STUDIES ON

THE LARVAL CESTODES OF ZOONOTIC IMPORTANCE IN KERALA WITH SPECIAL REFERENCE TO HYDATID

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THESIS
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DECLARATION

I hereby declare that the thesis entitled "STUDIES ON LARVAL CESTODES OF ZOONOTIC IMPORTANCE IN KERALA WITH SPECIAL REFERENCE TO HYDATID" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Mannuthy,
29-7-1978.

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CERTIFICATE

Certified that the thesis entitled " STUDIES ON LARVAL CESTODES OF ZOONOTIC IMPORTANCE IN KERALA WITH SPECIAL REFERENCE TO HYDATID" is a record of research work done independently by Sri.J.Abraham under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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C O N T E N T S

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INTRODUCTION

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A large number of pathogens infect man and animals, directly or indirectly, resulting in morbidity and mortality. Domestication of various species of animals and their close association has considerably increased the risk of man contracting diseases from these animals. Peridomestic and wild animals also contribute to such infections. The diseases which are thus shared by man and animals in nature, collectively known as 'Zoonoses', are recognised as public health problems in every country. In spite of the scientific advancement and better animal husbandry practices, human health is inextricably related to the health of animals and hence an animal population free from diseases is essential.

Larval infections of Taeniid cestodes are important zoonoses of man, domestic and wild animals, having a cosmopolitan distribution. Among them, hydatidosis and cysticercosis have attracted the attention of public health authorities as diseases detrimental to human health and as a serious threat to the meat trade and industry. The cystic phases of these cestodes, by their ability to localise and develop in different organs and tissues, necessitate massive condemnation of visceral organs of food animals and thus incur a considerable financial loss. The absence of proper

chemotherapy for cystic infections, risk and expenses on surgical intervention have heightened their global importance.

Hydatid disease, a cyclozoonotic infection of world-wide importance is mainly caused by Echinococcus granulosus and E.multilocularis, although other species with sylvatic cycles exist in various parts of the world. The former produce a unilocular and latter an alveolar type of cyst. Echinococcus granulosus is maintained by a variety of canine - ungulate cycle involving both domestic and sylvatic species. Dogs, foxes, jackals, hyenas and coyotes which act as definitive hosts serve as sources of infection to the human population and other animals. The wide range of host spectrum in different geographic regions contribute highly to the perpetuation and spread of the disease. There are also reports that hydatid disease has appeared in many countries previously free of it, because of the importation of animals (Matossian et al., 1977). Human infection is principally found to occur in localities where dogs harbour the adult worms and the domestic ruminants act as reservoirs of larval stage. The cystic stage, found in different organs, produce varying manifestations and greatly affect the productivity of domestic animals. The hydatid cysts are relatively slow growing and development of symptoms take a longer period with the result that many of the human

cases go unnoticed. Barnett (1939) has noted that a hydatid cyst is as old as the patient. Echinococcus multilocularis is principally maintained by a fox - microtine rodent cycle and have a restricted geographical distribution.

Hydatid disease is widely prevalent in India as reported by many investigators. The neighbouring states of Andhra Pradesh, Karnataka and Tamil Nadu are endemic areas of hydatidosis as evidenced by reports (Reddy et al., 1968; Hegde et al., 1974; Sreemathi et al., 1977). The fact that the majority of animals slaughtered in different slaughter houses in Kerala come from these neighbouring states is important in this context. Schwabe (1968) considered the incidence of the disease in animals in any one place as an 'index of environmental contamination'. The data on the prevalence of this disease in the state of Kerala is scanty. Hence a detailed investigation on the incidence of hydatidosis in the domestic animals slaughtered in different slaughter houses of Kerala has been taken up.

Studies on the incidence alone does not depict a true picture of the potential source of dissemination of hydatid disease. The hydatid cyst may be sterile or fertile and the rate of fertility varies in different species of animals and with their location in the host. Sterile cyst has no importance in the dissemination of the disease and

it could be termed as the 'dead end of the parasite'. A fertile cyst with live protoscolices poses an indirect threat to the human health, as it is essential for the completion of the life cycle. It has also been established that the antigenicity of the cyst-fluid has a relation to the fertility of the cyst. Hence it was proposed to conduct a study on the fertility of hydatid cysts encountered in various species of animals examined at slaughter.

Hydatid cyst-fluid is known to contain a complex mixture of substances derived from the host and by the metabolism of the parasite. Constituents like nitrogen and protein have a close relation to the antigenicity of the cystic fluid and those like sodium and potassium are associated with respiratory metabolism of the parasite and permeability of the cyst wall. The permeability of the cyst wall is an important consideration for the growth of the cyst, chemotherapy and immunity. A perusal of the available literature shows that information on this aspect is scanty and hence it was proposed to conduct a preliminary study on some of the constituents, viz; nitrogen, protein, sodium and potassium.

Immunodiagnosis of hydatid disease has been elaborately studied in various parts of the world with emphasis on human cases although several studies have been done in animals. Kagan (1968) while reviewing the merits and

reliability of various immunological tests has stated that agglutination test and intradermal test are the tests of choice in man, and for animals their reliability needs further evaluation due to the considerable variations observed. It is also reported that related cestode infections like cysticercosis due to Taenia hydatigena, T.saginata and T.ovis cross-react and give false positive results. An assessment on the reliability of indirect haemagglutination test and Casoni's intradermal test was made utilising the animals brought for slaughter in the slaughter houses.

Cysticercosis is an equally important zoonotic problem. It is caused by the larval stages of two closely related tapeworms of man viz., Taenia solium and Taenia saginata. Cysticercus cellulosae the larval stage of Taenia solium and Cysticercus bovis that of T.saginata utilise pigs and cattle respectively as their intermediate hosts. Intensification of animal production, development of meat industries and low standards of sanitation and food processing have contributed to the increased prevalence of cysticercosis in many countries. With more than 85% of the population consuming meat regularly or occassionally, the chances of human exposure is great in Kerala. Hence a study of the incidence of these infections in the animals slaughtered in Kerala has also been undertaken.

REVIEW OF LITERATURE

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Incidence of Hydatidosis in Animals

Echinococcosis/Hydatidosis is found in every continent of the earth where man has domesticated animal (Kagan, 1976). The earliest record of hydatidosis in India was made by Edwards (1927) who reported an incidence of 70 to 80% in cattle at Indian Veterinary Research Institute. Subsequently several reports of its incidence has been made from different parts of India. The incidence was found to vary from 12 to 63% in cattle (Sundaram and Natarajan, 1960; Endrajat, 1964; Krishnamurthy, 1968; Reddy et al., 1968; Sadananda, 1969; Pal and Sinha, 1970; Iyer, 1972; Pythal, 1974; Hegde et al., 1974 and Matossian et al., 1977). In buffaloes, an incidence of 11 to 40% has been recorded by Gill and Rao (1967); Rao (1968); Reddy et al. (1968); Sadananda (1969); Pal and Sinha (1970); Iyer (1972); Pythal (1974) and Matossian et al. (1977).

The incidence of hydatidosis in sheep has been found to vary from 0.5 to 21.75% (Maplestone, 1933; Chuttani and Chug, 1957; Sachdev and Talwar, 1960; Sharma and Chitkara, 1963; Reddy et al., 1968; Sadananda, 1969; Iyer, 1972; Pythal, 1974 and Matossian et al., 1977). Among goats, the disease was found to occur in 0.6 to 16.9% animals

(Maplestone, 1933; Chuttani and Chug, 1957; Sachdev and Talwar 1960; Endrajat, 1964; Reddy et al., 1968; Singh and Kuppuswamy, 1969; Pandey, 1971a; Iyer, 1972; Pythal, 1974 and Matossian et al., 1977).

The incidence was found to vary in animals from place to place and even in the same place, similar difference has been recorded from time to time and species to species (Iyer, 1972).

Cysticercosis

Cysticercosis caused by Cysticercus bovis and C.cellulosae is of great zoonotic importance and their incidence has been widely reported.

There are only very few records of the incidence of Cysticercus bovis in India. The earliest report of its incidence has been made by Moghe (1945) in Nagpur. Incidence of the same has been subsequently reported by Mudaliar and Alwar (1947); Thapar (1956); Krishnan and Ranganathan (1972); Nayak et al. (1973); Pythal (1974) and Ratnam (1977). According to Krishnan and Ranganathan (1972) the incidence of Cysticercus bovis in Indian cattle was one per cent in some regions of Tamil Nadu, 5.5% to 6.12% in Punjab and 4% in Assam. Nayak et al. (1973) has recorded 2.5% incidence in Madras and Pythal (1974) reported the highest incidence of 9.28% in Kerala. Regarding the incidence of Cysticercus cellulosae in pigs, Bisht (1977) has reported 1.5% to 3%

incidence among pigs slaughtered at Central Dairy Farm, Aligarh.

Incidence of Fertile Hydatid cysts in Infected Animals

A cyst with an active germinal epithelium, brood capsules and scolices is considered to be fertile.

Fertility of hydatid cysts encountered in Indian cattle was found to vary from 3 to 72% (Sundaram and Natarajan, 1960; Reddy et al., 1968; Reddy et al., 1970; Iyer, 1972; Rao and Mohiyuddin, 1974; Pythal, 1974 and Hegde, et al., 1974). The lowest percentage was reported from Bangalore (Rao and Mohiyuddin, 1974) and highest from Kerala (Pythal, 1974).

In buffaloes, the fertility of hydatid cysts was found to vary from 12.97 to 88.8% (Sundaram and Natarajan, 1960; Gill and Rao, 1967; Rao, 1968; Reddy et al., 1970; Iyer, 1972; Rao and Mohiyuddin, 1974 and Pythal, 1974). While Sundaram and Natarajan (1960) has found a low percentage of fertile cysts in Tamil Nadu, Rao (1968) has recorded the highest percentage of fertility of hydatid cysts from Uttar Pradesh.

In sheep, the percentage of fertile cysts has been reported to vary between 12.96 and 73.3 (Sundaram and Natarajan, 1960; Reddy et al., 1968 & 1970; Iyer, 1972 and Pythal, 1974). Highest incidence of fertile cysts has

been reported by Iyer (1972) whereas Sundaram and Natarajan (1960) has reported the percentage of fertile cysts to be low.

The occurrence of fertile hydatid cysts in goats was found to vary from 40 to 75% (Reddy et al., 1968; Singh and Kuppuswamy, 1969; Reddy et al., 1970; Iyer 1972 and Pythal 1974). The maximum fertility was reported by Singh and Kuppuswamy (1969) and the minimum by Iyer (1972).

Organ-wise incidence of fertile cysts has been reported by Gill and Rao (1967); Reddy et al. (1968); Singh and Kuppuswamy, (1969); Iyer (1972) and Pythal (1974). The percentage of fertile cysts in liver, as per the reports, was found to vary from 50 to 65.5 in cattle, 61.34 to 83.6 in buffaloes, 71.2 to 92.3 in sheep and 33 to 75 in goats. In lungs, the fertility was found to vary from 44.4 to 71.48% in cattle, 42.4 to 90% in buffaloes, 50 to 69.15% in sheep and 50 to 73.18% in goats.

Studies on some of the constituents of Hydatid cyst fluid

Whole hydatid cyst fluid has been recognised as a mixture of substances, some attributed to the host and others to the parasite. Basic studies on the metabolism of parasite involves analysis of the cystic fluid for its biochemical constituents. Further, hydatid fluid, which is extensively used as antigen for diagnostic procedures,

requires analysis of the antigenic components to help in standardization of the antigen. Kagan and Agosin (1968) emphasised the necessity of a reasonable knowledge of the chemical structure of hydatid fluid for proper evaluation of the antigen.

Carbone and Lorenzetti (1957) has reported a protein content of 17.3 to 227 mg/100 ml in hydatid fluid recovered from lungs and liver cysts of man, whereas the protein content of the cystic fluid obtained from sheep and cattle was 30.2 to 213 mg/100 ml and 23.7 to 166 mg/100 ml respectively. Magath (1959) has reported only 7.5 mg protein/100 ml of hydatid cyst-fluid collected from man. Pandey (1971b) has reported 56.25 to 73.75 mg protein/100 ml of hydatid fluid from goats.

Studies conducted by Sita Devi et al. (1971) revealed a significantly higher concentration of protein in hydatid fluid obtained from fertile cysts than that of sterile cysts collected from cattle and sheep. According to them, the protein content varies from 126.7 to 145.85 mg/100 ml in hydatid fluid recovered from fertile cysts in cattle, sheep and goat, and 19.4 to 87.6 mg/100 ml of sterile cyst-fluid of the above species. According to Kagan (1977) the biochemical constituents of the hydatid cyst fluid depend on their location in the body and that cysts in the liver are richer in protein than cysts in the lung.

The nitrogen content in the hydatid cyst-fluid was reported to vary between host species. While Goodchild and Kagan (1961) obtained 7, 34 and 29 mg nitrogen/100 ml of cystic fluid recovered from cattle, pig and man respectively, Kagan and Norman (1963) found corresponding values to be 3.2, 18 and 90 mg/100 ml. The nitrogen content of hydatid cyst-fluid of sheep origin was reported to be 116 mg/100 ml (Kagan and Norman, 1963). Pandey (1971b) who conducted biochemical analysis has recorded 59.5 to 90 mg of nitrogen/100 ml of hydatid fluid from goats.

Studies conducted by Schwabe (1959) revealed a mean sodium content of 124.9 mE/litre in bovine hydatid fluid. Rotunno et al. (1974) found that the sodium content of hydatid cyst fluid of mouse origin to be 112 mE/litre.

Indirect Haemagglutination Test

Extensive work has been carried out on immunodiagnostic aspects of hydatid disease as evidenced by the reports. The serology of hydatid disease can be traced back to the use of complement fixation test by Ghedini (1906). The use of tannic acid to alter the surface potential of erythrocytes to adsorb antigen was advocated by Boyden (1951). The technique of using tanned RBCs in haemagglutination was introduced as a test for hydatid disease by Garabedian et al. (1957).

The sensitivity of the indirect haemagglutination test in man has been reported to be high (Sorice, 1966; Zanussi et al., 1966; Hutchison, 1968; Apt and Knierim, 1970; Williams et al., 1971; Garabedian, 1971 and Mahajan et al., 1976).

A comparative study on the efficacy of indirect haemagglutination, complement fixation and intradermal tests in diagnosis of hydatid disease in man by Arabatizis and Papapanagoitou (1963) revealed high degree of positive correlation in the indirect haemagglutination test. They found better results when liver was involved compared to the involvement of lung. Similar observations on the comparative efficacy and high degree of positive correlation has been recorded by many workers (Addis and Mandras, 1958; Sweatman et al., 1963; Abou-Daoud, 1965; Hutchison, 1968; Kagan, 1968; Om Prakash and Vinayak, 1970; Mahajan et al., 1973 & 1976; Todoroff and Yurukova, 1974; Moch et al., 1974; Varela-Diaz et al., 1975 and Schantz et al., 1977). A combination of intradermal and indirect haemagglutination tests was found to be a more reliable diagnostic tool as reported by Mahajan et al. (1973); Schantz et al. (1973) and Spruance et al. (1974).

The sensitivity of indirect haemagglutination test in animal sera was not encouraging as inconsistent results have been reported. Pinelli (1961) reported that the test was not specific in the case of cattle and sheep. The

occurrence of false positive reactions in animal sera has been reported by Pauluzzi and Castagnari (1965); Iyer (1972) and Pythal (1974). Inconclusive and negative results had been recorded irrespective of whether the animals were infected or not in horse, pig and cattle (De Rosa et al., 1971). Cross reactions with other cestode infections of Cysticercus tennuicollis and Coenurus cerebralis in sheep yielded false positive results (Sweatman et al., 1963 and Kagan, 1968). Cross reactivity was observed in case of Schistosomiasis (Botros et al., 1973). Rydzewski and Kagan (1975) reported cross reactions of antigen of Taenia saginata with hydatid antibody. Kagan (1976) recorded cross reactions of hydatid antigen with cysticercus antibody.

Different types of antigen have been used in the indirect haemagglutination test. Kagan and Norman (1963) found that immunologic activity of hydatid fluid antigen varied from lot to lot and they observed the unsuitability of bovine hydatid fluid as antigen in serological tests. Sorice et al. (1966) recorded high sensitivity of the test by the use of globulin fraction of hydatid fluid as antigen. Orihara (1967) found that heating destroyed the protein fraction of hydatid fluid which acts as antigen in the indirect haemagglutination test. Fischman and Allen (1967) found that polysaccharide fraction of hydatid fluid did not react in indirect haemagglutination test.

Kagan (1968) recommended the use of whole hydatid fluid from sheep as antigen for indirect haemagglutination test. Hydatid fluid antigen was found to be superior to scolex antigen in serological tests (Garabedian, 1971). Varela-Diaz et al. (1974) detected the presence of specific antigens of Echinococcus granulosus in sheep hydatid cyst-fluid by immuno electrophoresis. The most reactive component which was considered to be specific for hydatid antigen as revealed by affinity chromatography had been found to be antigen 4 and 5 present in sheep hydatid fluid (Roberto pozzuoli et al., 1975). The use of sheep hydatid fluid as antigen for indirect haemagglutination test had been recommended by Bombardieri et al. (1974) and Mahajan et al. (1976).

Hariri et al. (1965) found that dilution of antigen gave better sensitivity in indirect haemagglutination test. Kagan and Norman (1976) strongly recommended the use of standardised antigens in serologic tests and determination of optimum dilution by box titration. Mahajan et al. (1976) obtained higher sensitivity by the use of diluted and standardised antigen. Kagan (1977) advised the use of antigen with 125 to 150 microgram N/ml and dilution of the same for obtaining maximum sensitivity.

Casoni's Intradermal Test

The intradermal test for the detection of hydatidosis in man and animals introduced by Casoni (1911) had been used

as a diagnostic procedure and as an epidemiological tool. The diagnostic feature of the test is an urticarial wheal and diffuse oedema at the site of injection of hydatid fluid (antigen) in patients (Rackemann and Stones, 1927). Studies conducted by several workers on proven cases of hydatidosis showed high degree of reliability of the test (Lass and Nitzani, 1954; Carta, 1956; Katsilambros, 1963; Arabatzis and Papapanagoitou et al., 1963; Panaitescu, 1964; Abou-Daoud, 1965; Mirdamadi and Sadatzadeh, 1968; Inanco and Lupasco, 1968; Reddy et al., 1968; Patricia Bradstreet, 1969; Baidaliev, 1970; Matus et al., 1973; Yarzabal et al., 1975; Garabedian and Arslanian, 1976 and Mahajan, et al., 1976). But conflicting reports on the sensitivity of the test had been made as evidenced by reports (Boko, 1958; Jolly, 1958; Garabedian et al., 1959; Abou-Daoud, 1965; Sorice, 1966; Cherubin, 1969; Gill, 1972; Iyer, 1972; Pythal, 1974 and Schantz et al., 1975). Hutchison (1930) had reported that a positive response in the test could not be considered as a conclusive evidence of the presence of the disease. Bulgakov (1958) has observed that, though Casoni's rection was specific for echinococcosis, if negative, it did not exclude the presence of hydatid cysts. Cross reactions were observed in patients with carcinoma (Arabatzis and Papapanagoitou, 1963 and Yarzabal et al., 1975). Similar cross reactions were reported in cases of schistosomiasis (Cherubin, 1969). Mansour Kamal (1963)

reported cross reactivity in cases of tuberculosis. Apt and Kneirim (1970) pointed out that the sensitivity of intradermal test varied with the localisation of cyst. Gill (1972) observed cross reactions in buffaloes harbouring infections of Fasciola gigantica, Gigantocotyle explanatum and Setaria digitata and recorded that the intradermal test had no diagnostic value. Cross reactions were also observed in cases of infection with Hymenolepis nana, Taeniasis and Fascioliasis (Schantz et al., 1975).

Casoni's test was used as an epidemiological tool by Wolfang and Pook(1956); Casley-Smith, (1959); Cameron (1960); Schwabe (1968); Reddy et al. (1968); Matus et al. (1973) and Alencar et al. (1975). According to Kagan (1968) casoni's intradermal test has tremendous potential for use both as a diagnostic and epidemiological tool, if standardised antigens are used and standardised methods of performing the test are adopted.

The antigen used in casoni's intradermal test is usually hydatid cyst-fluid of human or animal origin. The antigenicity of hydatid fluid was correlated to the presence of protoscolices by Fairley (1922). Carbone and Lorenzetti (1957) attributed the variation in antigenicity of hydatid fluid to fertility of the cyst. Deproteinised hydatid fluid was used as antigen by Boko (1958). Carta (1956) used lyophilised hydatid fluid as antigen and obtained good results.

Jolly (1958) obtained 37.8% positive results by using human hydatid fluid. Pauluzzi (1964) showed that hydatid sand, cystic membranes, lamellar and germinal membranes with or without scolices were antigenically rich. Fischman (1965) found human hydatid fluid to be superior as antigenic source, and that fertility of the cyst was related to the antigenicity. Hariri et al. (1965) tested several lots of hydatid fluid from man, sheep, donkey, cattle, camel and gerbil and found sheep hydatid fluid as the most suitable antigen since highest concentration of antigen was detected in the fertile hydatid cyst-fluid obtained from the livers of sheep.

Casoni's antigen, prepared by boiling hydatid fluid, was reported to be highly sensitive by Katsilambros (1965); Orihara (1967) and Yarzabal et al. (1975). Filtration and freezing was the technique adopted by Alencar et al. (1975) for the preparation of antigen. Garabedian and Arslanian (1976) reported that the antigen for casoni's intradermal test should not be filtered by seitz filter as it retains the reactive proteins. They suggested the antigen to be kept in waterbath at 60°C for one hour on three consecutive days, the sterility tested and then used.

According to Kagan (1968) the high degree of non-specific reactions in the intradermal test might be due to the use of non-standardised antigen and that the specificity

of the test increased as the concentration of antigen-nitrogen decreased. He recommended the use of hydatid fluid antigen standardised to contain 15 to 25 microgram nitrogen/ml. This recommendation was endorsed by Mahajan et al. (1976) and the World Health Organisation (Mann, 1978).

M A T E R I A L A N D M E T H O D S

MATERIALS AND METHODS

Incidence of Hydatid cyst and *Cysticercus bovis*

The incidence of hydatid cyst and *Cysticercus bovis* was studied in cattle, buffaloes, sheep and goats brought for slaughter at the following slaughter houses:

1) Municipal slaughter house, Trichur.
2) Municipal slaughter house, Alleppey.
3) Municipal slaughter house, Palghat.
4) Municipal slaughter house, Pollachi.
5) Corporation slaughter house, Trivandrum.
6) Panchayat slaughter house, Munnar.
7) Licensed slaughter house at Kundaly Estate, Munnar Panchayat.
8) Licenced slaughter house at Chenduvurrai Estate, Munnar Panchayat.

Incidence of Hydatid cyst:

In cattle, buffaloes, sheep and goats, the following organs were carefully examined for the presence of hydatid cyst: liver, lungs, heart, spleen and kidney. The right and left lungs were seperately examined for the presence of cyst.

Incidence of *Cysticercus bovis*:

The incidence of *Cysticercus bovis* was studied by thoroughly searching the heart, tongue, masseter muscles,

diaphragm, shoulder, lumbar and gluteal muscles in cattle. The muscles were incised and examined for the presence of bladder worms.

Fertility of Hydatid cysts

Fertility of all the hydatid cysts encountered was checked by aspirating the cystic fluid and/or opening the cyst wall and examining for the presence of brood capsules and protoscolices.

Studies on Total Nitrogen, Protein, Sodium and Potassium content of Hydatid fluid

The hydatid fluid for the analysis was collected aseptically from the cysts obtained from the infected animals during post-mortem and specifically marked as to its fertility and location. In all cases, the cyst-fluid was used for analysis on the same day or kept in sterile containers at $4^{\circ}C$ for not more than 48 hours.

Estimation of Total Nitrogen:

Estimation of total nitrogen was done using Kjeldahl method (Oser, 1965).

Reagents:

1) Concentrated Sulphuric acid.

2) Copper sulphate (AR).

3) Sodium sulphate (AR).

4) Sodium hydroxide solution 40%.

5) N/10 sulphuric acid.

6) N/10 sodium hydroxide.

7) Methyl red indicator.

Procedure:

To five ml of hydatid fluid taken in a Kjeldahl digestion flask, added 15 ml of concentrated sulphuric acid, 0.2 gm copper sulphate and five gm of sodium sulphate. A few glass beads were introduced to prevent spurting. The solution was first heated on a low flame till the whole solution became clear. It was then removed from the flame and allowed to cool to room temperature. The digest was then transferred to a 100 ml volumetric flask and made up the volume to 100 ml with distilled water.

Ten ml of N/10 sulphuric acid to which was added a few drops of methyl red indicator was taken in a small conical flask and placed under the dip tube of Kjeldahl distillation apparatus. Ten ml of the reconstituted digest was then transferred to the distillation chamber and sufficient quantity (15 to 20 ml) of 40% sodium hydroxide solution was added. The steam was allowed to pass through the mixture in the distillation chamber for five minutes. Before cutting off the steam supply, the flask containing the sulphuric

acid was lowered and tip of the delivery tube was washed with distilled water. The excess acid in the flask was then titrated against N/10 sodium hydroxide solution. The volume of N/10 sulphuric acid neutralised by ammonia was then calculated. One ml of N/10 sulphuric acid neutralised during the distillation is equal to 1.4 mg of nitrogen and total nitrogen content of the sample was calculated on the basis of this.

Estimation of Total Protein

Estimation of total protein was done using Biuret method (Gornall et al., 1949).

Reagents:

1) Standard solution (stock)

Bovine serum albumen (Sigma)	250 mg
Distilled water (glass distilled)	50 ml

2) Biuret reagent

Copper sulphate (AR)	1.5 g
Sodium potassium tartrate (AR)	6.0 g
Sodium hydroxide 10% solution	300 ml
Distilled water (glass distilled) to makeup	1000 ml

Procedure:

The standard solution was prepared by mixing one ml of the stock solution with four ml of biuret reagent and

five ml of distilled water. The unknown solution was prepared by treating two ml hydatid fluid with four ml of biuret reagent and four ml of distilled water. A blank was also prepared by mixing four ml of biuret reagent and six ml of distilled water. Spectronic 20 (Bausch & Lomb) was used for the calorimetric studies. The equipment was set at 540 wavelength and the blank solution was used to set at zero absorbance. The absorbance of the standard solution and the unknown smaples (hydatid fluid + biuret) were then recorded. From the readings obtained, the total protein content of the samples was calculated as follows:

$$\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times \frac{\text{Concentration of standard}}{\text{volume of unknown taken}} \times 100$$

= mg protein/100 ml.

Estimation of Sodium and Potassium

Sodium and potassium content of hydatid fluid was estimated by Flame photometry (Vogel, 1961).

Reagents:

1) Stock solution of sodium and potassium standard (2 mg Na and 1 mg K/ml)

Sodium chloride (AR)	5.0840 gm
Potassium chloride (AR)	1.9092 gm
Double distilled water to makeup	1000 ml

2) Working standards

Sodium - dilutions to give 2 to 20 ppm

potassium - dilutions to give 1 to 15 ppm

Procedure:

0.2 ml of hydatid fluid was diluted to 25 ml for estimation of sodium and 0.5 ml of hydatid fluid diluted to 25 ml for the estimation of potassium of each samples.

Systronic type 121 Mark II Flame Photometer was used for the estimation. The indane gas supply was turned on, the flame ignited and the air let in. An air pressure of 0.5 Kg/cm^2 and 0.6 Kg/cm^2 was employed for sodium and potassium estimation respectively. The gas pressure was adjusted while keeping distilled water at the spray intake to give a colour-free flame. A calibration curve was prepared for sodium and potassium separately using the working standards. The samples were also measured in the flame photometer as in the case of standards. With the help of calibration curve constructed for sodium and potassium, the concentration of the elements in the samples were interpreted independently.

Indirect Haemagglutination Test

The indirect haemagglutination test was done following the procedure of Garabedian et al. (1957) with suitable modifications.

Reagents:

1) Sheep erythrocytes

2) Tannic acid solutions (Merck) - 1:20,000

3) Antigen

Fertile hydatid fluid obtained from the liver cysts of sheep was tested at 0, 1:25, 1:50 and 1:100 dilutions in buffered saline, pH 6.4, to determine the optimum concentration of antigen for the test. The lowest concentration giving the highest titre with immune serum was found to be 1:25. Hence antigen dilution of 1:25 was used in the test.

4) Phosphate Buffered Saline (PBS)

a) Stock solutions

i) Disodium hydrogen phosphate 0.15 M Na_2HPO_4 solution

Disodium hydrogen phosphate (AR) - 21.3 gm
Distilled water to make - 1000 ml

ii) Potassium dihydrogen phosphate 0.15 M KH_2PO_4 solution

Potassium dihydrogen phosphate (AR) - 20.4 gm
Distilled water to make - 1000 ml

iii) Sodium chloride 0.15 M NaCl solution

Sodium chloride (AR) - 8.8 gm
Distilled water to make - 1000 ml

b) Phosphate Buffered Saline pH 7.2

Stock solution	i	- 76.0 ml
"	ii	- 24.0 ml
"	iii	- 100.0 ml

c) Phosphate Buffered Saline pH 6.4

Stock solution	i	- 32.3 ml
"	ii	- 67.7 ml
"	iii	- 100.0 ml

5) Normal Rabbit Serum as one per cent in phosphate buffered saline pH 7.2

6) Sodium Citrate solution 3.8%

Sodium citrate	- 3.8 gm
Distilled water to make	- 100 ml

Procedure:

Five ml of sheep blood was collected from healthy sheep in sterile tube containing six ml sodium citrate solution (3.8%). The blood was centrifuged at 2,000 rpm for 10 minutes, the supernatant plasma, and WBC layer were removed using a pipette. The erythrocytes were then washed three times in PBS pH 7.2 by centrifuging at 1,700 r.p.m. for five minutes every time. The packed cells were adjusted to a 2.5% suspension in PBS pH 7.2.

Tanning of the suspended cells was done by adding an equal volume of 1:20,000 tannic acid solution and incubating the mixture in a waterbath at 37^{o}C for 15 minutes.

The tanned cells were removed from the waterbath and centrifuged for five minutes at 1,700 r.p.m. After decanting the supernatant, the cells were washed once with PBS pH 7.2 and resuspended to a 2.5% suspension in PBS pH 6.4.

An equal volume of 1:25 dilution of antigen in PBS pH 6.4 was added to the tanned cell suspension and the mixture was incubated in a waterbath at 37°C for 15 minutes for sensitizing the tanned cells.

The antigen treated cells were removed from the waterbth and centrifuged for five minutes at 1,700 r.p.m. The packed cells were washed twice with one per cent normal rabbit serum in PBS pH 7.2.

After a final pack by centrifugation at 1,700 r.p.m. for 10 minutes, the sensitised cells were reconstituted to a 1.5% suspension in one per cent normal rabbit serum diluted in PBS pH 7.2.

The suspected sera were inactivated by incubating in a waterbath at 56°C for 30 minutes before utilising them for conducting the test. Serial dilutions ranging from 1:5 to 1:1,280 were made in agglutination trays, each well holding 0.5 ml of the diluted serum. One per cent normal rabbit serum in PBS pH 7.2 was used as diluent.

To each of the wells containing the sera, 0.05 ml of the sensitised cell suspension was added, the agglutination

trays were gently shaken and then allowed to settle for two hours at room temperature. Finally the trays were kept overnight in the refrigerator.

The readings were taken after 12 hours. Haemagglutination in the wells were recorded noting the formation of mat or carpet of cells covering the bottom of the well. The maximum dilution at which haemagglutination was observed was recorded in all sera samples.

The diluent, tanned cells, sensitised cells and negative sera were used as controls.

Casoni's Intradermal Test

Antigen:

1) Whole Hydatid cyst fluid (HCF)

Cystic fluid from fertile hydatid cysts collected aseptically from infected sheep livers was pooled, filtered to remove hydatid sand and other sediments and stored in deep freezer at $-10^{\circ}C$ until use. The nitrogen content of the cystic fluid was estimated by Kjeldahl method (Oser, 1965).

2) Standardised Antigen (SA)

The antigen was prepared by diluting HCF with normal saline to contain 25 microgram nitrogen/ml (Kagan et al., 1966).

Procedure:

The intradermal test was done on cattle brought for slaughter by injecting 0.2 ml of the whole hydatid fluid antigen on the right caudal fold and same quantity of standardised antigen on the left side. The thickness of the caudal folds was noted before the inoculation of the antigen by the use of a vernier calipers. The thickness was again noted 30 minutes post-inoculation. An increase in thickness of seven mm and above was considered as positive. The animals were examined at post-mortem for the presence or absence of hydatid cysts.

RESULTS

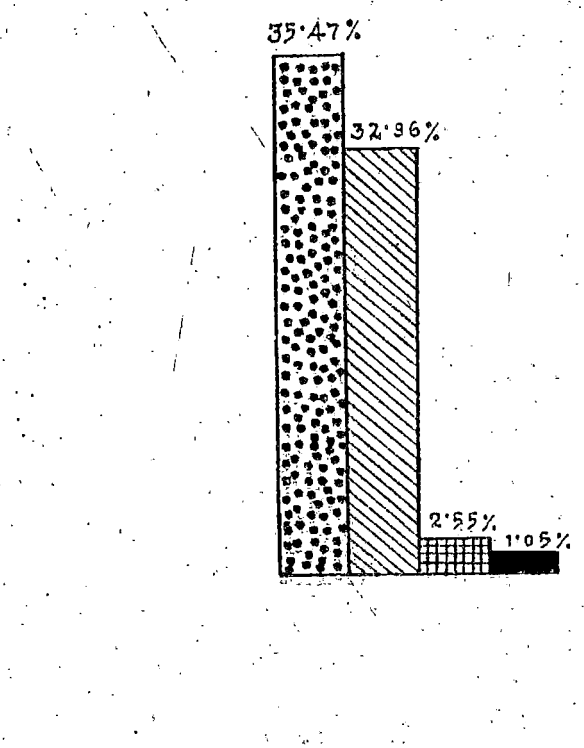
RESULTS

Incidence of Hydatidosis

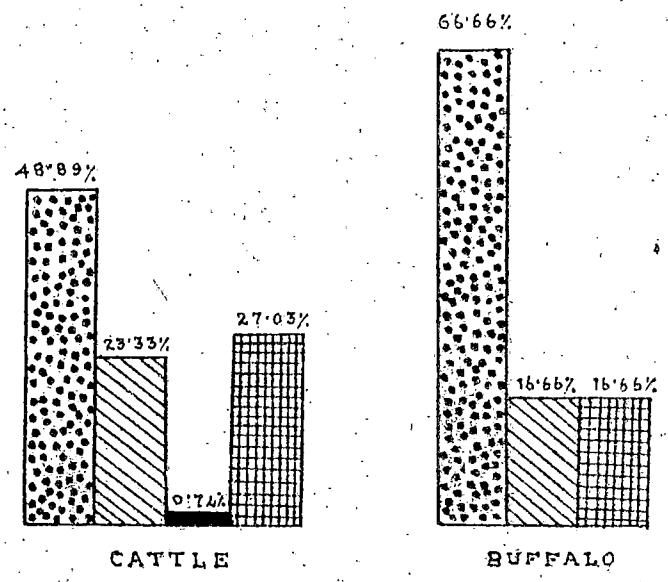
A total number of 2,602 animals (761 cattle, 91 buffaloes, 705 sheep and 1,045 goats) was examined for the presence of hydatid cysts. The incidence in these animals is shown in Tabel I. The overall incidence of hydatidosis was found to be 12.64%. Among the different species, cattle was found to be infected most frequently (35.47%) and was closely followed by buffaloes (32.96%). In sheep and goats the incidence was comparatively lower (2.55% and 1.05% respectively).

Data regarding the involvement of various organs with hydatid cyst are given in Table II and III. Lungs, liver, spleen and heart were found to be affected; the involvement of spleen and heart being noticed only in cattle. Hydatid cysts were found in one or more organs in the same animal. In all animals, the lung and liver were the most frequently affected organs. In cattle, buffaloes and goats, the involvement of lungs alone was higher (48.89, 66.66 and 63.64% respectively) than liver alone (23.33, 16.66 and 36.36% respectively) whereas in sheep, the liver alone was involved in 55.55% and lung alone in 38.88% cases. Only in two out of 270 positive cases (cattle) the spleen was found to be exclusively affected (0.74%).

INCIDENCE OF HYDATIDOSIS IN DIFFERENT SPECIES OF ANIMALS



INCIDENCE OF HYDATID CYST IN DIFFERENT ORGANS OF AFFECTED ANIMALS



In cattle, the involvement of more than one organ in an animal was noticed in 73 out of 270 positive cases (27.03%); the lung, liver and spleen in one; lungs and spleen in three; lungs, liver and heart in three and lungs and liver in 66 animals. In buffaloes and sheep, the lungs and liver were simultaneously affected in five out of 30 (16.66%) and one out of 18 (5.55%) cases respectively. Among 11 goats found positive, none was found harbouring cysts in more than one organ. In cattle, buffaloes and goats, the cysts were more often seen in lungs (75.92, 83.33 and 63.64% respectively) than liver (49.25, 50.00 and 36.36% respectively) when the total involvement of any organ was taken into account. The frequency of infection was found to be higher in the right lung of cattle, buffaloes and sheep (62.10, 58.80 and 58.34% respectively) and in goats both the lungs were equally affected.

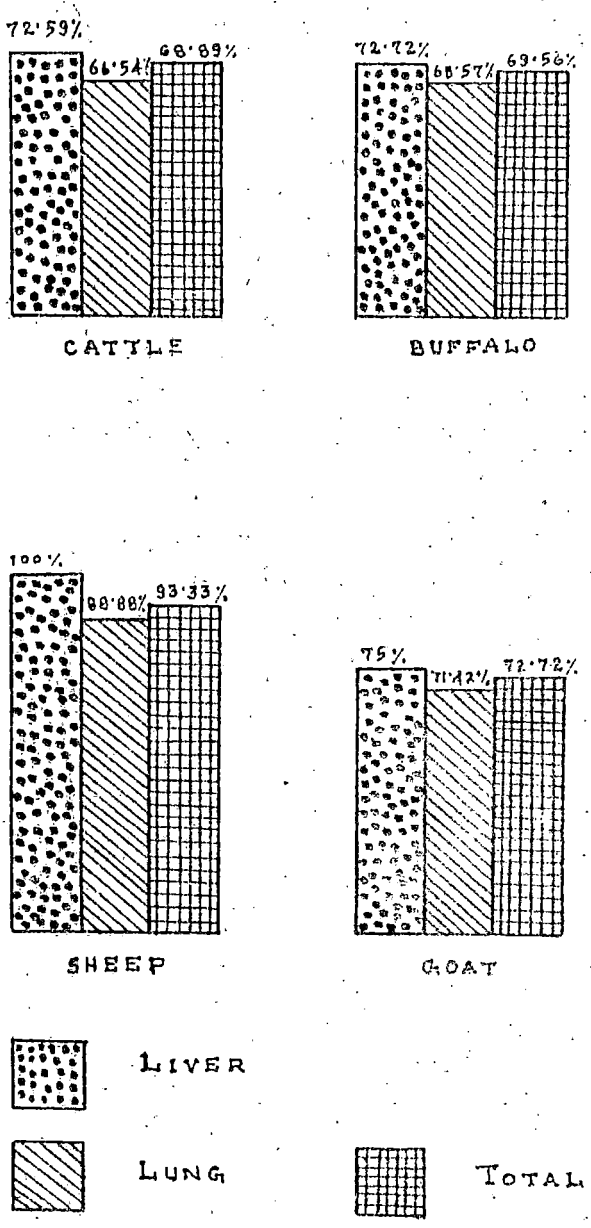
Incidence of Cysticercus bovis

Out of 761 cattle examined for the presence of bladder worms, 6 were found (0.74%) affected.

Fertility rate of Hydatid cyst

The percentage of fertile cysts found in various organs of different species of animals examined is furnished in Table IV. In all the species of animals, the liver cysts

Percentage of fertile cysts and its organwise distribution in Cattle, buffaloes, sheep and goats.



were found to be more fertile than the lung cysts, the percentage of fertility being 72.59 in cattle, 72.72 in buffaloes, 100 in sheep and 75 in goats. The fertility of hydatid cysts in the lungs of sheep was higher (88.88%) than in other animals. All the cysts recovered from the heart of cattle were found fertile whereas only 75% of the cysts found in spleen were fertile. The overall percentage of fertile cysts in cattle, buffaloes, sheep and goats were 68.89, 69.56, 93.33 and 72.72 respectively.

Analysis of some of the constituents of Hydatid cyst fluid

The result of the estimation done on sterile and fertile hydatid cyst-fluid from liver and lungs of infected animals for nitrogen, protein, sodium and potassium are tabulated (Table V). The nitrogen content of the fluid recovered from sterile and fertile hydatid cysts of liver was found to be 61.9 mg and 57.74 mg per 100 ml respectively. The corresponding values for cystic fluid obtained from lungs were 49.78 and 61.37 mg/100 ml respectively. The protein content was found to be higher in fertile cysts than in the sterile cysts of both liver and lungs. The mean protein content of cystic fluid from fertile liver cysts was 161.38 mg/100 ml and that of lungs 88.85 mg/100 ml. The sodium and potassium content of the cystic fluid was found higher in sterile cysts than fertile cysts of both

liver and lungs. The fluid from sterile cysts of liver had an average of 1,589 ppm of sodium and 312 ppm of potassium whereas the sterile lung cysts had an average sodium and potassium content of 1,639 ppm and 304 ppm respectively.

Indirect Haemagglutination Test

The results of the indirect haemagglutination test conducted on sera samples from 102 positive animals are shown in Table VI. Thirtyeight sera samples gave a titre of 1:40 or below (37.25%) and in 64 samples the titre was 1:80 or above (62.75%). The highest titre obtained was 1:640 from two sera samples (1.96%). Out of 37 sera collected from cattle harbouring lung cysts alone, 22 samples (59.45%) gave a titre of 1:40 or below. Only five samples from this group gave a titre of 1:160.

Among 32 sera samples collected from cattle harbouring liver cysts alone, nine sera (28.12%) gave low titres of 1:40 or below and 23 sera samples (71.88%) gave 1:80 or above. The maximum titre of 1:320 was obtained from two samples (6.25%) from among this group. Out of 33 sera samples tested from animals harbouring cysts in more than one organ (mainly liver and lung) only seven (21.21%) showed low titres of 1:40 or below and 26 (78.99%) samples 1:80 or above. The highest titre of 1:640 recorded in the test was obtained from two samples (6.60%) collected from cattle

harbouring cysts in the liver, lung and heart. Twentyfour sera samples collected from uninfected animals gave a titre of 1:40 or below.

The result of the protein content of 23 hydatid-cyst fluid and the indirect haemagglutination titre obtained from 23 sera samples collected simultaneously from the same infected animals are shown in Table VII. Fifteen cystic fluid samples were found to contain 100 mg protein/100 ml or above (Group A) and eight samples 83 mg protein/100 ml or below (Group B). In Group A, 12 sera samples (80%) gave a titre of 1:80 or above and only three sera samples (20%) showed titre of 1:40. In Group B, only one (12.5%) of the corresponding sera sample showed a titre of 1:80 and the rest seven sera samples (87.5%) showed a titre of 1:40 or below.

The hydatid cysts recovered from 92 cattle were examined for their fertility and the sera obtained from these were tested by indirect haemagglutination. The agglutination titre in relation to the fertility status is tabulated (Table VIII). Among 56 cattle which harboured fertile cysts, sera samples of 14 of them (25%) gave a titre of 1:40 or below and 42 (75%) gave titres 1:80 or above. Out of 36 sera samples from cattle which harboured sterile cysts, 17 samples (47.22%) gave a titre of 1:40 or below and only 19 samples (52.78%) gave titres 1:80 or above

Casoni's Intradermal Test

The results of the Casoni's test are shown in Table IX. Ninetythree cattle were tested using both whole hydatid cyst-fluid and standardised antigen. Fiftyseven cattle were subsequently found to harbour hydatid cyst at post-mortem. Fiftysix out of 57 positive cases gave positive reactions (98.24%) to both antigens. One animal which was affected did not react to the test with either antigen.

Out of 36 cattle found negative at post-mortem, positive reactions to the test was noticed in 4 cattle (11.11%) by whole hydatid cyst-fluid antigen whereas in the test using standardised antigen, false positive reaction was noticed only in two cases (5.5%).

Table I. Incidence of hydatidosis in different species of animals slaughtered in Kerala

Species	Number of animals examined	Animals infected	
		Number	Percentage
Cattle	761	270	35.47 **
Buffaloes	91	30	32.96 **
Sheep	705	18	2.55
Goat	1,045	11	1.05
Total	2,602	329	12.64

** Significant at 1% level compared to incidence in sheep and goats.

Table II. Organ-wise incidence of hydatid cyst in different species of animals

Species	Total number	Lung alone		Liver alone		Spleen alone		More than one organ involved		Total involvement of lungs		Total involvement of liver	
		No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage
Cattle	270	132	48.89*	63	23.33	2	0.74	73	27.03	205	75.92	133	49.25
Buffalo	30	20	66.66*	5	16.66			5	16.66	25	83.33	15	50.00
Sheep	18	7	38.88	10	55.55			1	5.55	8	44.44	11	61.11
Goat	11	7	63.64	4	36.36					7	63.64	4	36.36

* Significant at 5% level compared to involvement of liver

Table III. Showing distribution of hydatid cyst in right and left lung

Species	Total number of cyst examined	Right lung		Left lung	
		Number	Percentage	Number	Percentage
Cattle	275	173	62.10**	102	37.90
Buffalo	34	20	58.80	14	41.20
Sheep	12	7	58.34	5	41.66
Goat	10	5	50.00	5	50.00

** Significant at 1% level than left lung.

Table IV. Fertility rate of hydatid cysts in various organs and different species of animals

Location of the cyst	Cattle			Buffalo			Sheep			Goat		
	Total	Fertile	Percentage	Total	Fertile	Percentage	Total	Fertile	Percentage	Total	Fertile	Percentage
Liver	135	98	72.59	11	8	72.72	12	12	100	4	3	75.00
Lungs	275	183	66.54	35	24	68.57	18	16	88.88	7	5	71.42
Heart	4	4	100									
Spleen	4	3	75.00									
Total	418	288	68.89	46	32	69.56	13	28	93.33	11	8	72.72

Table V. Comparision of some of the constituents of sterile and fertile hydatid cyst fluid from lungs and liver of cattle

Constituent	Liver-sterile		Liver-fertile		Lung-sterile		Lung-fertile	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Nitrogen	47.6-77.4	61.9	47.6-72.7	57.74	19.6-67.2	49.78	33.6-95.2	61.37
Protein	55.5-150.0	95.32	66.6-416.6	161.38*	33.3-111.1	61.76	50.0-183.3	88.85*
Sodium	1525-1700	1589*	1525-1587	1552	1500-1813	1639*	1475-1613	1537
Potassium	260-390	312*	270-295	277	250-360	304*	240-315	265

Nitrogen and protein expressed as mg/100 ml

Sodium and potassium expressed as parts/million

* Significant at 5% level

Table VI. Indirect haemagglutination titre of 102 sera samples obtained from infected cattle

Organ affected	upto 20	40	80	160	320	640	Total
Lung	8	14	10	5	-	-	37
Liver	4	5	9	12	2	-	32
Lung & liver	2	5	7	10	7	2	33
Total	14	24	26	27	9	2	102

Titers 1:80 or above considered as positive.

Table VII. Showing the relation of protein content of cystic fluid and indirect haemagglutination titre

Serial number	Protein content of hydatid fluid in mg/100 ml	Haemagglutination titre
1	16	1:10
2	33	1:20
3	50	1:20
4	50	1:20
5	66	1:40
6	66	1:40
7	66	1:40
8	83	1:160
9	100	1:40
10	100	1:40
11	100	1:40
12	100	1:320
13	100	1:80
14	116	1:80
15	116	1:80
16	133	1:80
17	133	1:160
18	150	1:80
19	183	1:80
20	183	1:160
21	183	1:160
22	200	1:640
23	416	1:160

Table VIII. Fertility of the cyst in relation to indirect haemagglutination titre

Fertility status	Titers upto 20	40	80	160	320	640	Total
Fertile	7	7	16	18	6	2	56
Sterile	6	11	8	9	2	-	36

Table IX. Showing the result of Casoni's test in cattle using whole hydatid fluid and standardised antigens

Antigen	Total number of animals tested	Reaction of the test in 57 cattle found positive at post-mortem		Reaction of the test in 36 cattle found negative at post-mortem	
		Positive	Negative	Positive	Negative
Whole hydatid fluid	93	56	1	4	32
Standardised antigen	93	56	1	2	34

D I S C U S S I O N

DISCUSSION

Incidence of Hydatidosis

The incidence of hydatidosis in animals has been studied by many workers. The present investigation has revealed a significantly higher incidence of hydatidosis in large animals (cattle and buffaloes) than in small animals (sheep and goats). Similar higher incidence in large animals compared to smaller animals has been reported by Endrajat (1964); Reddy et al. (1968); Sadananda (1969); Iyer (1972) and Pythal (1974). Higher rate of incidence in large animals could be attributed to the chance of acquiring infection through the larger quantum of food consumed by them and the longer span of life.

Among cattle and buffaloes, hydatid infection is found more frequently in the former. This observation is in agreement with that of Sundaram and Natarajan (1960); Reddy et al. (1968); Iyer (1972) in Kerala and Pythal (1974). But Maqsood (1946) in Lahore and Iyer (1972) from Calcutta have reported higher frequency of occurrence of hydatidosis among buffaloes. The difference in the incidence among cattle and buffaloes was, however, not found to be significant.

The previous reports from Kerala show a lower rate of incidence in cattle (22.9% by Iyer, 1972 and 31.43% by

Pythal, 1974) than the present observation (35.47%). There appears to be a steady increase in the frequency of hydatidosis among the animals slaughtered in Kerala. Since majority of these animals are brought for slaughter from adjoining States, the above findings are to be viewed as an increase in the environmental contamination in those areas. The possibility of an increased source of infection to the definitive hosts and thereby to man and domestic animals in Kerala is to be reckoned with.

The incidence of hydatidosis in buffaloes is found to be 32.96%. Similar reports have been made by Gill and Rao (1967); Rao (1968) and Pal and Sinha (1970). Compared to the previous reports from Kerala by Iyer (1972) and Pythal (1974), the present incidence is found to be higher.

The incidence in sheep and goats is comparatively low and is in agreement with reports from other parts of the country (Maplestone, 1933; Chuttani and Chug, 1957; Sachdev and Talwar, 1960; Chitkara, 1957; Endrajat, 1964; Sadananda, 1969 and Iyer, 1972). Higher rates of incidence have been reported by a few workers (Reddy et al., 1968; Iyer, 1972 in Calcutta and Pythal, 1974). Most of the goats slaughtered are of local origin. The slight increase in the incidence noted in the present study (1.05%) compared to the observation made by Iyer [(1972), (0.69%)] may be

considered as an index of increased environmental contamination.

Organ-wise involvement shows that, in cattle, buffaloes and goats, the lung and liver are most commonly affected with hydatids and between these two organs, lung is more often involved. This finding is in agreement with the reports of Chuttani and Chug (1957); Sundaram and Natarajan (1960); Sharma and Chitkara (1963); Gill and Rao (1967); Rao (1968); Reddy et al. (1970); Pandy (1971a) and Hegde et al. (1974). El-kordy (1946) and Pythal (1974) observed that hydatid cysts occurred more frequently in liver than lungs of sheep, cattle and buffaloes. In the case of sheep, the liver appears to be more frequently involved than lung and in this respect, the present observation is in accordance with that of the latter authors. Gill and Rao (1967) have stated that the focus of infection is largely dependent on the host.

The high incidence of hydatid cysts in the lungs of cattle and buffaloes suggest that lung is a major predilection site. The lung is found to be more frequently involved even when the total involvement of all organs is taken into account. Reddy et al. (1970) have suggested that inhalation may be another mode of transmission of the infection to the animals. The present investigation shows a higher frequency

of occurrence of hydatid cysts in the right lung than the left in the case of cattle, buffaloes and sheep. In cattle, the frequency was found to be statistically significant. This finding is in agreement with the observations of Yamashita et al. (1957); Gill and Rao (1967) and Pandey (1971a). Contrary to this, Rao and Mohiyuddin (1974) found the left lung more frequently affected. The higher frequency of occurrence of hydatid cyst in the right lung may be due to, the supply of greater volume of blood through a wider and longer right pulmonary artery and a larger area of right lung than the left (Getty, 1976).

Between spleen and heart, the former appears to be more frequently involved. This is in agreement with the findings of Gill and Rao (1967); Reddy et al. (1970) and Pythal (1974).

Incidence of Cysticercus bovis

The present investigation shows an incidence of 0.74% among cattle slaughtered in Kerala. An investigation made by Pythal (1974) showed an incidence of 9.28%. Krishnan and Ranganathan (1972) has recorded only one per cent incidence in the neighbouring State of Tamil Nadu while Nayak et al. (1973) has observed 2.5% of its incidence in the same region. The incidence of Cysticercus bovis is thus found to vary from place to place and from time to time. This

may be due to factors connected with extent of human infection, the mode of sewage disposal, the environmental sanitation and the grazing facilities for cattle in the area.

Fertility rate of Hydatid cyst

Fertile cysts are found most frequently in sheep, followed by goats, buffaloes and cattle in that order. This is in agreement with the findings of Reddy et al. (1968 & 1970) and Iyer (1972). It has been reported that hydatid cysts in cattle are generally sterile (Sundaram and Natarajan, 1960; Gemmell, 1960; Anantharaman, 1966; Kagan, 1968; Hegde et al., 1974 and Rao and Mohiyuddin, 1974). Higher rates of fertility in cysts of cattle have been recorded by other investigators (Gill and Rao, 1967; Reddy et al., 1968 & 1970; Iyer, 1972; Pythal, 1974 and Matossian et al., 1977). The present finding is in agreement with those of the latter investigators. Gill and Rao (1969) were of openion that fertility is related to the host-parasite interaction.

In all cases, liver cysts are found to be more fertile than lung cysts though the difference is not statistically significant. Similar findings have been reported by Reddy et al. (1968 ' 1970) in cattle, buffaloes and goats; Singh and Kuppuswamy (1969) and Iyer (1972) in cattle, buffaloes and goats. Higher incidence of fertile cysts in the lung has been reported by Reddy et al. (1970) in sheep;

Pandey (1971a) in goats; Iyer (1972) in sheep and Pythal (1974) in cattle, buffaloes and goats.

Analysis of some of the constituents of Hydatid fluid

The mean total nitrogen content of the hydatid cyst-fluid does not show any significant difference either between the affected organs studied or fertility status. Goodchild and Kagan (1961) and Kagan and Norman (1963) have reported a low nitrogen content in bovine cystic fluid (3.2 to 7 mg/100 ml). The present study revealed a higher nitrogen content (57.63 mg/100 ml) in the cystic fluid. This may be due to the higher fertility of the hydatid cysts encountered in Indian cattle. Pandey (1971b) has reported a nitrogen content of 59 to 90 mg/100 ml of cystic fluid recovered from goats.

The protein content of the cystic fluid shows a significantly higher value in the fertile cysts than sterile ones. The protein content is found to vary between the cystic fluids of lung and liver. The higher protein content of fertile cyst-fluid from liver was found to be statistically significant. Carbone and Lorenzetti (1957) have reported the protein content in hydatid cyst-fluid to vary from 23.7 to 166 mg in cattle, 30.2 to 213 mg in sheep and 17.3 to 227 mg/100 ml in man. In goats, the protein content of cystic fluid ranges from 56.25 to 73.75 mg/100 ml

according to Pandey (1971b). The present observation of a significantly higher protein content in the fertile cystic fluid is in agreement with the findings of Sita Devi et al. (1971), though the values are found to be different. The maximum protein content is found to be in the fertile liver cyst. This difference in the protein content of fertile cystic fluid from liver and lungs is not seen reported by Sita Devi et al. (1971). According to Kagan (1977), fertile hydatid cysts in the liver has a higher protein content and the present finding is in agreement with that. The higher concentration of protein in fertile cystic fluid may be due to absorption of protein molecules by the active larva (Hustead and William, 1977).

The present study indicate that, though the total nitrogen content may be the same, the distinguishing difference between sterile and fertile hydatid cyst-fluid is in their protein-nitrogen content. The absence of significant difference in the total nitrogen content of sterile and fertile cyst-fluid may be attributed to the concentration of non-protein nitrogen. Schwabe (1959) has reported that urea permeates through the laminated membrane of the cyst readily and higher concentration of urea cause withdrawal of the germinal membrane resulting in differential permeability. In a sterile cyst, differential permeability for urea may not be effected through the germinal membrane.

The mean sodium content of hydatid cyst-fluid has been reported to be 171 mE/litre by Mazzocco (1923); 114 mE/litre (Schwabe, 1959) and 112 mE/litre (Rotunno et al., 1974). The present study indicate a mean sodium content of 1,576 ppm (68.5 mE/litre) in bovine hydatid cyst-fluid. The sodium content in the cystic fluid is found to be significantly higher in sterile cysts while no appreciable difference is noticed in relation to the organ involved. Reports do not show any observation in this regard.

The potassium content of hydatid cyst-fluid of bovine origin is found to be 298 ppm (7.6 mE/litre). This observation is in agreement with the report of Mazzocco (1923). Similar to sodium concentration, potassium content is also found to be significantly higher in sterile cysts, though there is no difference in relation to the organ involved. Schwabe (1959) found that the laminated membrane of hydatid cyst is readily permeable to potassium also and may cause withdrawal of germinal membrane from laminated membrabe. Thus various biochemical constituents of the cystic fluid may be associated with the permeability of cyst wall as suggested by Codounis and Polydorides (1936). Further, higher concentration of potassium and urea, as mentioned earlier, in hydatid cyst may have a bearing on the sterility of hydatid cyst.

Indirect Haemagglutination Test

IHA test in cattle in relation to the organ involved:

Twentyfour sera samples obtained from cattle, which were later found to be not harbouring hydatid cysts, showed haemagglutination titre upto 1:40 in 22 samples (91.66%). Only 8.44% of the sera samples showed a titre of 1:80. None of these samples gave a titre of above 1:80. Hence titres of 1:40 or below cannot be considered for diagnostic purposes since the test is found to give false positive reaction with less than 10% chance of error at 1:80 titre.

Haemagglutination titre of sera samples from 102 proven cases of hydatidosis showed a titre of 1:80 or above in 62.75% and the rest showed a titre of 1:40 or below. The reliability of the test can be assumed to be 62.75% at a titre of 1:80 or above. Pinelli (1961) found the test not specific in cattle and sheep, and Pauluzzi and Castagnari (1965) reported that the test was inconclusive for bovine and swine. Iyer (1972) obtained 52.5% reliability at a titre of 1:80 or above. Though higher titres have been obtained in human cases, the agglutination titre in positive animal sera samples were generally found to be low (Iyer, 1972 and Pythal, 1974).

In the present study, when the cyst is found in the lung, reliability of the test is only 40.55% and the maximum

titre obtained is also lower. This is in agreement with the reports of Todoroff and Yurukova (1974) and Yarzabal et al. (1975) in human pulmonary hydatidosis. When the liver is involved, the reliability of the test is increased (71.88%). This finding is in agreement with the reports of Jolly (1958); Arabatzis and Papapanagoitou (1963); Abou-Daoud (1965); Apt and Knierim (1970) and Mahajan et al. (1973) in human infections, and in animals by Iyer (1972) and Pythal (1974). Reliability of the test is dependent on the organ affected. According to Kagan (1963) the site of the cyst in the body affects the titre. The low titre in pulmonary hydatidosis may be related to the immunological mechanism of the host and permeability of the cyst membrane to antigen according to Kagan et al. (1966) and Apt and Knierim (1970). When both liver and lung are involved simultaneously, reliability of the test is found to increase (78.99%). Kagan (1976) feels that sensitivity of the test may be related to pathogenicity of infections and the difference in the biological interactions of the host with local strain of Echinococcus granulosus.

Haemagglutination titre in relation to fertility of the cyst:

A titre of 1:80 or above is seen in 75% of the sera samples obtained from animals harbouring fertile cysts. When the cysts are sterile, only 52.78% gave a titre of

1:80 or above. This increased sensitivity can be attributed to enhanced antigenicity of the fertile cyst. Fairley (1922) has correlated the presence of protoscolices in cystic fluid to antigenicity and Carbone and Lorenzetti (1957) has attributed the variation in the potency of antigen to fertility. Sensitiveness of the test was found to be more in human patients harbouring fertile cyst (Abou-Daoud, 1965). Similar relationship between the fertility, antigenicity and antibody formation has been suggested by Kagan et al. (1966) and Pauluzzi (1969).

Haemagglutination titre in relation to protein content of the cyst-fluid:

The results show a relationship between protein content of the cystic fluid and agglutination titre in sera obtained from the same animal. It has been reported by Pirosky et al. (1948) that the antigenic property of the various fractions isolated from the cystic fluid is more or less directly related to their protein content. Ballad et al. (1977) has observed that the protein fraction of the cystic fluid is the most immunogenic constituent. Experimental studies on rats by Senutaite (1976) has also proved that hydatid protein antigen stimulate antibody production. In the present study, higher titres are observed in sera samples obtained from animals harbouring hydatid cyst with more than 100 mg protein/100 ml in the cystic fluid.

Casoni's Intradermal Test

Positive reaction was noticed in 98.24% of the infected cattle while 11.11% of the non-infected group also gave similar reaction with whole hydatid fluid antigen. Boko (1958) has reported positive reactions in all 10 infected cattle. Pythal (1974) reported 75% positive reaction in infected cattle. When standardised antigen is used, no difference is seen in the reactivity of the infected group whereas false positive reactions are noticed only in 5.5% in the non-infected group. The only positive animal which did not react to the test, harboured a small lung cyst. False negative reaction has also been observed by Iyer (1972). In the non-infected group which gave positive reaction, two out of four cattle harboured Fasciola infection in the liver. Similar cross reactions has been observed in buffaloes by Gill (1972) and in man by Schantz et al. (1975). Standardised antigen having low nitrogen content as advocated by Kagan et al. (1966) appears to reduce false positive reaction in cattle also. The false positive reactions even with whole hydatid fluid antigen is seen only in 11.11%. This may be due to the comparatively low total nitrogen content (1.12 mg/ml) of the whole hydatid fluid antigen. High sensitivity of the test has been reported by Mirdamadi and Sadatzedeh(1968)

and Mahajan *et al.* (1976) by utilisation of standardised antigen containing 25 microgram N/ml in human patients. Hence low nitrogen content reduces false positive reaction and increases the specificity of the test.

S U M M A R Y

SUMMARY

Hydatidosis is a major public health and economic problem throughout the world. An assessment of this problem in domestic animals in an area would give an index of environmental contamination. Hence a study on the incidence of hydatid cyst among animals slaughtered in Kerala was undertaken. Investigation was made on the incidence of hydatidosis in animals slaughtered, in relation to species, organ involved, fertility of the cysts, biochemical constituents, viz; Nitrogen, Protein, Sodium and Potassium; and diagnostic tests: indirect haemagglutination, and intradermal tests. The incidence of cysticercosis in these animals was also simultaneously studied considering its importance on human health.

Data on incidence were collected by examining animals at post-mortum for the presence of hydatid cysts and Cysticercus bovis, in different slaughter houses of Kerala. The fertility of hydatid cysts was studied by examining the cysts for the presence of brood capsules and protoscolices. Estimation of total nitrogen was done by Kjeldahl method, protein by Biuret method and sodium and potassium by Flame Photometry. Indirect haemagglutination test was done on sera samples collected from infected and uninfected cattle brought for slaughter.

Casoni's intradermal test was done using whole hydatid fluid and standardised antigen on cattle and the results were confirmed during post-mortem.

The studies on the incidence of hydatidosis revealed a high rate of infection in cattle and buffaloes (35.47 & 32.96% respectively) than sheep and goats (2.55 & 1.05% respectively). This difference in cattle and buffaloes might be due to the higher chance of acquiring infection through consumption of larger quantum of food and the longer life-span. The studies indicate the possibility of an increased contamination of the environment by the parasite.

The organ most frequently involved in cattle, buffaloes, and goats was the lung while in sheep, liver was more frequently involved. The overall involvement of lung and liver shows that they serve as the chief predilection sites of hydatids in cattle, buffaloes, sheep and goats.

The frequency of infection was found to be higher in the right lung than the left in cattle, buffaloes and sheep.

The incidence of Cysticercus bovis was found to be 0.74% in cattle.

The fertility of hydatid cysts in cattle, buffaloes, sheep and goats was found to be 68.89, 69.56, 93.33 and 72.72% respectively. The high rate of fertility of cysts in Indian cattle and buffaloes shows that these animals may also act as congenial intermediate hosts of the parasite. Fertile cysts were found more frequently in the liver than lungs in all the species of animals studies.

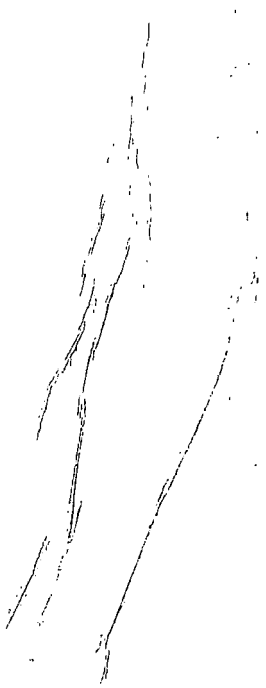
The total nitrogen content of cystic fluid from sterile and fertile hydatid cysts did not show any significant difference whereas the protein content was significantly higher in fertile cyst-fluid than that of sterile cysts. Higher concentration of sodium and potassium was found in sterile cyst-fluid. The possible reason for these differences is discussed.

The results of indirect haemagglutination test showed that a titre of more than 1:80 is diagnostic in cattle and is related to the fertility status of the cyst and the protein content of the cystic fluid. In pulmonary hydatidosis the haemagglutination titre was found to be poor, the reliability increased when the liver was affected and better titres and reliability obtained when more than one organ was involved.

In the case of intradermal test the reliability was found to be higher in infected animals. False positive

reactions were noticed in 11.11% of animals not harbouring hydatid cysts, with whole hydatid fluid antigen and 5.5% with standardised antigen. Antigen of low nitrogen content increases the specificity of the test.

The studies indicate an interrelationship between organ affected, fertility, biochemical constitutents and sensitivity of the diagnostic tests in hydatidosis.



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STUDIES ON

THE LARVAL CESTODES OF ZOONOTIC IMPORTANCE IN KERALA WITH SPECIAL REFERENCE TO HYDATID

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ABSTRACT

A study was made on larval cestodes of zoonotic importance in Kerala with special reference to hydatid.

The investigations included incidence of hydatidosis in animals slaughtered in Kerala in relation to the species, organ involved, fertility status; some of the biochemical constituents viz: total nitrogen, protein, sodium and potassium and diagnostic tests; indirect haemagglutination and casoni's intradermal test. Data on incidence of Cysticercus bovis was also collected simultaneously. Indirect haemagglutination test was carried out on sera samples collected from cattle and the observations studied in relation to the infection-status ascertained at post-mortem. Intradermal test was done on cattle brought for slaughter and results were confirmed during post-mortem.

A high rate of hydatid infection was noticed in cattle and buffaloes (35.47 & 32.96% respectively) than in sheep and goats (2.55 & 1.05% respectively). There is a possibility for an increased environmental contamination in Kerala by the parasite. Lung and liver serve as the chief predilection sites of hydatids in these animals and right lung was found to be more frequently

involved than the left. The fertility of hydatid cysts was high in all animals (68.89 to 93.33%) and fertile cysts were more often encountered in the liver. Cattle and buffaloes also were found to serve as congenial intermediate hosts of the parasite.

Total nitrogen content of hydatid cyst-fluid has not shown any difference between sterile and fertile cysts; protein content was significantly higher in fertile cyst-fluid and sodium and potassium significantly higher in sterile cyst-fluid.

Sensitivity of indirect haemagglutination test was poor in pulmonary hydatidosis but better when liver was affected and best when more than one organ was involved. The use of standardised antigen having low nitrogen content is found to be better in intradermal test as it reduces false positive reactions. The studies indicate an inter relationship between organ affected, fertility, biochemical constituents and sensitivity of diagnostic tests in hydatidosis.

Incidence of Cysticercus bovis in cattle slaughtered was found to be 0.74%.