Peters & Van Slyke,1958) and pH by Beckman electric pH meter. Total solid (dry matter) was determined by evaporating a weighed amount of the material to dryness at 105°C in hot-air oven for 24 hours. The inorganic substances (ash) were estimated by keeping a weighed and evaporated amount of the hydatid fluid in Muffle furnace at 500°C for 5 hours. The organic substance was calculated by

finding the difference between total solid and inorganic substances. Total nitrogen was determined by digestion of the hydatid fluid or membrane in micro-Kjeldahl apparatus by Arnold Gunning method, then distillation and titration by Meeker & Wagner (1940) method. For estimation of non-protein nitrogen (N.P.N.) the fluid was deproteinised by Somogyi's (1930a,b) method and then digested, distilled and titrated as above. The protein content was calculated on the basis of total nitrogen and non-protein nitrogen.

4. Experimental animals:- 9 pups, 2 cats, 12 rabbits and 12 guineapigs were used in this experiment. The details of the experimental design is summarised in table no.1a,b,c. The pups and cats were dewormed before starting the experiment.

5. Collection, fixation and preservation of the parasites:- After autopsy, the intestine was removed, cut into pieces of 1 foot each, opened and scraped. The worms were collected and counted after repeated washing and decantation. They were then fixed in hot 10% formol-saline and preserved.

6. Examination of worms:- The parasites were stained by Semichon's carmine, acetic alum carmine, Gower's carmine & haematoxylin stains. The scolices from hydatid cysts and rostella detached from adult worms, were mounted in Berle's mounting medium for examination of hooks. Five large and five small hooks were measured from each scolex. The measurements taken were the total length, handle length, blade length and width.

TABLE No. 1(a)

Experimental design (Dog and cat).

Sl. No.	Animal No.	Approx. age (months)	Sex	Weight (lb)	Infective material: Source	Infective material: Dose	Infective material: Administration.	Date of infection	Date of autopsy	Age of infestation (days)
1	Dog No.1	1	Female	6.5	Scolices from hydatid cysts from lungs & liver of goats.	25000 viable scolices.	Oral	18.4.66	16.5.66	28
2	-do- 2	1	"	5.0	"	"	"	18.4.66	30.5.66	42
3	-do- 3	1½	Male	7.0	"	"	"	16.4.66	4.6.66	49
4	-do- 4	2	"	10.5	"	"	"	11.4.66	10.6.66	60
5	-do- 5	2	"	11.0	"	"	"	11.4.66	25.6.66	75
6	-do- 6	2	Female	11.5	"	"	"	11.4.66	10.7.66	90
7	-do- 7	1	Male	7.0	"	"	"	11.4.66	6.8.66	117
8	-do- 8	1	Female	6.0	"	"	"	16.4.66	19.8.66	125
9	-do- 9	1½	"	7.5	"	"	"	11.4.66	22.8.66	133died
10	Cat No.1	1	Female	4	"	"	"	9.7.66	6.8.66	28
11	-do- 2	1	"	4	"	"	"	9.7.66	20.8.66	42

TABLE No. 1(b)

Experimental design (Rabbits).

Sl. No.	Age	Infective material			Date of infection	Date of autopsy	Age of infection (in days)
		Source	Dose	Administration.			
1	Adult	Mature eggs from gravid worms collected from dog no.6(table 1a) which was fed with scolices of hydatid cysts from goats.	2000 eggs	Orally by a medicine dropper.	10.7.66	25.7.66	15
2	"		"	"	"	9.8.66	30
3	"		"	"	"	9.8.66	30
4	"		"	"	"	8.9.66	60
5	"		"	"	"	8.9.66	60
6	"	"	"	"	"	23.9.66	75
7	"	"	"	"	"	23.9.66	75
8	"	"	"	"	"	8.10.66	90
9	"	"	"	"	"	18.10.66	100
10	"	"	"	"	"	18.10.66	100
11	"	"	"	"	"	7.11.66	120
12	"	"	"	"	"	7.11.66	120

TABLE No. 1(c)

Experimental design (Guineapigs)

Sl. No.	Age	Infective material			Date of infection	Date of autopsy	Age of infection (days)
		Source	Dose	Adm.			
1	Adult	Scolices from hydatid cysts from lungs & liver of goats.	2000 hydatid scolices suspension + 5000 units of penicillin G.sodium + 50 mg.of streptomycin sulphate.	I/p	4.4.66	4.4.66	Died immediately after injection due to shock.
2	"	"	"	"	4.4.66	4.4.66	-do-
3	"	"	"	"	4.4.66	8.4.66	4
4	"	"	"	"	4.4.66	11.4.66	7
5	"	"	"	"	6.4.66	21.4.66	15
6	"	"	"	"	6.4.66	6.5.66	30
7	"	"	"	"	6.4.66	5.6.66	60
8	"	"	"	"	9.4.66	23.6.66	75
9	"	"	"	"	9.4.66	8.7.66	90
10	"	"	"	"	30.4.66	13.8.66	105
11	"	"	"	"	30.4.66	28.8.66	120
12	"	"	"	"	30.4.66	28.8.66	120

OBSERVATIONS

O B S E R V A T I O N S

INCIDENCE

Hydatid cyst in goats(Table no.2,3,4 & 5; figs.1,2,3,4,5,6 & 7; graph 1 & 2):

The overall incidence of hydatids in goats was found to be 6.5% with a range of 0.33 - 8.5% in different age groups. The organs infected were the lungs, liver and spleen in order of preference. The tabular statements show that with the age of the animals there is increase in the overall incidence, the organwise infection and the number of cysts in affected animal in each organ. The maximum number of visible cysts recorded from the lungs, liver and spleen were 41, 110 and 13 respectively. In 40 cases where both the lungs were affected, observations were made on the number of cysts on either side of the lung and while 235 cysts were recorded from right lungs only 158 were found in the left lungs; the average for each being 5.875 and 3.95 respectively. The data were statistically analysed by applying paired t-test and it was concluded that the average number of cysts was significantly greater in the right lungs than in the left lungs. The maximum number of cysts in a right lung was recorded to be 25 and in a left lung as 18.

Most of the cysts were spherical in shape. The characters of hydatid cysts - fertility, vesicles, degeneration and size - are summarised in table no.5. On no occasion

The accessory hooks which occur between the large and small hooks in a more posterior position are either small or large in size and of different shapes (fig.11).

Strobila:

Number and arrangement of segments(table no.9 & 10): The number of segments in gravid worms is 3 in 83.7% and 4 in 16.3% of the worms examined and the sexually mature segment is the penultimate one, while the last one is gravid. In four segmented worms the mature segment is preceded by two immature segments. The specimens of different ages show variations in number and arrangement of segments according to the stage of maturity (figs. 18 & 19).

Total length of the parasite (table no.11): The length varies with the age and rate of development of the worms. The average length of the gravid worms is found to be 2.62 mm.(range 1.55 to 4.10 mm.).

Size and shape of the segments(table no.12 & 13):- The length and breadth of terminal and sub-terminal segments is summarised in table no.11 and the ratio of the terminal segment to total length of the worm and ratio of mature segment to gravid segment of the worm in table no.13. The terminal segment in gravid specimens is always more than 1 mm. and forms about 50% of the total length. The average ratio of mature : gravid segment is 1:2.1 (range 1:1.4 to 1:3.2).

The shape of the segments varies according to the maturity of the worms.

Size of scolex, rostellum and sucker:

The average measurements are : scolex, 417.5 μ, rostellum 73.6 μ and sucker 90.5 μ. The range and mean from different dogs are presented in table no.14.

Position of genital pore (table no.15 and figs. 13 to 19):

In mature segments the genital pore is very near to the middle of the segment. In gravid segments it lies posterior to the middle of the segment.

Male genitalia:

The range and mean of total number of testes and testes anterior or posterior to the genital pore from each experimental dog is summarised in table no.16. The overall average of total number of testes is 45, 27 of which lie anterior and 18 posterior to the genital pore, i.e. number of testes anterior to genital pore is more than posterior to it (figs.14,15,16 & 17) but in some cases they are distributed equally in two halves (fig.13). Usually the posterior testes are placed on lateral side of female genitalia extending upto the level of the vitelline gland. In some cases they hardly reach the anterior border of the ovary (fig.16) whereas in others they extend behind the vitellaria also (figs.13 & 14).

The cirrus sac is either elongated (figs.13,14,15 &

16) or pear shaped (fig.16) and lies either horizontal to the long axis of the segment or slightly tilted anteriorly from the genital pore (figs. 15 & 17) . It extends more than half way across the mature segment and less than half way in the gravid segment. The measurements are 158 x 65 μ (range 120 to 200 x 40 to 80 μ) in mature segment and 170 x 70 μ (range 150 to 220 x 50 to 85 μ) in gravid segment.

Female genitalia:

The ovary consists of two lobes joined by an isthmus. The lobes have radiating branches but in some specimens neither the lobulation nor the branching of ovarian lobes is clearly marked (fig.13). Vitellaria are posterior to ovary. The gravid uterus has small lateral sacculations. Its shape is largely affected by the number of eggs inside. The eggs are slightly ovoid and measure 40 x 37.5 μ (range 38 to 42 x 35 to 39 μ).

B I O L O G Y

Number and distribution of worms in intestine of experimental dogs (table no.17):

All the dogs which were fed with 25,000 scolices of hydatid cysts from goats were infected. The worms were distributed upto 4th feet of small intestine in all cases and their concentration was maximum in the IInd feet. The maximum and minimum number of worms recovered from dogs were 10288 from dog no.6 autopsied at 90 days and 12 from dog no.4 autopsied at 60 days after the infection.

TABLE No.6

Number of rostellar hooks

Source	No. of scolices examined.	Total no. of large & small hooks		Scolices with							
		Range	Mean	Uneven no. of hooks		Paired large hooks		Paired small hooks		Tiny hooks	
				Number	%	Number	%	Number	%	Number	%
Goat (Ist batch)	45	29-41	34.8	10	22.2	10	22.2	5	11.1	8	17.8
Goat (II batch)	41	30-42	33.5	10	24.4	10	24.4	7	17.0	5	12.2
Dog no.1	30	28-43	34.2	6	20.0	5	16.5	4	13.3	3	10.0
Dog no.2	30	29-42	33.8	7	23.3	6	20.0	5	16.6	4	13.3
Dog no.6	40	28-41	35.1	8	20.0	6	15.0	3	7.5	2	5.0
Dog no.7	40	29-43	36.0	7	17.5	8	20.0	5	12.5	5	12.5

TABLE No.7

Size of large hooks (in μ)

Source	No. of hooks examined.	Total length		Handle length		Blade length		Width	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
Goat (I batch)	50	22.7-27.2	24.7	6.0-10.6	8.1	9.1-13.6	12.1	6.0-10.6	8.6
Goat (II batch)	50	22.7-28.7	25.8	6.0-12.1	8.3	9.1-15.1	12.1	7.6-12.0	9.4
Dog no.1 (infected from goat II)	40	27.2-28.7	27.6	7.6- 9.1	8.6	12.1-13.6	13.0	9.1-10.6	9.8
Dog no.2 (infected from goat II)	30	27.2-30.2	28.8	7.6-11.5	9.8	10.5-16.6	12.2	10.6-13.5	11.0
Dog no.6 (infected from goat I)	50	27.2-39.3	32.6	10.6-19.6	13.6	10.6-13.6	12.2	10.6-16.6	13.0
Dog no.7 (infected from goat I)	50	27.2-31.7	29.6	9.1-12.1	11.0	9.1-12.1	10.9	9.1-13.6	11.6

TABLE No.8

Size of small hooks (in μ)

Source	No. of hooks examined	Total length		Handle length		Blade length		Width	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
Goat (I batch)	50	18.1 -24.2	20.9	6.0-10.6	8.9	6.0-10.6	8.0	4.5-9.1	7.0
Goat (II batch)	50	16.6-24.2	21.1	4.5-12.1	8.5	6.0-12.0	8.6	6.0-9.0	7.7
Dog no.1 (infected from goat II)	40	19.6-24.2	22.5	7.6-10.6	8.6	7.6-10.6	8.6	7.6-10.6	8.8
Dog no.2 (infected from goat II)	30	19.6-25.7	23.0	9.1-12.1	9.2	7.6-10.6	8.6	9.1-13.0	9.4
Dog no.6 (infected from goat I)	50	19.6-31.7	23.0	7.6-15.1	10.0	6.0-9.1	8.3	9.1-13.6	11.6
Dog no.7 (infected from goat I)	50	15.1-25.7	22.3	6.0-12.1	9.8	6.0-9.1	7.7	7.6-12.1	9.8

TABLE No.9

Number of segments per specimen (expressed as a percent of the number examined).

Dog No.	No. of worms examined.	All specimens examined				No. of worms examined.	Gravid specimens only	
		I seg.	2 seg.	3 seg.	4 seg.		3 seg.	4 seg.
1	10	60	40	-	-	-	-	-
2	30	-	66.7	30	3.3	4	75.0	25.0
3	12	-	50.0	50.0	-	-	-	-
4	7	-	85.7	14.3	-	-	-	-
5	32	-	68.8	31.2	-	-	-	-
6	50	-	12.0	86.0	2	39	97.4	2.6
7	41	-	19.5	53.7	26.8	24	54.2	45.8
8	37	-	5.4	89.2	5.4	25	92.0	8.0
Total	219	2.7	33.8	56.7	6.8	92	83.7	16.3

TABLE No.10

Position of the sexually mature segment
(expressed as % of the no. examined).

Dog No.	No. examined.	All specimens			Number examined	Specimens with ova only.
		No mature segment.	2nd last	Last		2nd last
1	12	91.7	-	8.3	-	-
2	30	13.3	10.0	76.7	4	100
3	9	22.2	-	77.8	-	-
4	7	57.1	-	42.9	-	-
5	32	40.6	-	59.4	-	-
6	50	2.0	80.0	18.0	39	100
7	39	5.1	69.2	25.7	23	100
8	37	-	70.3	29.7	25	100
Total	216	17.1	44.4	38.5	91	100

TABLE No.12

Measurement of the terminal & subterminal segments.

Dog No.	Terminal segment				Subterminal segment			
	Length		Breadth		Length		Breadth	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
1	0.31-0.55	0.42	0.20-0.34	0.28	0.10-0.17	0.13	0.14-0.21	0.17
2	0.34-1.66	0.80	0.20-0.72	0.38	0.17-0.86	0.30	0.14-0.45	0.22
3	0.38-1.07	0.63	0.17-0.38	0.27	0.10-0.38	0.27	0.14-0.21	0.14
4	0.31-0.86	0.52	0.20-0.34	0.25	0.14-0.28	0.24	0.14-0.17	0.15
5	0.24-0.69	0.46	0.17-0.38	0.24	0.14-0.34	0.21	0.10-0.24	0.16
6	0.48-1.76	0.98	0.28-0.62	0.43	0.20-0.83	0.42	0.17-0.34	0.25
7	0.38-2.03	1.20	0.20-0.56	0.36	0.14-1.04	0.59	0.17-0.34	0.22
8	0.79-1.72	1.17	0.24-0.62	0.42	0.34-1.0	0.58	0.14-0.41	0.24
Total	0.24-2.03	0.86	0.17-0.72	0.36	0.10-1.04	0.40	0.10-0.45	0.21

TABLE No. 13

Ratio of terminal segment to total length of the worm, length of mature : gravid segment & percentage of gravid worms.

Dog No.	Ratio of terminal to total length of the worm (%)		Length of mature : Length of gravid segment.		Percentage of gravid worms.
	Range	Mean	Range	Mean	
1	42.8-56.0	49.4	-	-	-
2	35.7-60.0	52.6	1:1.9-1:2.5	1:2.1	13.3
3	37.0-51.7	46.7	-	-	-
4	39.1-53.2	44.2	-	-	-
5	35.0-56.2	44.1	-	-	-
6	43.8-57.1	47.5	1:1.6-1:2.9	1:2.3	76
7	37.1-55.0	46.6	1:1.4-1:2.4	1:1.9	58.5
8	37.9-58.0	45.9	1:1.4-1:3.2	1:2.1	62.5
Total	35.0-60.0	47.3	1:1.4-1:3.2	1:2.1	-

TABLE No.14

Size of scolex, rostellum and sucker(in μ)

Dog No.	Scolex			Rostellum			Sucker		
	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean
1	12	270 - 480	362.4	11	45 - 75	65.4	11	60 - 90	64.0
2	24	315 - 555	419.4	27	45 - 90	68.3	24	60 - 97.5	80.5
3	9	300 - 480	360.0	19	60 - 90	76.6	10	75 - 90.0	85.5
4	7	315 - 450	292.1	7	45 - 75	66.4	7	75 - 90.0	85.5
5	20	240 - 465	342.8	21	45 -105	62.9	20	75 - 90.0	79.5
6	21	300 - 485	417.8	22	60 -105	77.9	22	7 5- 120	96.0
7	22	405 - 585	493.0	21	67 - 90	78.3	21	90 - 150	117.0
8	20	375 - 570	471.0	20	60 -120	89.5	15	90 - 120	105.0
Total	135	240 - 585	417.0	138	45 -120	73.7	130	60 - 150	90.5

TABLE No.15

Distance of genital pore from anterior margin of the segment (Expressed as % of total length of the seg.)

Dog No.	Mature segment			Gravid segment		
	No. examined.	Range	Mean	No. examined.	Range	Mean
2	13	47.8-65.2	53.8	3	54.2-57.9	56.1
3	6	46.7-57.9	51.2	-	-	-
4	2	47.4-52.0	49.7	-	-	-
5	8	38.5-53.3	47.8	-	-	-
6	30	41.0-59.0	48.9	16	49.0-59.4	57.4
7	15	43.4-66.6	52.5	4	57.1-65.4	59.7
8	15	41.6-67.8	54.8	13	51.1-66.6	56.8
Total	89	38.5-67.8	51.3	36	49.0-66.6	57.3

TABLE No.16

Number and distribution of testes.

Dog No.	No. examined.	Total		Anterior		Posterior	
		Range	Mean	Range	Mean	Range	Mean
2	7	36 - 62	46.1	20 - 45	29.3	12 - 30	16.9
3	2	31 - 68	49.5	19 - 36	27.5	14 - 32	23.0
5	6	32 - 43	37.6	15 - 31	22.5	12 - 20	15.2
6	15	31 - 61	43.3	10 - 36	22.1	14 - 30	21.2
7	8	29 - 52	35.2	16 - 45	23.9	9 - 17	11.3
8	20	35 - 66	47.4	23 - 42	31.0	10 - 26	18.2
Total	57	29 - 68	45.0	10 - 45	27. 0	9 - 32	18.0

TABLE No.17

Number and distribution of worms in small intestine recovered from the experimental dogs.

Dog No.	Length of s. inten-tine (in ft)	No. of worms recovered from each one ft. of small intestine								Total No. of worms.
		I	II	III	IV	V	VI	VII	VIII	
1	7	67	330	125	13	12	-	-	-	547
2	5½	70	798	290	125	-	-	-	-	1283
3	6½	20	40	5	5	-	-	-	-	70
4	6¾	4	6	2	-	-	-	-	-	12
5	5½	45	475	200	30	-	-	-	-	750
6	6	1750	4333	2300	1550	205	150	-	-	10288
7	8½	50	200	250	150	85	75	100	-	910
8	7¼	210	360	25	15	5	6	-	-	621
Total		2216	6542	3197	1888	307	231	100	-	14481

Growth and development:

Rostellar hooks:- The proportional increase of large and small hooks with the age of the worm is summarised in table no.18 below and graph no.3 & 4. It is the handle and the guard which increase with the age of the worm, the blade remaining almost constant throughout the life of the parasite.

TABLE No.18

Proportional growth of hooks at different days of infestation from experimental dog.

Days of infestation	Total length	Handle	Width
(A) Large hooks			
Cystic hooks	1	1	1
28 days	1.07	1.03	1.04
42 "	1.10	1.20	1.05
90 "	1.32	1.70	1.50
117 "	1.20	1.35	1.34
(B) Small hooks			
Cystic hooks	1	1	1
28 days	1.06	1.01	1.14
42 days	1.09	1.08	1.18
90 "	1.10	1.12	1.65
117 "	1.07	1.10	1.40

From the table above it is clear that the maximum growth occurs at 90 days of infestation and large hooks increase more rapidly than the small hooks.

Strobila:- The growth is rapid upto 42 days, slow between 42 to 75 days and again followed by a gradual development upto 117 days. The 117 days represents the peak of the growth period of the worm. The increase, in length of the worm, its proglottids and scolex, rostellum and sucker(table no.19 below) follows more or less the same pattern. The segmentation has proceeded only upto 2 at 28 days, 3 upto 75 days (except in dog no.2 - fig.19) and 4 at 90 days and onward (fig.18).

TABLE No.19

Proportional growth in size of scolex, rostellum and sucker at different days of infestation.

Dog No.	Days of infestation.	Scolex	Rostellum	Sucker
1	28	1	1	1
2	42	1.16	1.04	1.26
6	90	1.15	1.19	1.50
7	117	1.36	1.21	1.83
8	125	1.30	1.35	1.64

Genitalia:- The testes, vas deferens, ovary and vagina are discernible at 28 days but they are not yet fully mature. All the genital organs are fully mature at 42 days of infestation. The gravid segment is first visible at 42 days. The cirrus sac and eggs do not show any significant difference in size with the age of the worm.

Prepatent period:

It was found to be 70,60,90 & 80 days in dog nos. 6,7,8 & 9 respectively.

Development in cats:

Both the cats fed with the same number of scolices as dogs (25000) were found to be infected when autopsied at 28 and 42 days after exposure. The important characters of the worms and their comparison with the worms of similar age from dogs are presented in table no. 20, page no.36. As a whole the growth in cat is very slow, the worms are stunted and even the genital primordia are not visible at 42 days after infection.

Larval development in rabbits and guineapigs:

None of the 12 rabbits fed with eggs of E.granulosus of goat-dog origin and autopsied at different days between 15 to 120 days (table no.1b) showed the development of hydatid cyst. All the 12 guineapigs injected I/p with cystic scolices from goats (table no.1c) and autopsied at different days between 4 to 120 days proved refractory to secondary echinococcosis.

Biochemical studies:

The results of biochemical studies on hydatid fluid

are summarised in table no.21, page 37. The whole hydatid membrane on analysis gave 11.5% (range 10.7 to 12.5%) total solid on wet weight basis and 5.3 gm.% total nitrogen content on dry matter basis.

TABLE No.20

Comparison between the worms from cat & dog.

Sl. No.	Character		28 days infestation.		42 days infestation.	
			Cat	Dog	Cat	Dog
1	No.of worms recovered		123	547	80	1283
2	No.of segments per specimen(expressed as % of the no. examined.	Unsegmented	50	0	37.5	0
		1 segmented	50	60	56.3	0
		2 "	0	40	6.3	66.7
		3 "	0	0	0	30
3	Total length of worms(m.m.)	Unsegmented	0.29	-	0.45	-
		1 segmented	0.38	0.84	0.46	-
		2 "	-	0.87	0.57	1.36
4	Size of terminal segment(m.m.).	Length	0.14	0.42	0.14	0.80
		Breadth	0.07	0.28	0.10	0.38
5	Size of scolex (μ)		260	362	353	419
6	Size of rostellum (μ)		45	65	66	68
7	Size of sucker (μ)		57	64	81	82
8	Genital organs		Absent.	Present, partially mature.	Absent.	Present completely mature with gravid seg.

TABLE No.21

Biochemical observations on hydatid fluid from lungs of goats.

Sl. No.	Observation	Range	Mean
1.	Specific gravity	1.010-1.011	1.01075
2.	pH	6.6 - 8.0	7.3
3.	Total solid (dry matter) % W/W	1.54 - 2.11	1.84
4.	Inorganic substances(Ash) %	30.01-38.0	33.28
5.	Organic substances %	62.12-69.99	66.72
6.	Total Nitrogen (mg./100 ml)	59.5- 90.0	75.33
7.	Non-protein nitrogen (mg./100 ml.)	50.5- 78.2	64.9
8.	Protein nitrogen(mg./100 ml)	9.0- 11.8	10.43
9.	Protein (mg./100 ml.) (Protein nitrogen x 6.25).	56.25-73.75	65.20

PATHOGENICITY

Hydatid cysts in goats:

The pathological changes in liver, lungs and spleen from naturally infected goats were studied. The degree of changes was dependant upon the severity of the infection.

Liver:- In heavy infections, the liver looked more like a cystic mass, the size was greatly enlarged and only fine strands of liver parenchyma appeared between the cysts. At places the cysts looked like bunch of grapes (fig.2).

Microscopically the focus is characterised by the cyst wall and changes around it. The cyst wall is composed of 2 layers - (i) a thin inner nucleated germinal layer of embryonal cells and (ii) a thick outer laminated layer of faintly stained hyaline connective tissue (fig.24). In fertile cysts brood capsule with scolices inside it, are attached to the germinal layer (figs.7 & 24). Around the cyst wall proper, there is an outer capsule of dense fibrous connective tissue formed as a result of destruction of liver cells and subsequent replacement by fibroblasts. This capsule has an inner zone of mature connective tissue fibres with few nuclei and an outer more cellular zone of newly formed fibroblasts. The changes in liver parenchyma are of degenerative and proliferative nature. There is pericellular, monolobular and central

cirrhosis of the liver (figs.21,22 & 23). In central cirrhosis, the central vein is surrounded by a thick layer of connective tissue fibres around which there is a zone of diffuse haemorrhage. The architecture of the hepatic cords is disturbed, the central vein is no more central and in areas of heavy infection hepatic cords look like islands between cystic structures. The portal area as a whole shows proliferation of fibroblasts and has many newly formed bile ducts, some of which show fibrosis of outer layer and hyperplasia of the inner epithelial layer. The whole hepatic lesion is marked by intense cellular infiltration of mononuclear cells which consists chiefly of lymphocytes. At places the infiltration is focal and dense and is encapsulated by fibrous tissue (fig.20). Occasionally eosinophils, plasma cells and few giant cells have also been observed.

Lungs:- The changes in lungs are of similar nature as in the liver. The topography of the organ is affected according to the number and size of the cysts present (figs. 3,4 & 5). There are areas of collapse and emphysema evidensed by depression and elevation on the surface of the organ.

Microscopically typical hydatid lesion with cyst wall, adventitious fibrous capsule and mononuclear cellular infiltration is visible (fig.25). The vesicle shows herniation, around which cellular infiltration is more intense.

Collapsed areas are marked by approximation and layer formation of alveoli and emphysematous areas by over stretching, breaking down of alveolar walls and subsequant formation of large areas of inter communicating alveoli. The cellular reaction is more marked around peribronchial tissue. The fibrosis of alveolar walls is also present.

Spleen:- Macroscopic changes in spleen also depend upon the number and size of the cysts. Microscopically herniation of vesicle is evident. The changes around cyst wall are mild as compared to liver and lungs.

Clinical symptoms in experimental dogs:

Out of the 9 dogs experimentally exposed to 25000 scolices of hydatid cysts of goat origin, only one showed clinical symptoms of digestive disturbances - anorexia, emaciation, dysentery alternating with constipation - 70 days after exposure. This dog on autopsy, after 90 days of the infection, was found to harbour over ten thousand worms. None of the other dogs exhibited any clinical manifestation of the disease.

D I S C U S S I O N

INCIDENCE

The incidence of hydatid cysts in goats increases with the age. It is as low as 0.33% in animals below one year of age and as high as 8.6% in animals above three years of age. This variation is attributable to greater opportunity for infection and development rather than any increased age-susceptibility. The previous workers in India have not worked out the incidence separately for sheep and goats. However, an analysis of the observations of Sami (1938), Chhuttani & Chugh (1957) and Sharma & Chitkara (1963) indicates that probably these workers examined the young animals (sheep and goats), upto three years of age only, as the present observations for animals below one year correspond to those of Sami (loc.cit.) and the observations for 1-3 years to Chhuttani & Chugh (loc.cit.) and Sharma & Chitkara (loc.cit.). The overall incidence shown in the present study is significantly more than the previous reports (table no.22).

In the present investigation of all the infected animals 57.1% had cysts in lungs only, 15.2% in liver only and 25.9% in both the organs. These figures indicate the preponderance of lung infection and partially agree with those of Sharma & Chitkara (loc.cit.). As to the distribution of cysts in the animal and in different infected organs, multiple

TABLE No.22

Comparison of the incidence of hydatid cysts in sheep and goats & of adult *E.granulosus* in dogs in various studies in India.

Author	Year	Place	Hydatids in sheep & goats		Adult *E.granulosus* in dogs.	
			No. examined	%	No. examined	%
Pease	1902	Lahore	-	-	-	24.0
Maplestone	1933	Calcutta	-	-	100	2.0
Sami	1938	D.Ghazi Khan (W.Pakistan)	-	1.0	156	28.8
Maplestone & Bhaduri	1940	Calcutta	-	-	100	18.0
Chhuttani & Chugh	1957	Patiala	1000	2.8	245	13.9
Sharma & Chitkara	1963	Amritsar	1095	3.5	460	8.04
Present study	1966	Patna			20	10.0
		Goats below 1 year	302	0.33		
		" 1 - 3 years	176	3.40		
		" above 3 yrs.	1136	8.60		
		Total	1614	6.50		

cyst infection is more common and the lungs, spleen and liver have the number of cysts in the order mentioned. The right lung has been found to be a significantly more preferred site than the left lung. This observation is in agreement with those of Fairley & Wright - Smith (1929) from natural cases in ox, sheep and pig and of Yamashita et al. (1957) from three experimentally infected sheep.

The subdivision of a cyst by incomplete septa gives the appearance of its being made up of a number of loculi or alveoli which had led some (Barnett,1941; cited by Williams & Sweatman, 1963) to describe these as "alveolar" cysts. Williams & Sweatman (1963) have found that septa in cysts occur commonly in E.granulosus in cattle, sheep and swine. The present observations are in conformity with their findings. The fertility rate of cysts (77.7%) corresponds with that reported for pigs (Lapage,1962). From these observations it is concluded that goats are an ideal intermediate host of E.granulosus.

The incidence of adult stage in dogs was found to be 10% which agrees with the findings of Sharma & Chitkara(1963).

The incidence in dogs and goats in the same locality is indicative of its epidemiological importance. The poor hygienic conditions in abattoirs and meat markets, high fertility rate of goat hydatids and prevalence of infection in

local dogs are factors conducive to the endemicity and related dangers to human infection.

MORPHOLOGY

The important morphological characters observed in the present study of goat-dog material are compared with those of the other workers from other sources, in table no.23.

The number and size of the rostellar hooks in the present study on goat-dog material are consistent with the findings of the previous workers except with those of Hutchison & Bryan (1960) who reported greater dimensions of the hooks from pig-dog material. The small accessory hooks described by Vogel (1957) and Sweatman & Williams (1963) and the presence of large accessory hooks, uneven number, paired large and small hooks described by Sweatman & Williams (loc.cit.) have been observed in the present study also (figs.10 & 11). Cameron (1926), Rausch (1953) and Vogel (loc.cit.) consider the shape of the large hooks to be characteristic for a given species. Ortlepp (1937) considered the rugose nature of the hooks to be a characteristic feature of E.felidis. In the present study the shape of the hooks from the hydatid scolices obtained from goats and those of the adult obtained from experimental dogs show extreme variation not only between individual hosts but

also in the same host and even on the same scolex. These findings are in agreement with the observations of Sweatman & Williams (1963).

In the present goat-dog specimens, the number of segments in the gravid worms was either 3 or 4. This finding is in complete agreement with the observations of Petrov & Chertkova (1959) (cited in Rausch & Nelson 1963) and Euzeby (1960) (cited in Smith,1964) and partially with those of Rausch (1953),1956), Yamashita et al.(1956), Vogel(1957), Wright(1962), Nelson & Rausch (1963), Sweatman & Williams (loc.cit.) and Verster (1965) who reported the variable number of segments between two to five. In the present study the penultimate segment was always mature in the gravid worms, as also reported by Yamashita et al.(loc.cit.) and Vogel (loc.cit.). The length of the worms ranges from 1.6 - 4.1 m.m.(av.2.62 m.m.) which is shorter than those reported in literature for E. granulosus material from other sources. Rausch, Yamashita et al.,Vogel, Euzeby, Nelson & Rausch(all loc.cit.) found the genital pore to be near middle in the mature segment and posterior to middle in the gravid segment whereas Petrov & Chertkova and Wright (both loc.cit.) found it always posterior to the middle. In the present specimens the genital pore was observed to be near the middle in the mature segments and posterior to middle in the gravid segments.

The testes number from 29 to 68 (av.45), 10-45 (av.27) being anterior and 9-32 (av.18) posterior to the genital pore. They are distributed from behind the vitellaria to the anterior end of the mature segment. Similar arrangement has been reported in E.granulosus material from other natural and experimental cases (Yamashita et al.,Vogel, Wright, Nelson & Rausch; all loc.cit.). However, E.granulosus material originating from moose or reindeer origin has been described to have the testes distributed equally in two halves of the mature segment (Rausch,1953). Recently Verster(1965) has described the arrangement of testes as an important character for the identification of different species and sub-species of Echinococcus.

The ovary is found to be bilobed with radiating branches which confirms the findings of Sweatman & Williams (1963) and Verster (loc.cit.) but disagrees with those of Vogel(loc.cit.) who found it always kidney shaped in his pig-dog material. In the present goat-dog material uterus has always been found to be saccular with occasional lateral sacculations.

The morphological differences observed in the present goat-dog material may be attributed to the host and geographical variation.

BIOLOGY

Number and distribution of worms in small intestine of dogs:

Although all the 9 dogs used in this experiment were of the same age-group and breed and kept under similar managemental conditions, they exhibited extreme variation in individual susceptibility to infection. The number of worms recovered from these experimental cases was as low as 12 in one case and as high as 10288 in another. Similar variations have also been recorded by Verster (1965) with porcine, ovine and bovine materials. As there is no clear visible demarcation between duodenum, jejunum and ileum of dog, the worms were collected and counted from each portion of one foot length of the small intestine to find out their exact location. The worms extended upto more than half the length of small intestine with a maximum concentration in the second portion of one foot length. These findings are in agreement with those of Yamashita *et al.* (1956) and Sweatman & Williams (1963). Babero *et al.* (1963) had, however, found the worms distributed throughout the small intestine.

Growth and development:

Rostellar hooks:- Yamashita *et al.* (*loc.cit.*) in Australian sheep-dog material found that the small and the large hooks increased in length upto 375th day. Vogel (1957)

in pig-dog material from Germany showed that the hooks increased in size only between 51,64 and 81 days. Hutchison & Bryan (1960) in pig-dog material from Mississippi,observed no increase in the size of the hooks after 56 days. Verster (1965) in South African material from a wide range of experimental hosts found that the handle increased in size but blade remained almost constant throughout the life of the parasite but she was not able to determine the age at which the hooks ceased to grow.

In the present study on the goat-dog material both the large and the small hooks were found to increase in total length, handle length and width upto 90 days after infection but the blade length did not show any significant increase with the age of the worms. These observations confirm the findings of Vogel (1957). The pace of growth of hooks was found slower in the present goat-dog material than that of sheep-dog material reported by Yamashita et al.(1956).

Strobila:- The different parts of strobila were found to grow in size with the age of the worm. The segmentation of the body, the development of genitalia and proportionate increase of individual parts observed in this study have not been reported previously. However, Smith (1962) and Yamashita et al. (loc.cit.) carried out some preliminary experiments on growth rate. The present goat-dog material shows slower growth rate