

IMMUNODIAGNOSIS OF HYDATID DISEASE IN LIVESTOCK USING
AN INTRADERMAL TEST AND AN ENZYME IMMUNOASSAY

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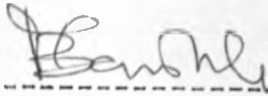
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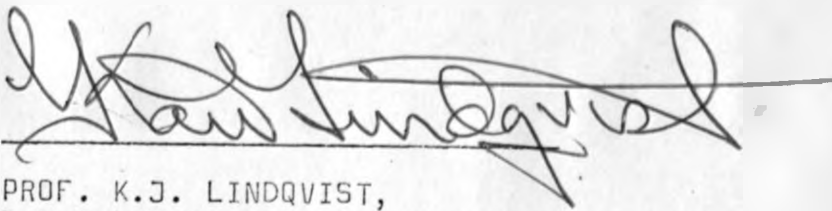
DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

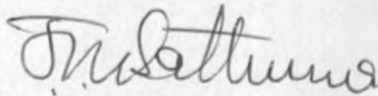


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(iii)

D E D I C A T I O N

Dedicated to my parents and family

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ABSTRACT

A variety of methods based on immunological principles have been tried for the diagnosis of hydatid disease but largely with unsatisfactory results. One of the oldest methods is the Casoni test which gives a reaction of immediate hypersensitivity when the antigen is injected intradermally. This test, however, has not given the specificity nor the sensitivity required for a diagnostic test. Recent attempts to prepare a defined Casoni antigen with higher specificity have resulted in a preparation which contains as its only in vitro - reactive immunological component the antigen B of Oriol et al. (1971).

In this study, a Casoni antigen was prepared by boiling hydatid cyst fluid to inactivate most of the antigens and subsequent absorption using insoluble immunosorbents to remove any remaining undesirable immunoreactive antigens. The final preparation was shown on immunodiffusion and crossed immunoelectrophoretic analysis to contain only antigen B. This was confirmed by the use of specific antisera.

Determination of the molecular weight of antigen B using Sephadex G-200 gel filtration indicated a major component with a molecular weight of approximately 180,000 daltons. Components of higher molecular weight

were also noted but they were thought to be aggregates of the 180,000 dalton component. The detection of antigen B activity in fractions of gel filtration was performed with an enzyme immunoassay and antiserum specific for antigen B as well as Casoni skin tests.

To elucidate if the Casoni antigen could elicit immediate hypersensitivity through non-immunological means, such as activation of the alternate complement pathway, various dilutions of the preparation were injected intradermally in a normal rabbit which had received Evans Blue intravenously. The Casoni antigen dilutions gave insignificant reactions, similar to those given by the injections of saline. When dilutions of antigen were injected intradermally in a sensitized goat, for the purpose of standardisation, distinct and well delineated skin reactions were obtained. A dilution of $\frac{1}{4}$ of the antigen was selected for use, as it was the highest dilution which gave distinct skin reactions which were only marginally smaller than the more concentrated dilutions. This preparation contained approximately 300 μ g protein per ml. Intradermal injections of 0.1ml thus contained 30 μ g protein and was used to study the reaction in 135 goats, of which 50 were found to have hydatid cysts at meat inspection, and in 64 cattle, of which 35 had hydatid cysts. The results were recorded as increases in skin thickness measured 30 minutes after the intradermal injection.

In the cattle population, animals with hydatidosis gave an increase in skin thickness ranging from 0.7mm to 10.3mm with an average of 3.79mm, while the normal cattle gave reactions ranging from 0.3mm to 3.5mm with an average of 1.98mm. The difference between the two means was found to be statistically (t-test) significant ($P < 0.05$). When a 3mm increase in skin thickness was regarded as positive for hydatidosis, a specificity of 97% and a sensitivity of 57% were obtained.

The results obtained with the goat population showed an almost complete overlap of skin reactivity between normal animals and animals with hydatidosis. The increase in skin thickness in the normal animals ranged from 0.2mm to 4.7mm with an average of 2.47mm while in animals with hydatidosis the increase varied from 0.3mm to 5.3mm. No significant difference was found between the means of normal animals and those with hydatidosis.

It can therefore be concluded that the Casoni test might be useful under specified circumstances in cattle, while virtually incapable of distinguishing between infected and non-infected goats. These findings are largely in agreement with those of other research workers who have used the Casoni test in animal populations, in spite of the use of an antigen preparation which, in vitro, showed the presence of only antigen B. It is possible that antigen B may not be the antigen which elicits the strongest and/or most specific immediate hypersensitivity reaction in

animals and further investigations may be warranted to elucidate this.

The unsatisfactory results with the use of a partly purified antigen preparation in the Casoni test, prompted investigations into the use of the so called "Arc 5" antigen of Capron et al. (1967) also referred to as antigen A, of Oriol et al. (1971) in an enzyme immunoassay. This antigen had shown considerable promise for use in the serological diagnosis of hydatid disease in man, although experience with its use in the Turkana population has been disappointing.

A specific antiserum against "Arc 5" antigen had already been prepared by injection of precipitin lines into goat No. 346, prepared by immunoelectrophoresis and later by immunodiffusion. The serum was absorbed for antibovine serum and antishoop serum antibody activity. Absorption was also carried out with immunosorbents made from saline extracts of common intestinal parasites. Goat No. 346 IgG was used in the preparation of the conjugate used in enzyme immunoassay. A total of 268 serum samples from cattle, of which 162 came from animals with hydatidosis, were titrated in an inhibition enzyme immunoassay designed to detect antibodies to "Arc 5" antigen only. Crude goat hydatid cyst fluid was used as antigen while goat No. 346 IgG - glucose oxidase was the conjugate.

Reference curves for a known positive serum and a known negative serum were established. From the positive reference curve, the 50% inhibition titres for each test serum was calculated. The test gave a sensitivity of 56% and a specificity of 59% when a 50% inhibition titre of 8 was considered significant. When a 50% inhibition titre of 12 was considered significant, however, a sensitivity of 21% and a specificity of 99% were obtained. It is concluded that because of the low sensitivity and/or specificity obtained, the test is unsuitable for serodiagnosis or seroepidemiological surveys in cattle.

In this study, the Casoni test using a partially purified hydatid cyst fluid antigen and an enzyme immunoassay based on the detection of antibodies to "Arc 5" antigen yielded unsatisfactory results, and none of these tests can therefore be recommended for the diagnosis of hydatid disease in livestock.

INTRODUCTION

Hydatidosis is a cyclo-zoonotic infection of global proportions. The disease is caused by the larval stages of E. granulosus a tapeworm of the dog and some wild carnivores. The disease affects both human and livestock populations. The prevalence of the disease both in domestic food animals and man varies from area to area and country to country but is particularly high where the livestock industry is the major type of agriculture (Matossian, 1977).

In East Africa the disease is endemic in the human population in some areas. In livestock a high percentage of animals can be infected. The major definitive host of the disease in East Africa is the domestic dog (Canis lupus f. familiaris) but several wild carnivores have been shown to harbour the adult worm. A survey carried out by Nelson and Rausch (1963) in and around Nairobi demonstrated a 50% infection rate in domestic dogs while in Turkaaa 70.4% of the dogs examined were positive. In Kajiado district 27.3% of the dogs were found to be infected (Eugester, 1978). The adult worm has also been found in jackals, wild hunting dogs and the spotted hyena (Macpherson, 1980).

Infection rates of hydatid cysts upto 30% in cattle, sheep and goats have been reported in Kenya (Nganga, 1972). All the infected organs are condemned as stipulated in the Kenya Meat Control Act (1972). This leads to an economic

loss to the farmer as well as loss of valuable protein to feed the growing population.

With regard to human hydatidosis the Turkana district of Kenya has the highest incidence in the world. An infection rate of 96 cases per every 100,000 per year has been reported by O'leary (1976).

In man, there is no effective chemotherapy but mebendazole shows some promise (Bekhti et al., 1977). Surgery, the alternative treatment is expensive, risky and not readily available.

Hydatidosis in livestock is only diagnosed at routine meat inspection after slaughter when organs are checked for the presence of hydatid cysts.

A highly sensitive and specific test would be of immense value in antemortem diagnosis of hydatid disease. This would help to pick diseased animals before slaughter. Such animals can then be slaughtered under strict supervision so that the condemned organs can be properly disposed of. This would break one important link in the life cycle of the parasite. The test would also be of great value in picking those animals that require treatment should a drug effective in treating the disease be developed.

Several immunodiagnostic tests have been developed for the diagnosis of hydatid disease especially in man (Rickard, 1979). These include skin testing and in vitro detection of humoral and cellular immune responses. These

methods usually suffer from lack of sensitivity and specificity (Schantz, 1973). Overcoming the problem of specificity and sensitivity must be the main objective of any test to be developed (Williams, 1982). The natural occurrence of human blood group substances in tissues of larval cestodes complicates the matter further. These have been shown to lead to non-specific reactions in human populations (Ben-Ismaïl et al., 1980). The problems therefore call for the use of selected and purified antigens in immunodiagnostic tests.

The intradermal test (IDT) is one of the tests that has been widely used for the diagnosis of hydatidosis in human populations since its introduction by Casoni in 1911. The use of the test in livestock has been limited. High sensitivity has been reported with various antigen preparations (Kagan et al., 1966; Roy et al., 1978; Williams, 1972; Conder et al., 1980) but the test has been reported to have low specificity (Kagan et al., 1966; Varela-Diaz and Coltorti, 1974; Schantz et al., 1975). Antigens used have ranged from whole hydatid cyst fluid to boiled hydatid cyst preparations adjusted to a certain nitrogen concentration (Kagan, 1968; Orihara, 1970; Williams, 1972). This has resulted in reported increased sensitivity and specificity.

The unsatisfactory results obtained with the use of the partly purified antigen preparation in the intradermal tests prompted investigations into the use of the so called "Arc 5" antigen of Capron et al. (1967) in an

enzyme immunoassay. Serum samples from cattle were titrated in an inhibition enzyme immunoassay designed to detect antibodies to "Arc 5" antigen only.

The aim of this study was to prepare partly purified antigen(s) from hydatid cyst fluid using various biochemical and immunological methods. The antigen(s) once made and identified were to be used in the intradermal test for the diagnosis of hydatidosis in livestock. It was hoped that this would lead to the development of a sensitive and specific, rapid and easily performed test.

2. LITERATURE REVIEW

2.1 DESCRIPTION OF THE DISEASE

In this description the term hydatidosis will be used to refer to the disease in the intermediate host while echinococcosis will refer to the presence of tapeworm in the definitive host.

Hydatidosis is a cyclo-zoonotic infection caused by the cystic stages of cestodes belonging to the genus Echinococcus. The disease has a world wide distribution and because of its zoonotic nature it represents a public health problem in certain areas.

At present, four species of the genus Echinococcus are regarded as taxonomically valid (Schantz, 1982). These and their synonyms are listed in Table 1. The four species are morphologically distinct both in adult and larval stages.

2.2 MORPHOLOGY OF E. GRANULOSUS

The adult worm varies between 2 - 7 mm in length and usually possesses three or occasionally four segments (rarely upto six). The scolex is pyriform and has the typical double row of hooks. The last segment is gravid. The cestode is a hermaphrodite. The gravid uterus is characterised by a number of well developed sacculations.

TABLE 1: SPECIES OF THE GENUS ECHINOCOCCUS RUDOLPHI
1801 AND THEIR SYNONYMS(Schantz, 1982)

Valid species	Synonyms
<u>E. granulosus</u> (Batsch, 1786)	<u>E. cameroni</u> ; Ortlepp, 1934 <u>E. intermedius</u> , Lopez Meyra and Soler, 1943 <u>E. longimanbrius</u> , Cameron, 1926 <u>E. minimus</u> ; Cameron 1926 <u>E. lyacontis</u> ; Ortlepp, 1934 <u>E. ortleopi</u> , Lopez Meyra and Soler, 1943 <u>E. felidis</u> ; Ortlepp, 1937 <u>E. pataonicus</u> , Szidat, 1960 <u>E. cepanzoi</u> , Szidat, 1960
<u>E. multilocularis</u> (Leuckart, 1863)	<u>E. sibiricensis</u> Rausch and Schiller, 1954
<u>E. olicarthus</u> (Diesing, 1863)	<u>E. cruzi</u> Brumpt and Joyeux, 1924 <u>E. pampeanus</u> Szidat, 1967
<u>E. vogeli</u> (Rausch and Bernestein, 1972)	<u>E. cruzi</u> Brumpt and Joyeux, 1924

2.3 LIFE CYCLE OF E. GRANULOSUS

E. granulosus requires two mammalian hosts for the completion of its life cycle. In contrast to other taeniids this worm has a relatively low host specificity in the larval stages and an enormous reproductive potential (Thompson, 1979). The intermediate hosts include a wide range of non-carnivorous animals while the final host is a variety of carnivores as indicated in Table 2.

The eggs of E. granulosus are highly resistant and may remain infective for a long time in a favourable environment. Dessication and high temperatures adversely affect the longevity of the eggs (Dunn, 1978).

The eggs are passed in the faeces of the definitive host and the intermediate host acquires infection by ingesting infective eggs. Following the action of enzymes in the stomach and small intestines, the eggs hatch leading to the release of the oncospheres from the embryophores. The presence of bile assists in activating the oncospheres. Once released, the oncospheres penetrate the wall of the small intestines and get into the liver approximately 12hrs after infection. The hooks are important in the penetration process but the precise role of the secretion produced by the oncosphere remains to be established (Heath, 1970).

TABLE 2: INTERMEDIATE AND FINAL HOSTS OF E. GRANULOSUS
INTERMEDIATE HOSTS

BOVIDAE	Sheep (<u>Ovis ammon</u> f. <u>aries</u>)
	Goat (<u>Capra aegagrus</u> f. <u>hircus</u>)
	Cow (<u>Bos primigenius</u> f. <u>taurus</u>)
	Buffalos (<u>Bulbalus arnee</u> , <u>Syncercaffer</u>)
	Bison (<u>Bison</u> spp)
	Kongoni (<u>Alcephalus buselaphus cokii</u>)
	Impala (<u>Aepyceros melampus</u>)
	Wildebeaste (<u>Connochaetes taurinus</u>)
	Grants gazelle (<u>Gazella granti</u>)
	Waterbuck (<u>Kobus</u> spp)
	Blue ducker (<u>Cephalophus monticola</u>)
	Ibex (<u>Capra Sibirica</u>)
	Mongolian gazelle (<u>Gazella outturossa</u>)
	Saiga (<u>Saiga tatarica</u>)

CERVIDAE	Reindeer, Elk, Wapiti(<u>Cervus elphus</u>)
	American deer (<u>Odocoileus</u> spp)
	Moose (<u>Alces alces</u>)
	Reindeer, Caribou (<u>Rangifer tarandus</u>)
	Roe deer (<u>Capreolus capreolus</u>)
	Axis deer (<u>Cervus rippon</u>)
	Fallow deer (<u>Dama dama</u>)

CAMELIDAE	Alpaca (<u>Lama funicoe</u> f. <u>pacos</u>)
	Camel(<u>Camelus dromedarius</u>)
	Bactrian camel (<u>Camelus bactrianus</u>)

GIRAFFIDAE	Giraffe (<u>Giraffa camelopardalis</u>)
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SUIDAE	Domestic and wild pig
	Warthog (<u>Phacochoerus aethopicus</u>)

EQUIDAE	Domestic horse, donkey, mule, wild ass, zebra (<u>Equus</u> spp.)
ELEPHANTIDAE	Elephant (<u>Loxodonta africana</u>)
HIPPOTAMIDAE	<u>Hippopotamus amphibius</u>
LEPORIDAE	European hare (<u>Lepus europaeus</u>)
PRIMATA	Lemur (<u>Lemur monqoz</u>) Macaqueus (<u>Macaca</u> spp., <u>Cynopithecus niger</u>) Baboon (<u>Papio cynocephalus</u>) Mandrill (<u>Mandrillus</u> spp) Orangutan (<u>Pongo pyomaeus</u>) Chimpanzee (<u>Pantroolodytes</u>) Gorilla (<u>Gorilla gorilla</u>) Man (<u>Homo sapiens</u>)
	Kangaroos and Wallabies (<u>Thylogale</u> spp; <u>Wallabia</u> spp; <u>Macropus</u> spp).

FINAL HOSTS

FAMILY	MEMBERS
CANIDAE	Domestic dog (<u>Canis lupus</u> F. <u>familiaris</u>) Wolf (<u>Canis lupus</u>) Coyote (<u>Canis latrans</u>) Dingo (<u>Canis lupus</u> f. <u>dingo</u>) Golden jackal (<u>Canis aureus</u>) Hunting dog (<u>Lycaon pictus</u>) Cape Silver fox (<u>Vulpes chama</u>) Gulpeo fox Magellan fox (<u>Dusicyon culpacus</u>) Raccoon dog (<u>Nyctereutes procyonides</u>)
HYNENIDAE	Spotted Hyena (<u>Crocuta crocuta</u>)
FELIDAE	Lion (<u>Panthera leo</u>) Leopard (<u>Panthera pardus</u>)

Compiled from:

- Nelson and Rausch (1963)
- Schantz and Schwabe (1969)
- Eugester (1978)
- Macpherson (1980)

The largest number of oncospheres is trapped in the liver while others may reach the lungs, kidney, spleen, eye orbit and other organs. Heath (1971) observed that ruminants have more cysts in the liver while in non-ruminants the cysts are predominantly found in the lungs.

At the final site the oncosphere develops into a fluid filled vesicle known as the hydatid cyst. The hydatid cyst grows slowly and takes about five months to produce protoscolices and brood capsules. A cyst does not always produce protoscolices and the potential to produce them is dependent on the host and site at which development is taking place (Thompson, 1979). The protoscolices produced have a dual potential. If the cyst bursts within the intermediate host, the protoscolices are capable of developing into secondary hydatid cysts. If they are ingested by a suitable definitive host, they develop into sexually mature adults.

The typical hydatid cyst in the intermediate host has a thick outer wall, a concentrically laminated layer and an internal germinal layer (Lascano et al., 1975) divided into the tegument, tegument cell region and an innermost layer bordering the cyst cavity.

2.4 SPECIATION IN E. GRANULOSUS

Numerous subspecies of E. granulosus have been described. These include:-

1. E. granulosus granulosus whose intermediate hosts are sheep, cattle, pigs, man and wild ruminants and final hosts are a variety of canids.
2. E. granulosus canadensis whose intermediate hosts are the reindeer, caribou and man. Canids are the final hosts.
3. E. granulosus equinus whose intermediate hosts are the equidae and the final hosts are canids.
4. E. granulosus borealis whose intermediate hosts are the red fox, many cervid and man. The final hosts are canids (Dunn, 1978).

The taxonomic validity of these subspecies is still a matter of controversy (Thompson, 1978). Ecological isolation, host specificity and morphologic characteristics are considered important in taxonomic considerations.

In Britain, two subspecies of E. granulosus, the sheep and horse strains were distinguished by Williams and Sweatman in 1963. The subspecies were distinguished primarily on morphologic grounds. Rausch (1967) considered these two subspecies invalid since there is no evidence of ecological isolation.

McManus (1981) carried out a biochemical study on the adult and cystic stages of E. granulosus of human and animal origin in Kenya. He found the sheep and human forms of hydatid cyst fluid to be very similar. He also indicated that cattle, goat and camel forms of E. granulosus were distinct biochemically from each other and from that of human and sheep types. He also noted that E. granulosus of sheep origin from Kenya and U.K. have the same biochemical composition. McManus suggested that there exists a complex strain picture in Kenya. He concluded that cattle, goat and camel strains are either non-infective or only poorly infective to man.

2.5 HYDATID DISEASE IN KENYA

The first recorded incidence of the disease was by Leese (1915) in camels. Walker (1925) reported that the disease occurred rarely in sheep, but Daubney (1926) reported the disease in both cattle and sheep. Records are available on the incidence of the disease in cattle, sheep, goats and pigs but no records are available on the prevalence of the disease in camels. Nothing has been recorded in this country concerning the disease in horses and donkeys (Macpherson, 1980).

Hydatid disease causes monetary loss due to condemned organs, carcasses and down-graded meat. Figures provided by the Veterinary Services Division (1980) for the condemnation of cattle and smallstock livers both from local and export slaughterhouses throughout Kenya indicate that 12,982 cattle and 4,016 smallstock livers were condemned in 1979 alone. Macpherson (1980) calculated this to be worth approximately KShs. 800,000. However, the records kept in slaughterhouses must be taken with considerable reservation.

Nobody in Kenya has tried to work out the loss in productivity of the animals affected by hydatid disease i.e. milk, wool or carcass weights as compared to healthy animals. In Russia, Vibe (1958) has reported the average decrease of production from naturally infected

sheep to be 10% for meat, 19% for fat, 57-62% for livers and lungs and 17% for wool. The average loss from naturally infected cattle was found to be 3.4% for meat, 19% for fat, 46% for livers and lungs and 12% for milk production. The number of lambs from every 100 ewes was decreased by twelve and number of calves from 100 heifers was reduced by 3. These represent quite a considerable loss.

Ginsberg (1956) reported a high prevalence of hydatid disease in cattle slaughtered at the Kenya Meat Commission abattoir at Athi - River. This was later confirmed by Froyd (1960) who found 25.5% of 1000 cattle at KMC infected. Mango (1971) in a provincial survey, reported 20.7% in 2196 cattle, 20.7% in 2240 sheep and 16.2% in 1228 goats. Eugester (1978) working in Kajiado district showed a prevalence of 46.7% in 1446 cattle, 9.5% in 44 sheep and 9.0% in 100 goats. Macpherson (1980) reported 80% infection in 10 camels, 10% in 20 cattle, 1.6% in 61 sheep and 0.8% in 844 goats in Turkana as indicated in Table 3. O'leary (1976) observed an infection rate of 40% in sheep, goats and camels in the same district.

In Kenya, the dog is regarded as the most important definitive host for E. granulosus (Nelson and Rausch, 1963; Eugester, 1978). Nelson and Rausch (1963) found 50% infection in 16 dogs in and around Nairobi and 70.4% in 27 dogs examined in the Turkana district. Eugester (1978)

reported a 27.3% infection rate in 165 dogs examined between 1974 and 1975 in Kajiado district. Wild carnivores have also been shown to harbour the adult worm. These include the black backed jackal (Thos mesomelas), the wild hunting dog (Lycaon pictus) and the spotted hyena (Crocuta crocuta). In Turkana the silver backed jackal and the golden jackal (Thos aureus) have been shown to harbour the adult worm (Macpherson, 1980).

The Turkana district has by far the highest human endemicity of the disease in the world. Schwabe (1969) estimated the incidence of hydatidosis in Turkana as 40 cases per every 100,000 persons. O'leary (1976) in a five year study of the disease in Turkana district put the figure at 96 cases for every 100,000 persons. Her study also showed that the majority of cysts (66%) in this population are found in the liver. More than 100 surgical operations on hydatidosis patients were performed at Kakuma and Lodwar hospitals in 1979 alone (AMREF, 1979).

Apart from the Turkanas, human hydatidosis is occasionally found in the Suk of West Pokot, Rendille, Shangilla and Masailand. Although the livestock in Masailand is heavily infested, only sporadic human cases are reported (O'leary, 1976).

TABLE 3: DOMESTIC LIVESTOCK EXAMINED FOR THE PRESENCE OF HYDATID CYSTS IN TURKANA (According to Macpherson 1980)

Host species	No. . examined	No. infected	% infected	Abattoir
CAMEL	10	8	80.0	LODWAR
CATTLE	8	1	12.5	LODWAR
	2	0	-	KAKUMA
	10	1	10.0	TOTAL
SHEEP	50	1	2	LODWAR
	10	0	-	KAKUMA
	1	0	-	NANAM
	61	1	1.6	TOTAL
GOAT	784	5	0.6	LODWAR
	32	2	6.3	KAKUMA
	15	0	-	LOKITAUNG
	12	0	-	LOKORI
	1	0	-	NANAM
	844	7	0.8	TOTAL

2.6 IMMUNOLOGICAL DIAGNOSIS

The immunological diagnosis of hydatid disease is based on the detection of humoral or cellular responses of the host against the parasite. Numerous studies have been carried out to develop tests with increased specificity and sensitivity. So far, most of these tests have not met the required criteria for suitable tests which according to Rickard (1979) are

1. High sensitivity
2. High specificity
3. Ease of performance
4. Persistence i.e. period which the test remains positive after the patient has been treated
5. Amount of antigen used.

2.6.1 IMMUNODIAGNOSTIC TESTS USED

The immunodiagnostic tests used in the diagnosis of hydatid disease have been extensively reviewed by Rickard (1979). These include:-

- (i) Intradermal test (IDT); Casoni, 1911
- (ii) Haemagglutination test (IHA); Garabedian, et al., 1957
- (iii) Latex Agglutination test (LA); Varela Diaz, et al.., 197
- (iv) Immuno-electrophoresis (IEP; Capron et al., 1967.
- (v) Indirect Fluorescent Antibody Test (IFAT); Ambroise - Thomas, 1976.
- (vii) Enzyme-Linked-Immunesorbent Assay (ELISA) Farag et al., 1975.
- (viii) Double Diffusion (DD); Varela-Diaz and Coltorti, 1978.

2.6.2 ANTIGENS USED

The most widely used antigen for routine immunodiagnosis of hydatidosis is hydatid cyst fluid (Rickard, 1979). The cyst fluid is usually obtained from E. granulosus cysts. Both untreated fluid and fluid concentrated and clarified in various ways has been used. The fluid is a mixture of substances of both host and parasite origin.

Whole concentrated and clarified hydatid cyst fluid has been used in intradermal test, IHA, LA, and IEP. Protoscolices are used as particulate antigen in IFAT. Several workers have tried to purify the antigens for use in these tests. Purification has been done using immunological methods. However, the advantage gained in terms of sensitivity and specificity does not warrant the labour involved (Rickard, 1979).

2.6.3 COMPOSITION OF HYDATID CYST FLUID

Chordi and Kagan (1965) analysed sheep hydatid cyst fluid by immunoelectrophoresis and found a total of 19 antigenic components. Ten of these bands were shown to be of parasitic origin while the rest were of host serum origin.

2.6.4. POLYSACCHARIDES IN HYDATID 'CYSTS

Glycogen is the main polysaccharide found in Echinococcus and other helminth parasites. Whether this glycogen is antigenic or not is a matter of speculation. Heidelberger, et al. (1954) used carefully prepared and highly purified samples of glycogen from *Ascaris* and found them to be definitely antigenic. Cmelik, (1952) isolated active polysaccharide- containing fractions in the laminated membrane. The purest fraction contained unidentified aldohexoses, deoxyntoses, glucosamine and had antigenic properties.

2.6.5. PROTEINS

There are several proteins in hydatid cyst fluid and parasite extracts. Some of these have been found to be of host origin and others definitely of parasite origin (Chordi and Kagan, 1965). Several methods such as the trichloroacetic acid method of Dennis (1957) and the ammonium sulfate precipitation of Castagnari and Sorice (1965) have been used for the precipitation of proteins.

2.6.6. LIPIDS

Agosin et al. (1957) reported that 13.6% of the dry substance of protoscolices is lipid in nature. Lipoproteins were also demonstrated by Chordi and Kagan (1965) in sheep cyst fluid by staining bands developed

on immunodiffusion with Sudan Black. Kilejian et al. (1962) characterised lipids in hydatid cyst fluid as lecithins in association with cholesterol. Lemaire and Ribere (1935) reported the presence of creatinine, lecithin and ammonium salts.

Hydatid cysts contain two major lipoprotein antigens. These have been given different notations by different investigators which tends to be confusing. Table 4 indicates the different notations and the authors who designated them. According to Williams (1982) the antigen 4 of Chordi and Kagan (1965), antigen 4 of Puzzuoli et al. (1974), antigen 4 of Oriol et al. (1971) and antigen A of Williams et al. (1971) all correspond to the Arc 5 of Capron et al. (1967). Williams further suggests that the antigen 5 of Chordi and Kagan and antigen 5 of Puzzuoli et al. (1974) corresponds to antigen B of Oriol et al. (1971).

The Arc 5 of Capron et al. (1967) has been characterised by Dottorini and Carmelo (1977). The antigen showed a molecular weight varying from 100,000 to 300,000 daltons after chromatographic analysis on Biogel P30. The antigen was for a long time considered to be specific for hydatid cyst infections until cross reactions with E. cellulosae (Taenia solium cysticercosis), E. tenuicollis (Taenia hydatigena cysticerci) and serum from a patient with multiple myeloma were reported by Schantz et al. (1980).

TABLE 4: NOMENCLATURE OF HCF LIPOPROTEIN ANTIGENS

AUTHOR	NOMENCLATURE
Chordi and Kagan (1965) Capron <u>et al.</u> (1967) Pozzuoli <u>et al.</u> (1974) Oriol <u>et al.</u> (1971) Williams <u>et al.</u> (1971)	Band 4 Arc 5 Band 4 Antigen A Antigen A
Chordi and Kagan (1965) Oriol <u>et al.</u> (1971) Puzzuoli <u>et al.</u> (1974)	Antigen 5 Antigen B Antigen B

Oriol et al. (1971) examined physicochemical characteristics of antigen A and B preparation by gel filtration, immunoelectrophoresis and chemical analysis. The degree of contamination by host serum proteins was measured with specific I¹²⁵-labelled antigen and found to be in the region of 1.6%. One component of the fraction, antigen B, was further purified by gel filtration on Sephadex G-200 and its approximate molecular weight calculated to be 160,000 daltons.

2.6.7. BLOOD GROUP SUBSTANCES

The presence of antibodies to blood group substance P in the serum of hydatid disease patients was first reported by Merrit and Hardy (1955). Cameron and Stavely (1957) reported the presence in the cyst fluid of a substance that specifically inhibited anti - P₁ and partially inhibited anti P + P₁ serum. Russi et al. (1974) isolated and characterised a blood P₁ active carbohydrate antigen of cyst membranes. Ben-Ismail et al. (1980) showed that the presence of anti - P₁ antibodies in sera of hydatid cyst patient leads to non-specific reactions in Indirect Haamagglutination.

2.6.8. TISSUE EXTRACTS OF PARASITE

Crude antigens are also obtained from the membrane layers of the cyst wall and protoscolices. Bozicevich (1951) reported that antigen(s) prepared from the germinal

layer and protoscolices gave increased specificity in complement fixation test.

2.6.9. METABOLIC ANTIGENS

Kagan and Norman (1961) speculated that metabolic products produced by hydatid cyst scolices accumulate in the cyst and are responsible for the serologic activity of these antigens. This would explain the assumed lack of antigenicity of sterile cysts.

2.7 CLASSIFICATION OF ALLERGIC REACTIONS

The following classification of allergic reactions has been proposed by Coombs and Gell (1957).

2.7.1 Type I - Reaction (Anaphylactic)

This type of reaction is initiated by antigen reacting with antibody associated with tissue cells and leading to the release of pharmacologically active substances.

2.7.2. Type II Reaction (Cytolytic or Cytotoxic)

This type of reaction is initiated by antibody reacting with either (i) an antigenic component of a tissue cell or (ii) an antigen or a hapten which has become

intimately associated with tissue cells.

2.7.3 Type III Reaction (Arthus reaction and Toxic Complex Syndrome)

This type of reaction is initiated by the combination of antigen with potentially precipitating antibody either (i) in tissue spaces with resulting microprecipitates accumulating around small vessels causing damage to cells secondarily, or (ii) in the bloodstream to form soluble circulating complexes which are subsequently deposited in blood vessel walls where they cause local inflammation.

2.7.4. Type IV Reaction (Delayed Hypersensitivity)

This response is initiated by the reaction between an allergen at a local site and specifically sensitized mono-nuclear cells. The specifically sensitized mono-nuclear cells infiltrate to the site where antigen is located and here they cause tissue damage.

2.7.5 Type V Reaction (Stimulatory)

This type of reaction occurs when non-complement fixing antibodies directed against certain cell surface components stimulate rather than destroy the cell. An example of this type is in thyrotoxicosis.

Another type of hypersensitivity not under Gell and Coombs classification is the Basophil Cutaneous Hypersensitivity reaction (CBH). CBH reactions are delayed time course immune responses in which basophils comprise a significant proportion of infiltrating cells (Mitchell et al., 1982). The CBH reactions differ from classic tuberculin type delayed hypersensitivity in certain aspects (i) the CBH is induced by immunizing procedures which avoid the use of mycobacterial adjuvants (ii) CBH reactions are relatively non-indurated (iii) they are characterised by extensive infiltration of basophilic leukocytes in addition to mononuclear cells (iv) are affected by lymphocytes which are difficult to tolerize (v) have a low avidity for antigen (vi) lack extensive fibrin deposits (vii) with many antigens can only be elicited at early intervals of immunisation (Dvorak and Dvorak, 1974). CBH are now recognised as a distinct form of immunologic response separate from delayed hypersensitivity (DH) and antibody mediated reactions.

2.7.6. IMMUNOGLOBULINS INVOLVED IN TYPE I REACTIONS

IgE is the formerly called reaginic antibody. It has a four chain structure. Its heavy chain contains four CH regions and one VH region. IgE has a M.W. of 196,000 daltons (Ishizaka, 1970). Small quantities of IgE 20 - 400 ug/ml are found in sera of most species (Jarrett, 1972).

Much of the IgE is bound to mast cells and basophils through its CH₃ and CH₄ regions. Since IgE bind to cells the IgE are also called cytotropic or cytophilic. If IgE binds to cells of the same species, the IgE are said to be homocytotropic. If they bind to cells of other species the IgE are said to be heterocytotropic (Ovary, 1958).

An increased production of IgE is a frequent concomitant with various parasitic diseases, especially intestinal parasitism (Jarrett, 1972). It has been suggested that parasite specific IgE may play a role in both protective immunity to helminth parasites and in the pathogenesis of certain clinical manifestations of these infections (Capron and Dessaint, 1977).

Different techniques have been used for assaying IgE levels in serum These include:-

1. Single radial - immunodiffusions (Rowe, 1969)
2. Double antibody radioimmunoassay (Gleich, et al., 1971)
3. Solid Phase Antibody Radio-immunoassay (Benninch and Johansson, 1971)
4. Enzyme-Linked Immunosorbent Assay (Hamilton, et al., 1971)
5. Solid Phase (Sepharose) Radioallergosorbent (RAST) (Yusuf et al., 1982).

Solid Phase Radioallergosorbent is now clearly established as the method of choice for serological determination of IgE antibodies because of high sensitivity, reproducibility and specificity.

IgG has also been shown to bind mast cells and participate in type 1 reactions. This is the heat stable IgG short-term homocytotropic antibody of Parish (1970).

2.7.7. CELLS INVOLVED

Mast cells are the major type of cells involved in immediate type hypersensitivity. These are large cells 15-20 um in diameter and distributed throughout connective tissue. These cells may carry surface receptors for the Fc portion of IgE and hence free IgE will adsorb onto them (Ishizaka, 1970). No visible reaction occurs on the mast cells due to the attachment of free IgE but when antigen binds to the Fab region of this fixed IgE and cross-links two IgE molecules, degranulation occurs, pharmacologically active substances are released. The released mast cells do not die but are difficult to identify due to lack of characteristic morphology.

Basophils are also involved in immediate type hypersensitivity. They have Fc receptors for IgE. The basophils are in circulation and may be passively sensitized with IgE. They respond to antigens in the same way as mast cells. A subpopulation of human peripheral blood lymphocytes (Gonzalez-Molina and Spiegelberg, 1977) and monocytes (Melewicz and Spiegelberg (1980) have also been shown to have Fc receptors for IgE.

1-2% of normal peripheral blood lymphocytes and 20% monocytes have Fc IgE receptors.

2.7.8 PASSIVE CUTANEOUS ANAPHYLAXIS

Antibody is first injected intradermally and a sufficient time interval is allowed for the fixation of antibody to cells. When subsequently an antigen mixed with Evans Blue is injected intravenously, the antigen reacts with the cell-fixed antibody leading to the release of pharmacologically active substances of anaphylaxis. This results in increased permeability of the vessel wall and extravasation of fluid from blood plasma into the tissues. The Evans Blue binds to serum protein and this combination of albumin and dye leaks out and stains the tissues. This is the so called Passive Cutaneous Anaphylaxis, PCA (Ovary, 1958)..

Several workers have tried to demonstrate passive cutaneous transfers between different species and within species using different antibodies and antigen.

Ovary (1958) demonstrated that PCA in the guineapig can be provoked by guineapig (i.e. homologous), rabbit and human antibody. He also noted that goat and horse serum does not give PCA in the guineapig.

Recently McGuire et al., (1979) attempted to demonstrate the function of bovine IgG₁ and IgG₂ in skin reaction in several species. They showed that both IgG₁ and IgG₂ antibodies to ovalbumin caused PCA reaction in bovine skin. However, in the rat skin, only IgG₁ mediated the reaction.

Gershwin (1981) demonstrated heterologous PCA with bovine IgE. She showed that rats, rabbits, guineapigs and dogs failed to develop PCA reactions when sensitized by intradermal injections of bovine IgE and challenged at intervals of 2 to 72 hours later by intravenous injection of ovalbumin and Evans Blue. In contrast, sheep and goats became passively sensitized by bovine IgE. Of the 5 sheep, 2 had positive PCA reactions after a 48hrs latent period and one gave positive reaction after a 24 hour period. There was no positive PCA in sheep after 6 and 72 hr latent period. Of the goats, 1 responded with positive PCA at 6, 24, 48 and 72 hr latent periods and the second goat only after 48 and 72 hours.

In the goat, differences have been shown to exist between IgG subclasses in their ability to induce PCA (Micusan and Borduas, 1977). Goat IgG₂ has only been shown to elicit PCA in the homologous species. IgG₁ subclass was shown to be effective in including both heterologous PCA in the guinea pigs and also a reverse Arthurs reactions.

2.8 THE INTRADERMAL TEST

This test has been used widely in the diagnosis of human hydatidosis since its introduction by Casoni in 1911.

The test is based on the appearance of an immediate type hypersensitivity reaction in the skin of hydatid disease patient as a result of an intradermal injection of antigen from cysts fluid (Rickard, 1979). The test, however, has not been used much in livestock and the literature on its use in this respect is scanty.

The test has several advantages in that it is easy to perform, the results are immediate, it requires small amounts of antigen and the sensitivity is high (Rickard, 1979). The disadvantages include the purity of antigens used (Schantz et al., 1975), the standardisation of these, and also low specificity (Kagan, 1968; Varela-Diaz and Coltorti, 1974). It is also difficult to agree on what is to be considered positive or a negative reaction (Williams, 1972). The intradermal injection has also been known to provoke anaphylactic shock in some individuals (Weiszer, 1972). Positive reactions persist long after cysts have been removed by surgery and may also be induced by previous intradermal testing of an individual.

Schantz et al. (1975) demonstrated non-specific reactions with intradermal tests in human patients with other helminth infections. These tests were carried out using a partially purified antigen solution prepared from boiled hydatid cyst fluid. Non-specific reactions were demonstrated in patients with Hymenolepis nana, Taenia spp; Fasciola hepatica, mixed parasitism and several non-helminthic conditions. Of the 18 patients who had non-helminthic conditions, one with a plasmacytoma gave a positive reaction as did a woman from whom an ovarian non-hydatid cyst had been removed by surgery.

Yarzabal et al. (1975) compared IDT with IEP in 47 surgically confirmed cases of human hydatid disease and 73 non-hydatid persons. For the IDT he used a boiled hydatid cyst fluid antigen preparation and whole cyst fluid antigen. The boiled HCF antigen gave a sensitivity of 80.9% in IDT, while the whole HCF antigen gave a sensitivity of 68.1%. Both antigens produced false positive reactions in 3 out of 30 negative controls. The IEP produced a sensitivity of 78.7%. Diagnostic sensitivity of both tests varied according to the localisation and condition of the hydatid cyst. A detectable immune response was more frequent in patients with lung cysts. The lowest diagnostic sensitivity was observed in patients whose cysts were intact and of the hyaline type.

Several workers have reported high sensitivity of the intradermal test using various antigen preparations in man. Roy et al. (1970) evaluated CFT, IDT and IHAT in India with 25 proven cases of lung cysts. The IDT was positive in 80% of these patients. Todorov (1970) in studies in Romania found a sensitivity of 86.6%. A sensitivity of 89% was reported by Lass et al. (1973) with the immediate reaction and 68% sensitivity with delayed reactions which were said to be completely specific.

Williams (1972) showed increased sensitivity of IDT using boiled hydatid cyst fluid adjusted to a low nitrogen concentration. He used an antigen solution containing 15ugN/ml of the antigen in IDT and obtained a sensitivity of 80% in human patients. The boiled hydatid cyst fluid contained only one reactive component of parasite origin and some host components.

Most investigators agree on the low specificity of IDT. Kagan (1968) reported false positive reactions ranging from 17-18% in human patients. Kagan et al. (1966) used a nitrogen content ranging from 12 ugN/ml to 405 ugN/ml and found many false positives in the control individuals. Thus, with antigen containing over 100 ugN/ml, 30-40% of the negative controls were positive. The specificity of the test increased as the concentration of antigen decreased.

Comparison has been done between IDT and other serological tests. Conder et al. (1980) compared IEP, IHA and DD with IDT in experimentally infected sheep. One flock was infected with E. granulosus eggs while the other was infected with T. hydatigena eggs. They found the DD to be as specific as the IEP while IHA sensitivity and specificity approached that of DD and IEP. The IDT supported the DD and IEP but demonstrated a lack of specificity. Using IDT, all four experimentally infected sheep showed a positive reaction after 30 minutes, 3 were positive after 1 hour and two remained positive at 4 hours. Wheal and flare reactions of more than 1.1 cm² were considered positive. One sheep showed an Arthus type reaction, while 3 showed delayed type hypersensitivity. Of the sheep infected with T. hydatigena eggs, one showed a positive reaction while the rest were negative.

Some investigators have disputed the conclusions that boiling of hydatid cyst fluid is accompanied by a relative increase in specificity of IDT (Yarzbal et al., 1975). Schantz et al., (1975) suggests that the thermostable component in hydatid cyst fluid is a group antigenic component shared by a variety of helminths. This may lead to cross-reactivity observed with other parasites. It has also been shown that hydatid cyst components as well as larval materials from other taeniid parasites contain anti-complementary substances which spontaneously activate complement via the alternate pathway (Rickard, 1979).

The enzymes most frequently used for labelling are alkaline phosphatase, horseradish peroxidase, α -D-galactosidase and glucose oxidase. Horseradish peroxidase is most favoured because of its low cost, easy conjugation and wide variety of substrates (Voller *et al.*, 1979). Enzymes used must be stable, highly reactive, available in purified form, form stable conjugates and be safe to use. A suitable substrate must also be available.

The objective of the conjugation process is to link the enzyme with antibody or antigen in such a way that each retains maximum reactivity. This is achieved by the use of cross-linking reagents with at least two active groups. The cross-linking can be accomplished in a one-step procedure or a two-step method. In the one-step procedure the enzyme, antibody and cross-linking reagent are mixed together. In the two-step method the enzyme is first reacted with the cross-linking agents. The activated enzyme is then reacted with antibody or antigen. Examples of cross-linking reagents are glutaraldehyde and sodium periodate.

Each of the enzymes used in the conjugation process requires a suitable substrate. The aim of the substrate is to provide a sensitive detection method for the enzyme in the conjugate.

Most investigators have used substrates that are initially colourless but on being acted upon by the enzymes give a strong colour. Examples of substrates are ortho-phenylenediamine (OPD) for horseradish peroxidase, p-nitrophenylphosphate for alkaline phosphatase and 2-2 azino-di-(3ethyl benzothiazolin sulfone-6) (ABTS) for glucose oxidase.

The results of ELISA tests may be read visually or with photometric equipment such as spectrophotometers or the specialized ELISA microplate readers.

TYPES OF ASSAY

These are extensively reviewed by Voller et al. (1979) and illustrated. They include:-

- (1) Competitive Method
- (2) Double Antibody Sandwich Method
- (3) Modified Double Antibody Sandwich ELISA
- (4) Inhibition ELISA for antigen
- (5) Indirect Method
- (6) Solid Phase Anti-IgM ELISA

The literature on the use of ELISA in the immuno-diagnosis of hydatid disease is scanty. Farag et al. (1975) used ELISA with purified 'Arc 5' antigen and Matossian et al. (1978) used crude hydatid cyst fluid.

The test was found to compare favourably with other tests in terms of sensitivity and specificity but seemed to offer no advantage over them. Iacona et al. (1980) using ELISA obtained a sensitivity of 64% in human serum samples. Njeruh and Lindqvist (1982) assayed for the presence of hydatid cyst antibodies in bovine serum samples with ELISA using antigen B of Oriol et al. (1971). They obtained 100% specificity and a sensitivity of 91.2%.

3. MATERIALS AND METHODS

3.1 HYDATID CYST FLUID

Organs condemned due to presence of hydatid cysts were collected from various slaughter-houses in and around Nairobi and transported to the laboratory where the cyst fluid was harvested. The cyst fluid was harvested by first washing the cyst under a running tap and then piercing the cyst with a wide-bore needle with a plastic tubing attached. The cyst was then squeezed to allow fluid to flow into a container.

The fertility of the cyst fluid was determined by microscopic examination for the presence of protoscolices. The fluid specimen were stored separately according to animal source, organ and fertility of the cyst. Whenever necessary, pools were made from various sources and organs. The fluid was stored either frozen at -20°C or at 4°C in presence of 0.1% Sodium azide as a preservative.

The fluid was filtered through Whatman No. 1 filter paper to remove debris, centrifuged in Heraeus Christ Labofuge II centrifuge at 2940g for 30 minutes. Final clarification was done by filtration through 0.3 micron Sartorius membrane filter and a pressure of approximately 50lbs/inch². Concentration of the fluid was done on a PM 10 Amicon filter with a cut-off point of 10,000 daltons (Amicon Corporation, Massachusetts, U.S.A.).

3.2 COLLECTION OF BOVINE SERUM SAMPLES

Bovine serum samples for use in ELISA were collected from the Kenya Meat Commission Abattoir at Athi River and slaughterhouses at Ongata Rongai. The serum was obtained from the clot from the heart of each animal. A thorough inspection of the organs was carried out for the presence of hydatid cysts and the findings recorded. Sodium azide was added as a preservative and the serum was kept either frozen or at +4°C.

3.3. PREPARATION OF AN ANTISERUM SPECIFIC FOR ANTIGEN A ("ARC5")

Antigen A, also called "Arc 5" (Capron et al., 1967) was for a long time thought to be specific for hydatid disease both in man and animals (Schantz et al., 1980).

Precipitin lines of the antigen were prepared in crossed immunoelectrophoresis (CIE) as described by Weeke (1973). The electrophoresis was carried out in an LKB, 2117 Multiphor electrophoresis equipment connected to a Lauda-MF. SSGERATE - WERK LAUDA (West Germany) cooler. One percent agarose (Type HSA, Litex, Denmark) in calcium lactate barbital, buffer, pH 8.6 (Appendix I) was used in the gel. In the first dimension run, electrophoresis of the antigen, concentrated

Hammerberg et al. (1977) showed that pronounced vascular changes occurred in the skin following intradermal inoculation of complement activating fractions of hydatid cyst fluid. He suggested that this may account for non-specific reactions observed in IDT.

Antigen B of Oriol et al. (1971) seems to be a very potent allergen in the skin test. Heat treated preparations of hydatid cyst fluid like those of Williams (1972) probably owe their effectiveness to the presence of antigen B. Purified antigen B has been shown to bind to IgE anti-bodies in the serum of human hydatid cyst patients (Dessaint et al., 1975).

2.9 ENZYME IMMUNOASSAY

Enzyme immunoassays were first described by Engvall and Perlmann (1971) and independently by Van Weeman and Schuurs (1971). These assays are characterised by the use of an enzyme labelled antibody or antigen to detect the interaction of the antibody with an antigen.

In ELISA, one of the reactants is absorbed onto a solid phase. The most frequently used solid phase is polystyrene. Polyvinyl, latex, glass and propylene have also been used (Voller et al., 1979). These solid phases are in the form of beads, tubes or wells in plastic trays.

bovine hydatid cyst fluid, was carried out at 4°C for 1hr with a potential difference of 10v/cm gel. In the second dimension electrophoresis calf No. 28 serum which is polyvalent for bovine hydatid cyst fluid antigens was incorporated in the upper gel. An intermediate gel containing neither antigen nor antiserum was also included. The second dimension electrophoresis was carried out at a field strength of 2v/cm for 24 hours at +4°C. The gel was pressed for 4 hrs and washed overnight in a 3% sodium citrate solution to remove non-precipitated proteins. After washing, the slides were pressed further for 1 hr. The precipitin line was identified on the plate and carefully cut out with a razor blade, taking caution to avoid other precipitin lines. Eighty of these lines were prepared and left in phosphate buffered saline, pH 7.4 for 7 days with daily changes to remove non-precipitated proteins. The washed precipitin lines were sonicated in a Braunsonic 1510 (B. Braun Melsungen AG) Ultrasonicator. Complete Freund's adjuvant (DIFCO Laboratories, Detroit, U.S.A.) was added and sonication continued to give a thick homogenous paste. This paste was injected into the superficial lymph nodes as well as intramuscularly into goat No. 346. The goat was bled after 3 weeks and boosted further with precipitin lines. This was continued until after 9 weeks a single distinct precipitin line was obtained on immunodiffusion with whole concentrated hydatid cyst fluid, as antigen.

Thereafter, booster doses were given at intervals of 2-3 weeks using precipitin lines prepared by immunodiffusion.

The antibody activity of goat No. 346 serum was compared using immunodiffusion tests with an anti-antigen A reference kindly supplied to Prof. K. Lindqvist by Dr. Capron. Concentrated fertile bovine HCF as antigen was placed in the middle well. The goat No. 346 serum showed one distinct precipitin line which gave a reaction of identity with Dr. Capron's anti-antigen A serum.

All batches of goat No. 346 serum were absorbed with insoluble sheep and bovine serum immunosorbents to remove possible antibodies to sheep and bovine gammaglobulins. The batches were also absorbed with insoluble immunosorbents prepared from saline extracts of Fasciola spp, Moniezia spp, and Haemonchus contortus to remove antibodies which may have resulted from natural infections with these parasites. To remove possible antibodies to blood group substances, the serum was absorbed with a pool of glutaraldehyde fixed erythrocytes of goat, sheep and cattle. The antiserum was stored at +4°C with 0.1% sodium azide or frozen at -20°C.

3.4 OTHER ANTISERA

Other antisera used in this project were produced by colleagues in the Immunology Unit of the Department of Public Health, Pharmacology and Toxicology. These include:-

- (i) Goat No. 880 serum - This was prepared by immunisation of goat No. 880 with precipitin lines of antigen B.
- (ii) Sheep No. 822 serum - Sheep antiwhole HCF was prepared by injecting whole hydatid cyst fluid in Freund's adjuvant. The serum is polyvalent for hydatid cyst antigens.
- (iii) Calf No. 28 serum - This serum was prepared by injections of whole bovine hydatid cyst fluid in Freund's adjuvant into the calf. The serum is polyvalent for bovine hydatid cyst fluid antigens.
- (iv) Rabbit No. 187 serum - This serum was prepared by injections of whole bovine serum into the rabbit. The serum is polyvalent for bovine serum antigens.

3.5 PREPARATION OF IMMUNOSORBENTS

Cyanogen bromide activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) insoluble immunosorbents were prepared for the removal of host components from antigen preparations. Rabbit anti-host (either bovine, goat or sheep) serum, was coupled to Sepharose 4B following the procedure recommended by the manufacturers.

Briefly, the method was as follows:- The gel was washed on a No. 3 sintered glass filter with 1mM HCL approximately 400 mls per gram of Sepharose, followed by several washes with saline. The protein was mixed with 0.1M bicarbonate buffer pH 8.3 (Coupling buffer). One volume of protein was mixed with 9 volumes of coupling buffer. The mixture was then added to the swelled gel and left on an end-over-end rotator for 2hrs at room temperature and 18hrs at +4°C for the coupling to occur. Excess proteins were removed by washing the gel with coupling buffer, pH 8.3 and the remaining active groups were blocked by 1M diethanolamine, pH 9.0 for 2hrs on an end-over-end rotator at room temperature. Excess diethanolamine was removed by washing several times with coupling buffer, pH 8.3 and 0.1M acetate buffer, pH 4.0 containing 0.5M sodium chloride followed by coupling buffer.

Finally, the immunosorbent was washed with distilled water several times followed by saline. Sodium azide was added to a final concentration of 0.1% as a preservative. The immunosorbent was regenerated immediately after use by washing twice with 0.2M glycine/HCL buffer, pH 2.5, centrifuged and supernant discarded and then washed several times with coupling buffer 0.1M NaHCO₃, pH 8.3 with 0.5M NaCl. A final wash with saline was done and the immunosorbent stored at +4°C in saline containing 0.1% sodium azide.

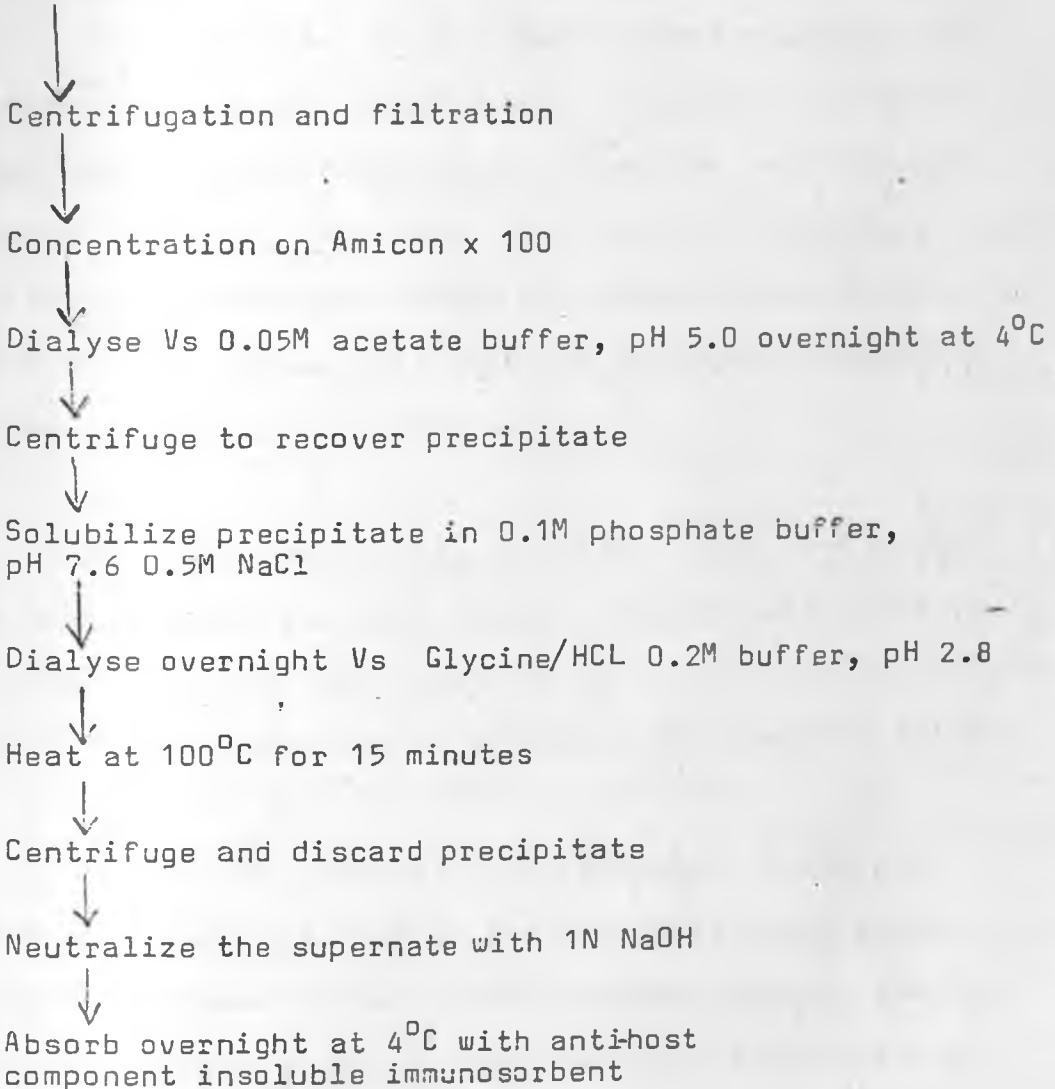
3.6 PREPARATION OF ANTIGEN B

The antigen was prepared by the method of Mussoni et al., (1978). Hydatid cyst fluid concentrated 100 x on an Amicon membrane filter with a cut off point of 10,000 daltons, was dialysed against 0.05 M acetate buffer pH 5.0 overnight at +4°C. The contents of the dialysis bag were centrifuged at 2940g to recover the precipitate. This precipitate was solubilized in 0.1 M phosphate buffer (pH 7.6) containing 0.5M NaCl and dialysed overnight at +4°C against 0.2M glycine/HCl buffer, pH 2.8. The material was heated at 100°C for 15 minutes, centrifuged and the precipitate discarded. The supernate was neutralized with 1N NaOH and examined for the presence of antigen B and host components in immunodiffusion tests using antiserum produced in goat No. 880 which is specific for antigen B, antiserum produced in sheep No. 822 which is polyvalent for HCF antigens and rabbit No. 187 which is antiovine serum. The antigen preparation which was shown in immunodiffusion to have host components was mixed with rabbit antiovine serum insoluble immunosorbent and left overnight in the cold (+4°C) on an end-over-end rotator to remove the remaining bovine components. The mixture was centrifuged to recover the antigen in the supernate. The preparation method is illustrated in Fig 1.

The antigen preparation was tested again in immunodiffusion and electrophoresis.

FIG 1: STEPS IN THE PREPARATION OF ANTIGEN B

CYST FLUID



3.7 TITRATION OF THE SKIN REACTIVITY OF ANTIIGEN B IN GOAT NO. 880

3.7.1 TITRATION IN THE FLANK

The left flank of goat No. 880 was clipped a day before the test was carried out. The hair was removed completely by applying a paste of barium sulfide and starch in water. The paste was made by taking equal parts of barium sulfide and starch and mixing these in water to form a thick paste. The goat was thoroughly washed to remove remnants of the paste.

The following morning, injection sites were marked on the flank of the goat using a felt pen and labelled. A similar pattern was drawn out on paper and a randomisation plan for the injection of dilutions and controls filled.

The protein content of the antigen B preparation had been determined earlier by the method of Lowry et al. (1951) and found to be 1.205ug protein per ml. Double dilutions of this antigen were made in sterile saline upto a dilution of 1:512.

The goat was given an intravenous injection of 20mls of sterile 2% Evans Blue to facilitate reading of the skin reactions. Intradermal injections of antigen dilutions and saline controls were carried out according to the randomisation plan. Four injections per dilution were carried out.

0.1ml of either antigen or saline was injected intradermally using 1ml tuberculin syringe fitted with a 26 gauge needle. The time of each injection was recorded on the randomisation plan. The diameter of the skin reactions appearing as well delineated, almost circular areas were measured using a Vernier Sliding Calipers after 15 minutes, 30 minutes and 1 hr.

3.7.2 CAUDAL FOLD INJECTION

0.1ml of antigen dilutions $\frac{1}{4}$ and $\frac{1}{16}$ were injected intradermally into the right caudal fold of the goat. 0.1ml of saline was injected into the left fold as a control. The thickness of the fold was measured using Vernier sliding Calipers and recorded after 15 minutes, 30 minutes, 45 minutes and 1hr.

3.7.3 EAR INJECTION

The right ear of the goat was injected intradermally with $\frac{1}{4}$ dilution of antigen while the left ear was injected with 0.1ml sterile saline as control.

3.7.4. PALPEBRAE INJECTION

0.1ml of the antigen was infused into the palpebral region of the right eye of the goat. A saline control was also infused into the palpebrum of the left eye and both palpebrae observed for any changes.

3.7.5 TITRATION OF SKIN REACTIVITY OF ANTIGEN B IN A NON-IMMUNUSED ANIMAL

A previously non-immused New Zealand White rabbit was used. The hair on the back was clipped and remnants removed using the barium sulfide starch mixture. Injection sites for control and antigen dilutions were marked on the back using a felt pen. The rabbit was blued by injecting 2.5mls of sterile 2% Evans Blue intravenously. 0.1ml of the antigen dilutions and sterile saline as control were injected. The diameters of the reaction sites were recorded.

3.8 DETERMINATION OF MOLECULAR WEIGHT OF ANTIGEN B

Determination of the molecular weight of the heated hydatid cyst antigen was carried out on a Sephadex G-200 column by the method of Andrews (1964).

The column was packed using the method of Hudson and Hay (1980). 17g of Sephadex G-200 powder (Pharmacia Fine Chemicals, Uppsala, Sweden) were suspended in 750mls of PBS, pH 7.4 and boiled for 3hrs. The gel was then allowed to cool to room temperature. It was then degased using a vacuum pump to remove all air bubbles present. A reservoir was fitted at the top of the column and the gel was poured into the column all at one time. The column outlet was left open during the packing for one day and a flow adapter was fitted. Packing was continued by

pumping PBS, pH 7.4 with 0.02% sodium azide until completely packed using an LKB peristaltic pump. The height of the packed column was 86cm while the diameter was 2.6cm.

3.8.2 CALIBRATION OF THE COLUMN

Calibration was done using markers of known molecular weight supplied by Pharmacia Fine Chemicals and serum proteins of known molecular weight. The markers used and the corresponding molecular weights are listed below.

Marker	Molecular weight
Blue Dextran 2,000	2,000,000
Bovine IgG	150,000
Bovine Serum Albumin (BSA)	67,000
Ovalbumin	43,000
Horseradish peroxidase	40,000

1mg Blue Dextran, 50mg bovine IgG, 50mg bovine serum albumin and 50mg ovalbumin were dissolved to a final volume of 3mls in saline. The protein mixture was then applied to the column using a peristaltic pump. The flow rate of the pump was adjusted to deliver 8 drops per minute. The Fraction Collector (LKB Bromma, Sweden) was adjusted to deliver 60 drops per tube. Phosphate buffered saline, pH 7.4 with 0.02% sodium azide was the eluting buffer.

After all the fractions had been collected, their optical densities were read on a Beckmann Spectrophotometer Model 25 at 280 nm. From these readings a curve was made showing peak tubes for each protein standard used. By accurate measuring of the volumes of the fractions the Void volume (V_0) of the column and the Elution volume (V_e) for each of the standards was obtained. The column was washed overnight with the eluting buffer to remove protein remnants. An identical mixture of protein standards was applied to the column and the void and elution volumes obtained. The average void and elution volumes for the two sets of protein standard were taken. These are listed in table 5.

From the formula

$$K_{av} = \frac{V_e - V_0}{V_t - V_0}$$

where

V_0 = void volume

V_e = elution volume

V_t = Total bed volume

K_{av} = Fraction of stationery gel volume which is available for diffusion of a given solute (Gel filtration: theory and practice. Pharmacia Fine Chemicals).

the Kavs of the standards used were calculated. The Kavs were then plotted on a semilog scale against the corresponding molecular weights. The best line which joins the points on the graph was drawn to give the calibration curve as illustrated in Fig 7.

3.8.3 APPLICATION OF ANTIGEN B PREPARATION ON THE COLUMN

The antigen B preparation was concentrated approximately 100 x on an Amicon membrane filter with a cut off point of 10,000 daltons. 3 mls of this sample was applied on the column with the same pump and fraction collector adjustments. Detections of antigen B in the fractions was done by ELISA and by skin tests in goat No. 880.

TABLE 5: ELUTION VOLUMES(V_e) AND K_{av} s OF PROTEIN STANDARDS

SUBSTANCE	V_e	K_{av}	M.W.
IgG	220	0.213	150,000
BSA	280	0.413	67,000
Ovalbumin	303	0.490	43,000
HRPO	310	0.513	40,000

3.9 DETECTION OF ANTIGEN B IN SEPHADEX G-200 COLUMN FRACTIONS USING ELISA

The detection was done using goat No. 880 serum as the first antibody and sheep antigoat IgG Horseradish peroxidase as the conjugate. The test was carried out in microtitre plates (Falcon, Div, Becton, Dickson and Company, Oxnard, California, USA).

The plate was first coated with 100 μ l per well of each of the fractions in duplicates. Dilution of unfractionated antigen were also included. The plates were incubated overnight at room temperature in a humid chamber. The fractions were flipped from the plates which were washed 5 times at 5 minutes intervals using the Enzyme Immunoassay Wash (Appendix II). Each time the plate was flooded with the wash solution making sure to fill all the wells and left for 5 minutes. The plate was then flipped off and tapped on tissue paper.

Goat No. 880 serum diluted 1:800 in serum diluent (Appendix III) was added in volumes of 100 μ l per well using the Multi-Channel pipette (Titertek, Finland). The plate was shaken for 1 minute on a Micro-shaker (Dynatech Instruments, Inc., Santa Monica, California, U.S.A.) and incubated for 2hrs at 37°C in a humid chamber. After incubation the plate was washed 5 times as described earlier. 100 μ l of 1/100 dilution of antigoat IgG - HRPO conjugate was added to each well. The plate was

shaken and incubated for 1hr at 37°C in a humid chamber. The plate was then washed and rinsed with distilled water.

Finally 100µl of the substrate Ortho-phenylenediamine (Appendix IV) per well was added. The plates were shaken and incubated at room temperature in the dark. The reaction was monitored with 410nm filter on a Minireader (Dynatech Instruments, Inc, Santa Monica California, U.S.A.) The reactions were stopped after 1hr by the addition of 50µl of 2N H₂SO₄ to each well. The plates were shaken and the optical densities read with 490nm interference filter. The optical densities obtained were plotted against fraction volumes.

3.10 DETECTION OF ANTIGEN B IN SEPHADEX G-200

COLUMN FRACTIONS USING SKIN TEST IN GOAT NO. 880

The right flank of goat No. 880 was clipped a day before the test. The hair was removed by applying a paste of barium sulfide and starch in water. The goat was thoroughly washed to remove remnants of barium sulfide.

The following morning injection sites were marked on the goat using a felt pen. An injection plan was drawn out on paper. The fractions were then injected in duplicates from tubes 34 to 65. 0.1ml of each fraction

was injected intradermally using a 26 gauge needle. 0.1ml sterile saline was injected as the control. Double dilutions of unfractionated antigen B preparation were also injected. The thickness of each injection site was recorded after 30 minutes.

3.11 INTRADERMAL TESTING OF CATTLE FOR HYDATID DISEASE

The IDT screening of cattle for hydatid disease was carried out at the Kenya Meat Commission Abattoir at Athi River. Attempts were made to select animals from high incidence and also low incidence areas.

The animals to be screened were put in a crush and identified by marking numbers on the forehead and rump using an indelible ink plastic marker. The site of injection was the caudal fold. The left caudal fold was used as the control site while the right side was used as the test site. The test and control sites were first marked with a felt pen. The thickness of the folds at the sites was then taken before injection and recorded. 0.1ml of antigen dilution and 0.1ml of sterile saline was injected on the right and left sides, respectively at the point marked with the felt pen. The injection was done intradermally using a 1ml tuberculin syringe fitted with a 26 gauge needle. A time lapse of 30 minutes was allowed and thickness on both sites measured and recorded.

The animals were then followed to slaughter and carcass and organs tagged with the same number as appearing on the skin of the animals. A thorough inspection of the carcass and organs was carried out and any parasites or pathological changes observed were recorded.

3.12 INTRADERMAL TESTING OF GOATS FOR HYDATID DISEASE

The IDT of goats with antigen B was carried out at Ongata Rongai, Ngong Cooperatives slaughterhouse in the outskirts of Nairobi. Attempts were made to select goats from high incidence areas like Turkana and Masailand and also flocks from low incidence areas. A total of 135 goats were skin - tested.

The goats were first identified by tagging with a number on the hindleg before test and slaughter. The site of injection was the caudal fold. The left side was used as control site while the right was the test site. The point of injection on both folds was marked with a felt pen and the fold thickness taken using a caliper and recorded. 0.1ml of antigen dilution and 0.1ml of sterile saline as control were then injected intradermally on the right and left caudal fold, respectively using a 1ml tuberculin syringe fitted with a 26 gauge needle. The time of injection was recorded. After 30 minutes the thickness of the right and left folds was measured and recorded.

The goats were then followed to slaughter and the carcass and organs marked with the same number as that on the hindleg. A thorough inspection of the carcass and organs was carried out during meat inspection especially checking for the presence of hydatid cysts. Any other parasites or pathological changes were recorded.

3.13 ENZYME IMMUNOASSAY (EIA)

3.13.1 PREPARATION OF CONJUGATE

The goat No. 346 IgG -glucose oxidase conjugate was prepared in our laboratory by the method of Wilson and Nakane (1978). A chequer-board titration was carried out to determine the optimal conjugate dilution to use in the test.

3.13.2 PROCEDURE FOR THE INHIBITION ENZYME IMMUNOASSAY

The microtitration procedure was used for the screening of bovine serum samples for the presence of antibodies to "Arc 5" antigen. For each test serum sample two dilutions of 1:10 and 1:50 were run in duplicates. On each plate a known positive serum (calf No. 28) and a known negative serum were included. Crude fertile goat hydatid cyst fluid clarified by

centrifugation and filtration was used as the antigen. The antigen was diluted 1/20 in phosphate buffer with 0.4M NaCl, 2% Polyethylene glycol (PEG) and 0.02% sodium azide.

The procedure was as follows:-

- 1) Microtitre plates (Falcon, Div, Becton Dickson and Company, Oxnard, California, U.S.A.) were coated with goat HCF 1/20 as described above. The plates were incubated overnight at room temperature. The plates and their contents were then kept at -20°C .
- 2) The plate to be used was then kept out for the contents to thaw and washed 5 times at 5 minutes intervals using the Enzyme Immunoassay washing solution as described earlier.
- 3) Dilutions of test serum samples were added in quantities of 100 μl per well in duplicates using a micropipette. Similar quantities of the positive and negative serum samples were also added in duplicates. The plate was incubated overnight at room temperature in a humid chamber.
- 4) The plate was flipped dry and not washed. Goat No. 346 IgG - glucose oxidase conjugate diluted 1/40 in KCL/EDTA + 0.5% tween without PEG was added in volumes of 100 μl per well. 2% normal bovine serum,

2% normal sheep serum, 2% normal rabbit serum and 2% normal human serum were previously added to the conjugate to absorb any possible activity to any of these hosts. The plates were incubated for 1hr at 37°C.

- 5) The plate was washed again as described and finally rinsed with distilled water and dried with tissue paper. 100µl of the substrate ABTS (Appendix V) were added to each well. Maximum colour development occurred between 30 and 50 minutes and optical densities were read at 410nm on the Minireader.

CONTROLS INCLUDED

1. Zero% - Inhibition

In one column of wells, no serum was added. The optical density values represent zero percent inhibition.

2. Conjugate controls

The wells only received the conjugate and substrate. This was to check whether the conjugate binds non-specifically to the plate.

3. Substrate controls

Only the substrate was included in this control.

3.13.3 STANDARD CURVE FOR THE ENZYME IMMUNOASSAY

Each plate contained dilutions of known positive sera and known negative sera. The known positive sample was calf No. 28 serum. The dilutions of calf serum samples run were 1:200, 1:400, 1:800, 1:1600, 1:3200 and 1:6400. The negative serum sample dilutions included were 1:10, 1:50 and 1:250. Each dilution was run in duplicate.

To establish the standard curves, average ODs for 15 assays of each of these dilutions both for positive and negative sera were used. The average OD was then plotted versus the serum dilution on semi-log scale. From these standard curves it was then possible to determine the 50% inhibition titres for all the test serum samples. This was done by:-

- a) Calculating the % inhibition of each dilution of serum sample using the formula.

$$\% \text{ INHIBITION} = \frac{\text{OD}_z - \text{OD}_s}{\text{OD}_z} \times 100$$

where OD_z = optical density at 0% inhibition

OD_s = optical density of serum sample

- b) From the standard curve obtain the dilution of the reference positive serum giving 50% inhibition and the dilution of reference serum having the same % inhibition as given by serum sample dilution.

- c) Get the reciprocals of these serum dilutions and obtain the 50% inhibition titre using the formula

$$50\% \text{ Inhibition titre} = \frac{a}{b} \times c$$

where

a = Reciprocal dilution of reference serum giving 50% inhibition

b = Reciprocal dilution of reference serum having same % inhibition as given by the serum sample dilution

c = Dilution of test serum sample.

4. RESULTS

4.1 ANTIGEN B PREPARATION

Boiling of hydatid cyst fluid rendered all except one of the hydatid cyst fluid antigens inactive in the immunological tests employed in this study. The preparation was shown in immunodiffusion and immunoelectrophoresis to contain only one hydatid cyst fluid antigen. In immunodiffusion sheep, No. 822 serum showed only one precipitin line which was identical with goat No. 880 precipitin line. Rabbit antiovine serum (Rabbit No. 87) did not detect any bovine serum components in this preparation.

4.2 TITRATION OF ANTIGEN B IN GOAT NO. 880

Well delineated blue spots were obtained on the flank of the goat. The diameters of these blue spots were measured. The average diameters were calculated for each dilution of antigen. These are indicated in Table 6. The skin reactions reached a maximum diameter after 30 minutes after which there was no further increase. This titration however did not give a good dose response but a dilution of $\frac{1}{4}$ of the antigen was selected for use in the skin test as it was the highest dilution which gave reactions which were just slightly smaller than those given by the more concentrated dilutions. Reaction sites are illustrated in Figures 2, 3, and 4.

TABLE 6: DIAMETER OF REACTION SITES OF ANTIGEN B
TITRATION IN GOAT NO. 880

DILUTION	DIAMETER (mm)
UNDILUTED	13.0
1:2	12.1
1:4	12.0
1:8	10.7
1:16	11.3
1:32	11.0
1:64	10.2
1:128	6.5
1:256	5.9

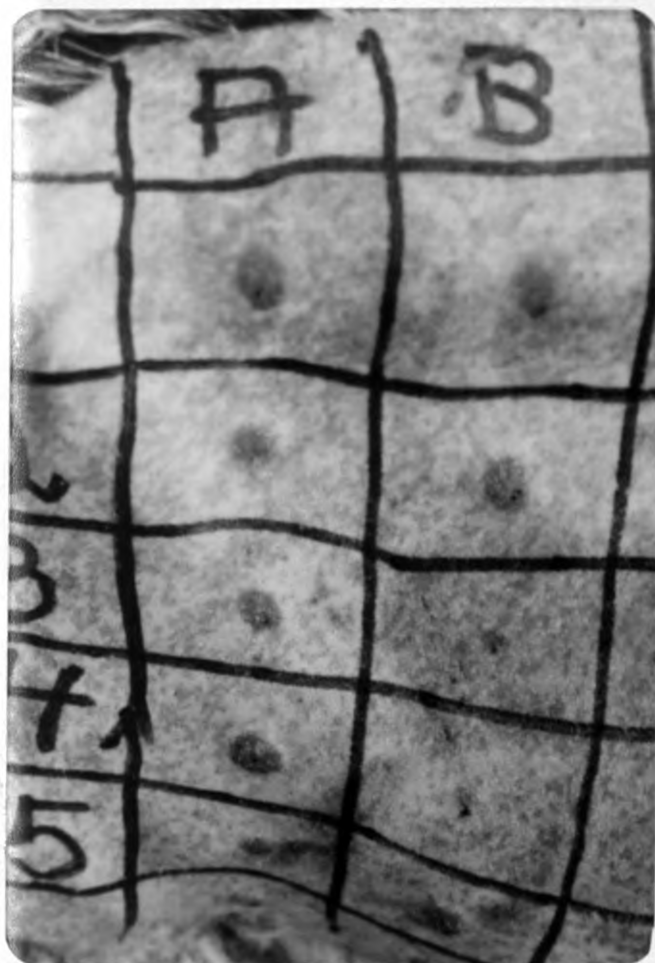


Fig 2: Intradermal reactions resulting from injections of various dilutions of antigen B into goat No. 880. The reaction sites were randomised in an experiment designed to establish the optimal dose for routine use.



Fig 3: Intradermal reactions resulting from the injections of various dilutions of antigen B into goat No. 880. The photograph was taken at an angle which allowed clear visualization of the marked oedematous swellings.

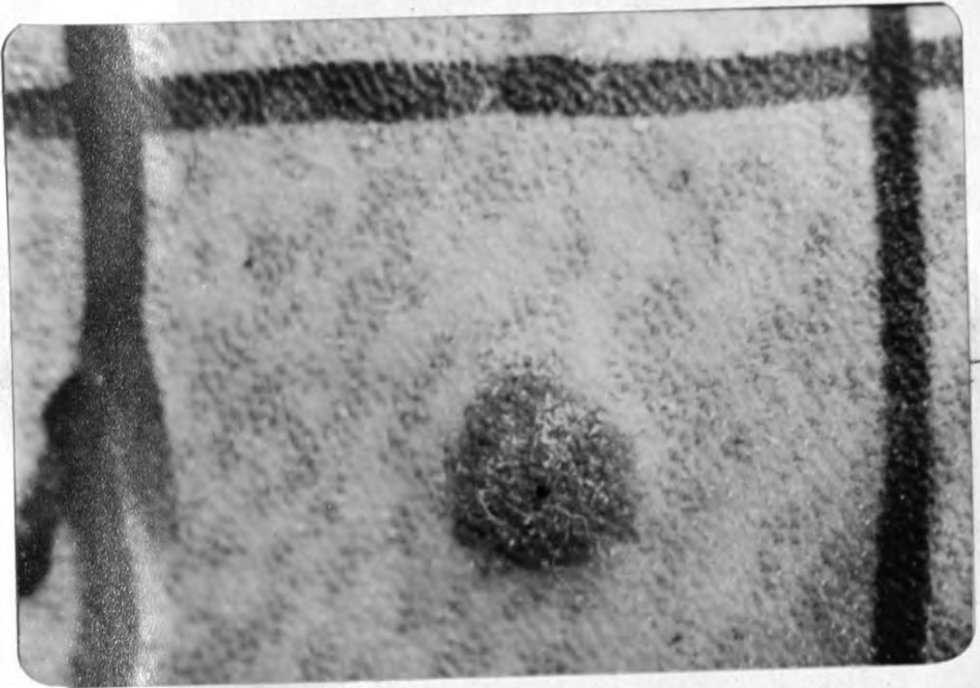


Fig 4: A close-up photograph of reaction resulting from an intradermal injection of antigen B. The increased vascular permeability is clearly shown by the extravasation of Evans blue.



Fig 5: The intradermal reaction resulting from injections of $\frac{1}{4}$ dilution of an antigen B preparation in the right caudal fold of goat No. 880. The left fold was injected with sterile saline as negative control.

4.3 REACTIVITY OF ANTIGEN B IN A NON-IMMUNISED ANIMAL

The reactions obtained with antigen dilutions injected were similar to those obtained by injection of the sterile saline controls. These reactions also showed no dose response. All the reactions were in the range of 1-2mm.

4.4 RESULTS OF

1. CAUDAL FOLD INJECTION IN GOAT

After 30 minutes, the $\frac{1}{4}$ dilution of antigen gave a skin thickness of 7.0mm while the sterile saline control gave a thickness of 2.5mm. The $\frac{1}{16}$ dilution of antigen gave a skin thickness of 5.5mm while the control gave a thickness of 3.0mm. The caudal fold reactions is illustrated in Fig. 5.

2. EAR INJECTION IN GOAT

The right ear that was injected intradermally with $\frac{1}{4}$ dilution of antigen showed extensive oedema in the subcutis while very little oedema was observed on the left ear where the sterile saline control was injected.

3. PALPEBRAL INJECTION IN GOAT

No change was observed in the palpebral region of the eye that received the dilution of antigen. The reaction was similar to that of the control eye that received sterile saline. No inflammatory reaction was observed.

4.5 RESULTS OF DETECTION OF ANTIGEN B IN SEPHADEX
G200 COLUMN FRACTIONS

(a) ELISA

Three peaks of antigen activity were obtained. The first peak coincided with an elution volume of 148mls, the second with an elution volume of 188 mls and the third peak with an elution of 205mls.

(b) Skin tests

Two peaks of skin reactivity were obtained (Fig. 6). The first peak coincided with an elution volume of 148mls and the second with an elution volume of 205mls.

4.6 RESULTS OF MOLECULAR WEIGHT DETERMINATION

The elution volume for the antigen peak was found to be 205 mls. The K_{av} for the antigen was calculated using the formula

$$\begin{aligned} K_{av} &= \frac{V_e - V_o}{V_t - V_o} \\ &= \frac{205 - 156}{478 - 156} \\ &= 0.152. \end{aligned}$$

From the standard curve the corresponding molecular weight to K_{av} of 0.152 was read off. This gave a molecular weight of approximately 180,000 daltons.

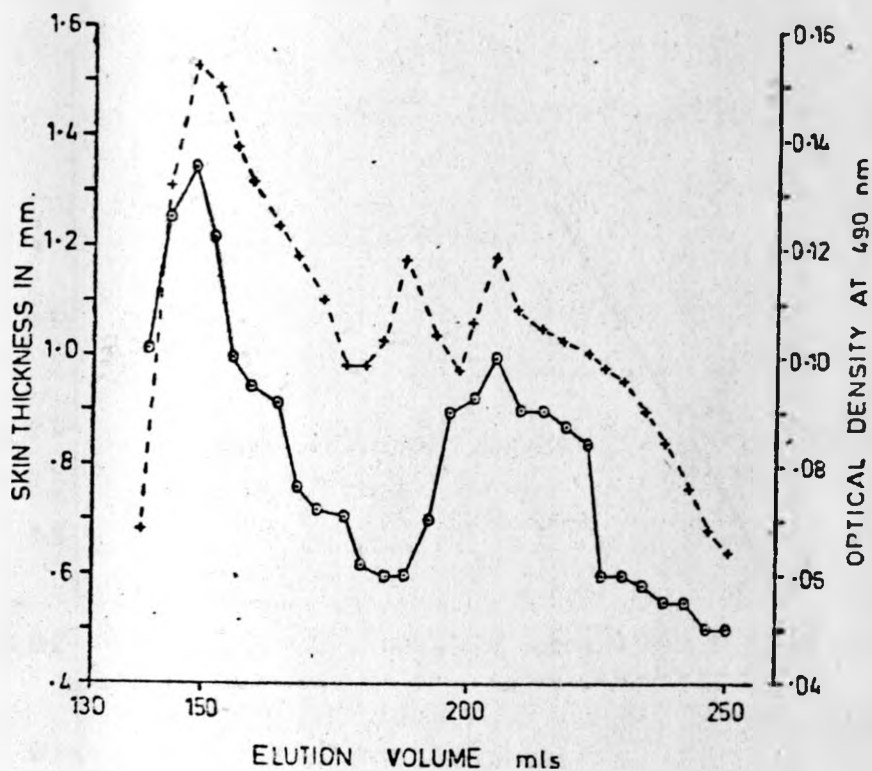


Fig 6: Detection of antigen B in Sephadex. G-200 gel-filtration fractions by

(i) —○—○— Skin test on goat No. 880

(ii) - -x- -x- ELISA optical densities

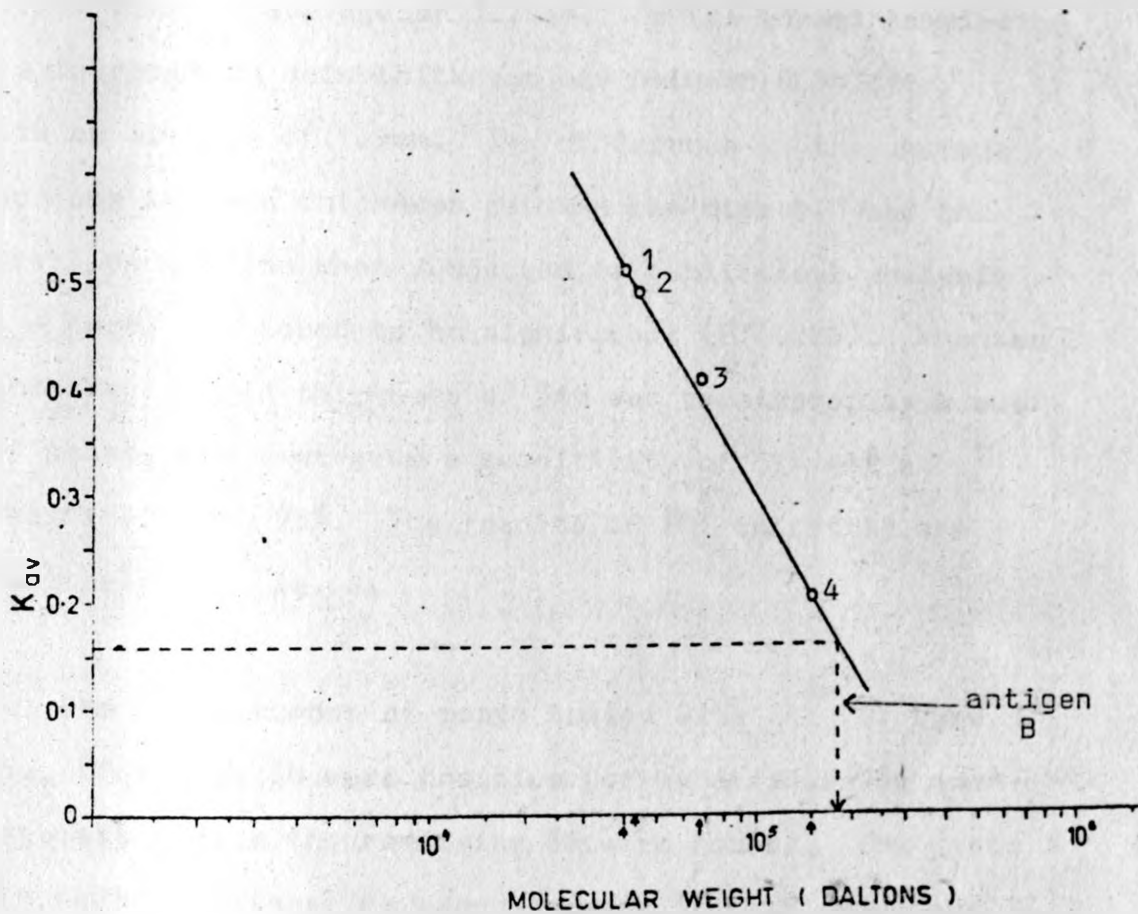


Fig 7: Standard curve for Molecular Weight determination obtained by using HRPD (1), Ovalbumin (2), BSA(3) and IgG (4).

4.7 RESULTS OF IDT IN CATTLE AND GOATS

A total of 64 cattle were screened with IDT. Of these 35 were positive for hydatidosis on meat inspection while 29 were normal. The increase in skin thickness of the caudal fold in the positive animals ranged from 0.7mm to 10.3mm with an average of 3.79mm. In the normal population the increase in skin thickness was between 0.3-3.5mm with an average of 1.9mm. The difference in the average increase in skin thickness between the diseased and the normal population when subjected to statistical analysis (t - test) was found to be significant ($P < 0.05$). When an increase in skin thickness of 3mm was considered as a cut off point, the test gave a sensitivity of 57% and a specificity of 97%. The results of IDT in cattle are illustrated in Fig. 8.

The total number of goats tested with the IDT were 135. Of these, 50 were positive for hydatidosis on meat inspection while the remaining 85 were normal. The goats with hydatid disease gave an increase in skin thickness ranging from 0.3mm to 5.3mm with an average of 2.5mm while the normal goats gave an increase ranging from 0.2mm to 4.7mm with an average of 2.47mm. When the differences between the mean increase in skin thickness in the positive and the normal population were subjected to statistical analysis (t - test), no significant difference was found. Results of IDT in goats are illustrated in Fig. 9.

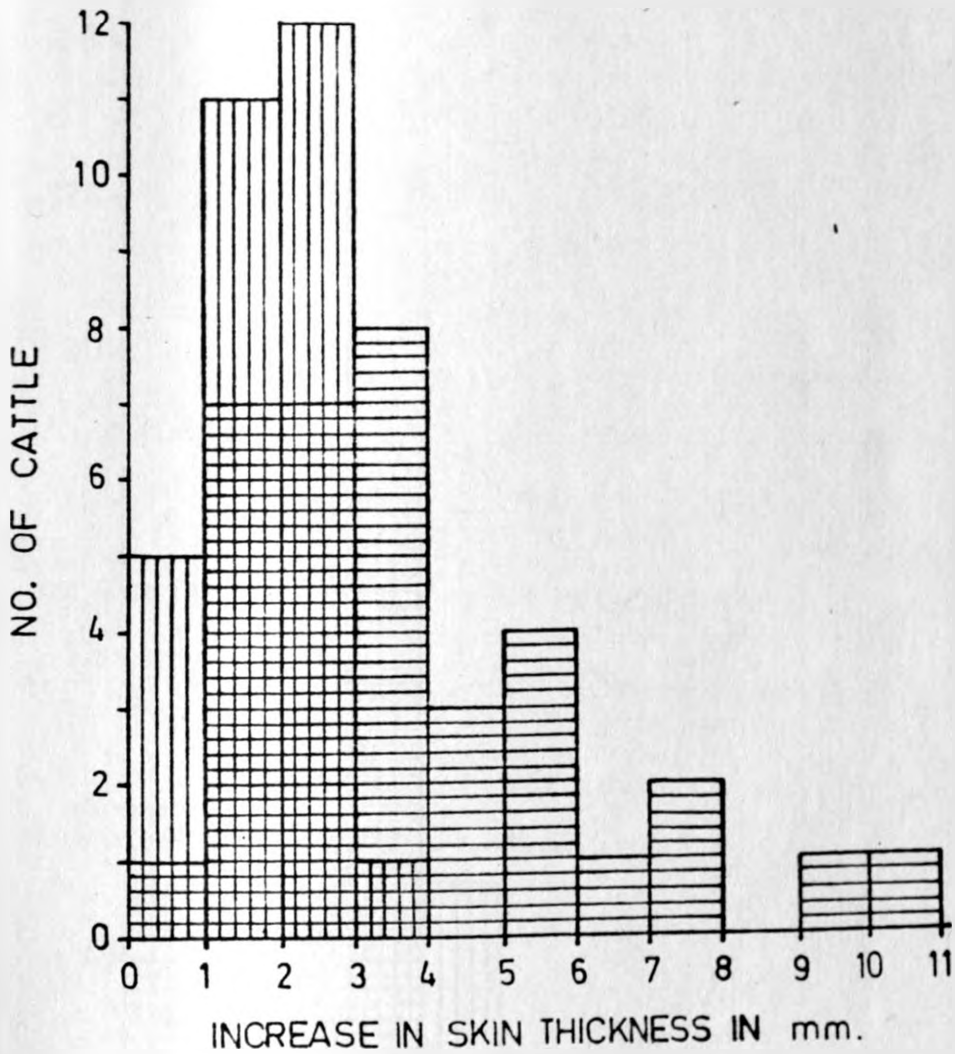


Fig 8: Results of intradermal tests in cattle with or without hydatid cysts, as determined by routine meat inspection procedures.

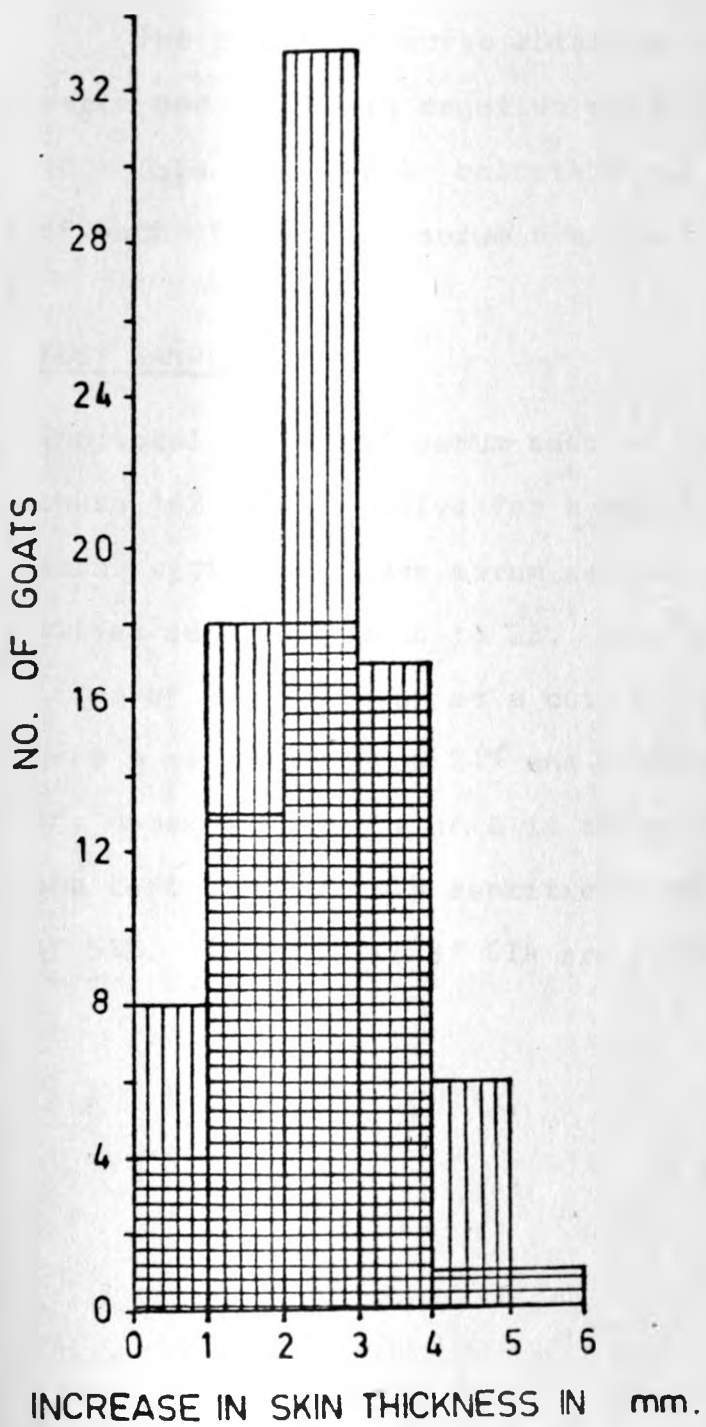
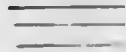



Fig 9: Results of intradermal tests in goats with  or without  hydatid cysts, as determined by routine meat inspection procedures.

4.8 RESULTS OF ENZYME IMMUNOASSAY

STANDARD (REFERENCE) CURVE

The reference curve obtained for the known positive serum and the known negative serum is illustrated in Fig. 10. This was used to calculate the 50% inhibition titres of each of the test serum samples.

TEST SAMPLES

The total number of serum samples titrated was 268. Of these 162 were positive for hydatid disease while 106 were negative. These serum samples gave 50% inhibition titres ranging from 4 to 28. When a 50% inhibition titre of 12 was taken as a cut off point, the test gave a sensitivity of 21% and a specificity of 99%. If, however, a titre of 8 is taken as the cut off point the test would give a sensitivity of 56% and a specificity of 59%. The results of EIA are illustrated in Fig. 11.

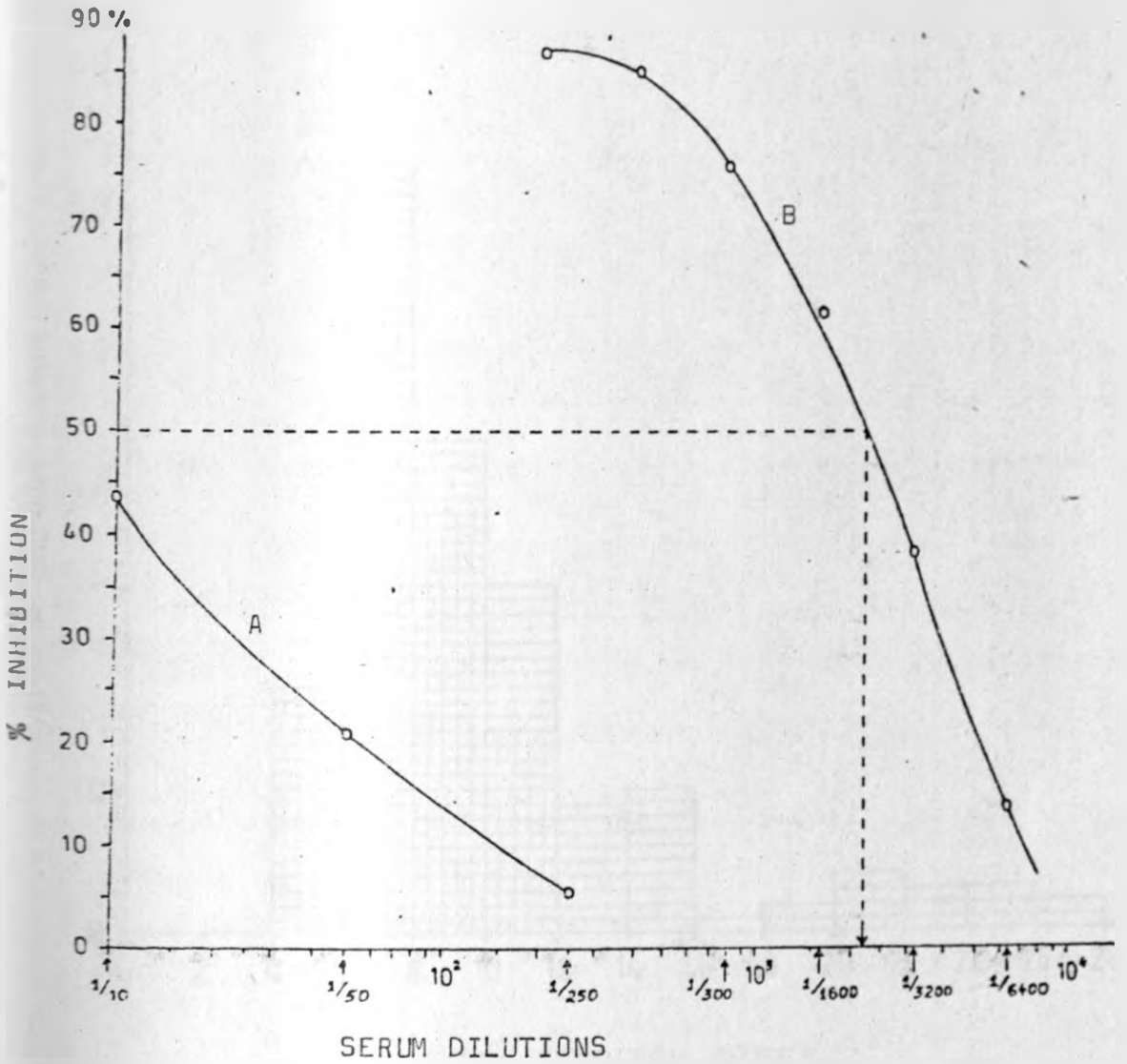


Fig 10: Enzyme Immunoassay Reference Curve.

A: Curve given by known hydatid disease negative serum.

B: Curve given by known hydatid disease positive serum.

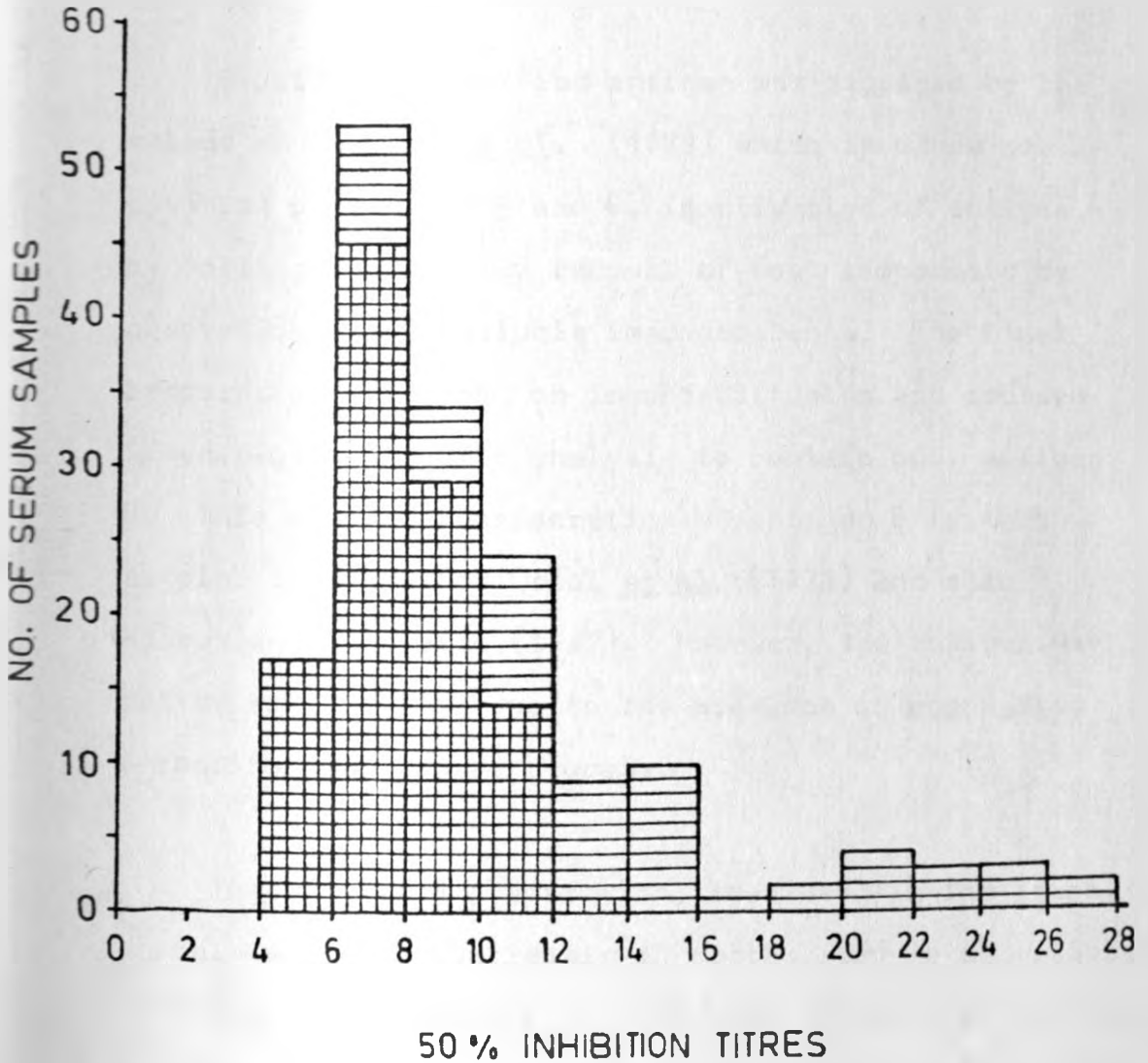




Fig II: Results of Enzyme Immunoassay on cattle serum samples positive  or negative  for hydatid disease as determined by routine meat inspection procedures.

5. DISCUSSION

The aim of the first part of this project was to prepare from hydatid cyst fluid a partially purified antigen suitable for use in IDT for the diagnosis of hydatid disease in livestock.

A partially purified antigen was prepared by the method of Musiani et al. (1978) which involves precipitation of antigen A and B, inactivation of antigen A by boiling and finally removal of host components by absorption with insoluble immunosorbents. The final preparation was shown on immuno-diffusion and crossed immunoelectrophoretic analysis to contain only antigen B. This method of preparation of antigen B is much simpler than that of Oriol et al. (1971) and also Njeruh and Lindqvist (1982). However, the antigen may not be equally pure due to the presence of aggregates formed in the heating process.

The antigen B preparation, when used in the intradermal test for hydatidosis in cattle, gave a sensitivity of 57% and a specificity of 97% when an increase in skin thickness of 3mm and above was considered positive. The results obtained with goats showed an almost complete overlap of skin reactivities between the normal animals and those with hydatidosis. The difference between the average increases in skin thickness of the hydatidosis population of goats and the normal population was not statistically significant.

The difference in reaction between cattle and goats as indicated in the results could have been just a random occurrence. The possibility that there may be an inherent difference in skin reactivity of the two species to antigen B should also be considered.

Most of the literature available on IDT concerns its use in the human population where results indicate high sensitivities (Roy et al., 1970; Lass et al., 1973) but most investigators agree on its low specificity. Different antigen preparations varying from whole hydatid cyst fluid to boiled hydatid cyst fluid have been used. Several workers have suggested that boiling of hydatid cyst fluid improves both the sensitivity and specificity of IDT (Williams, 1972; Yarzabal, 1975). It was anticipated that this could be confirmed in this study but this proved not to be the case. Unacceptably low sensitivity and specificity were obtained.

Several factors could have led to the results obtained. The purity of the antigen used in the IDT has always been a major problem (Rickard, 1979). Further purification of the antigen preparation may be necessary. Although the antigen preparation showed only one immunoreactive component in vitro when tested with various specific sera, it would still contain denatured components which may give rise to non-specific skin reactions. The concentration of antigen used may also have resulted in low sensitivity and specificity. An antigen dilution of $\frac{1}{2}$ which contained

30ug protein in 0.1ml was used. This was arrived at by titration of antigen on a goat which had been hyperimmunized with antigen B. The hyperimmune goat showed good skin reactivity, but a hyperimmune goat cannot be considered representative of or even remotely similar to naturally infected animals. It would therefore not be justified to conclude that naturally infected animals should be expected to exhibit skin reactions of sufficient intensity for diagnostic purposes. Obviously, a more rational approach to the standardization of the skin test antigen and to examine the anaphylactic antibody responses of animals would be to use experimentally infected animals and/or animals known to have hydatid cysts. In this study, it was not possible, for practical reasons and also because of the hazards involved in infecting animals with E. granulosus eggs, to use such animals for the standardization and evaluation of the skin test antigen.

The animals used in this study all come from endemic areas where the chances of having been exposed to hydatid disease are high. It may also not be possible at meat inspection to detect all the small cysts in the liver and lungs and a positive animal may be passed as negative. The chances of having animals at an early phase of infection where macroscopically visible cysts have not formed, but the immune response has been elicited may also account for the results.

Antigen B has been shown to cross-react with some parasites that are common in cattle and goats. Njeruh and Lindqvist (1982) have shown that antigen B cross-reacts with some helminth parasites, namely Avitellina spp, Desofohanostomum spp, I. saqinata, C. tenuicollis and Moniezia spp. These parasites are quite common in cattle and goat populations where the test was performed. The presence of these parasites may give rise to non-specific skin reactions.

In man, the IgE antibody class is known to be the major immunoglobulin class responsible for immediate type hypersensitivity reactions, although IgG has been shown to take part (Parish, 1970). In animals, however, the situation is not clear as to what immunoglobulin class is responsible. Anaphylactic antibodies are definitely present in animals as demonstrated in the case of Nippostronoylus brasiliensis infections where worms are rejected by the host and after several infections no worms are allowed to establish in the gut (Dunn, 1978). Another example is the self-cure mechanism observed in smallstock infected with Haemonchus contortus where worms are expelled from the gut due to what has been considered an anaphylactic type reaction (Dunn, 1978).

The presence and possible role of homocytotropic antibodies have not yet been clearly defined in most species (Tizard, 1977). It appears that most investigators have had difficulties in their attempt to define

immunoglobulins corresponding to IgE in man (Jarett, 1973). It is known that immunoglobulins of other classes may elicit or participate in anaphylactic reactions in several species of animals. The possibility thus exists, that in the common domestic animals, immunoglobulin classes other than IgE may play the major role.

The findings in this study are largely in agreement with those of other workers who have used the Casoni test in animal populations. Recent reports on the evaluation of IDT in cattle and sheep showed unsatisfactory sensitivity and unacceptable lack of specificity (Alencar Filho, 1978; Jercic et al., 1979). Moreira et al. (1978) tested a herd of 169 cattle and concluded that, although easy to perform, the IDT is not a useful test in cattle. It is possible that antigen B is not the antigen that elicits the strongest and/or the most specific immediate hypersensitivity reaction in animals and further investigations of this question are warranted.

The molecular weight of antigen B was estimated to be 180,000 daltons. The detection of the antigen in the Sephadex G-200 column fractions was done with ELISA and by skin testing. The first peak both for the ELISA and skin reactivity which coincided with the void volume is thought to comprise aggregates of the 180,000 daltons component. The second peak in ELISA corresponds with low skin reactivity. The third peak in ELISA

corresponds with the second peak of skin reactivity. Oriol et al. (1971) reported a molecular weight of 160,000 daltons while Williams (1982) reported a molecular weight of 150,000 daltons for antigen B. It is suggested from this study that the best fraction to use for IDT from a Sephadex G-200 column with the same specifications is one coinciding with the second peak of skin reactivity (i.e. molecular weight of 180,000 daltons).

The unsatisfactory results obtained with the use of a partly purified antigen preparation in the Casoni test, prompted investigations into the use of the so called "Arc 5" antigen of Capron et al. (1967) also referred to as antigen A of Oriol et al. (1971) in an enzyme immunoassay. Enzyme immunoassays have shown promise when used by other research workers. Farag et al. (1975) used ELISA with purified "Arc 5" antigen and obtained a high sensitivity. Njeruh and Lindqvist (1982) obtained absolute specificity and a sensitivity of 91.2% using antigen B.

In this study, however, when a cut off point of a 50% inhibition titre of 12 was considered positive for hydatid disease in cattle, an unacceptably low sensitivity of 21% was obtained while a specificity of 99% was realised. When a 50% inhibition titre of 8 was considered positive the sensitivity increased to 56% at the expense of the specificity which was reduced to 59%.

The detection of antibodies to "Arc 5" may have proved difficult in this case for several reasons. Possibly the level of anti - "arc 5" antibody in cattle is low. The "arc 5" antigen is usually present in very low quantities in hydatid cysts. The ratio of "arc 5" antigen to antigen B in hydatid cysts is usually 1:10 (Musiani et al., 1978). In man, antigen B appears to escape much more easily through the laminated membrane but the immune response it elicits is less effective than that of "arc - 5" antigen (Williams, 1982). In cattle, however, the situation is not clear.

The cattle population used in the study came from an endemic area where chances of having been infected are high. These animals, although not showing any hydatid cysts at meat inspection would have detectable antibody titres. These may give serologically positive results that would be erroneously classified as "non-specific". Recently, "arc - 5" antigen has been shown to be present in certain other parasites (Schantz et al., 1980). If any of these are present in the animals used in the test, this would also affect the sensitivity and specificity of the test.

In the Turkana population immunodiagnosis for hydatid disease has shown disappointing results giving low sensitivity and specificities (Chemtai et al., 1981). Similar findings are reported by Huldt et al. (1973) with the Lapps in Sweden. These low sensitivities

and specificities have been thought to be results of the presence of inhibitors and immune complexes in serum samples. Although no attempts were made to check for inhibitors and immune complexes in the cattle serum samples used in the enzyme immunoassay, their presence and subsequent effect on the results cannot be ruled out.

The fact that the conjugate was made from hyper-immune serum resulted in the production of a conjugate with high avidity antibodies. The high avidity antibodies of the conjugate interfered with the competitive reaction by displacing low avidity antibodies that are attached to the antigen. It might have been better in the present study to use, for preparation of the conjugate, antibodies from early bleedings which have been shown to have a low avidity.

CONCLUSIONS

It is concluded from the results of this study that the Casoni test might be useful in cattle under specified circumstances while it is virtually incapable of distinguishing between infected and non-infected goats. Although the test can be used in cattle under such circumstances, the sensitivity and specificity are too low to warrant the use of this test for routine serodiagnosis or seroepidemiological surveys.

The results indicate that further purification of the antigen B preparation used may be necessary. A better method for the standardisation of the antigen to be used in the IDT is also necessary. It is also concluded that although antigen B is a potent allergen, it does not seem to be specific for hydatid disease and further investigations may be warranted to elucidate this aspect.

The enzyme immunoassay using anti "arc - 5" specific serum has shown unsatisfactory sensitivity and specificity. This test can not therefore be recommended for use in a serodiagnostic or seroepidemiological work in cattle.

REFERENCES

- African Medical Research Foundation. Annual Report (1979).
- Agosin, M., Von Brand, T., Rivera, G.F. and
McMahon, P. (1957). Studies on the metabolism
of E. granulosus II: Some observations on the
carbohydrate metabolism of hydatid cyst scolices.
Exp. Parasitol. 6: 586-593.
- Alencar Filho, R.A. DE (1978). Diagnostico immunologico
da hidatidose animal - resultados preliminares.
Arg. Inst. Biol. (S. Paulo) 45: 101.
- Ambroise - Thomas, P.(1976). Immunofluorescence in the
diagnosis of, therapeutic follow-up and sero-
epidemiological studies of some parasitic diseases.
Trans. Roy. Soc. Trop. Med. Hyg. 70: 107-112.
- Andrews, P. (1964). Estimation of the molecular weights
of proteins by Sephadex Gel-Filtration.
Biochem. J. 91: 222-232.
- Bekhti, A., Schaaps, P., Capron, M., Dessaint, J.P.,
Santoro, F. and Capron, A. (1977). Treatment of
hepatic hydatidosis with mebendazole: Preliminary
results in four cases. Brit. Med. J. 2: 1047-1051.
- Ben-Ismail, R., Carne, E. Niel, G. and Gentilini, M.
(1980). Non-specific serological reactions with E.
granulosus antigens: Role of Anti-P₁ antibodies.
Amer. J. Trop. Med. Hyg. 29: 239-245.

- Sennich, H. and Johansson, S.G.O. (1971). Structure and function of human immunoglobulin E.
Adv. Immunol. 13: 1-4.
- Bozicevich, J. (1951). Quoted in Echinococcus antigens.
Bull. Wld. Hlth. Org. 1968. 39: 13-24.
- Cameron, G.L. and Stavely, J.M. (1957). Blood group P substance in hydatid cyst fluids.
Nature 179: 147-148.
- Capron, A., Vernes, A. and Biguet, J. (1967).
Le diagnostic immunoelectrophoretic de l'hydatidose.
Lecyste Hydatique du foie journee lyonnaises
de hydattidoque. P. 27-40. Simep, Lyon.
- Capron, A. and Dessaint, J.P. (1977).
IgE and cells in protective immunity.
Path. Biol. 25: 287.
- Casoni, T. (1911). La diagnosi biologica dell' Echinococcosis
Umana mediante l'Intradermoreazione.
Fol. Clin. Chem. Micro 4, 5.
Cited In: Clinical Aspects of Immunology P. 186
(Eds. Gell, Coombs and Lachman).
Blackwell Scientific Publications Ltd.
- Castagnari, L. and Sorice, F. (1965). Quoted in
Echinococcus antigens.
Bull. Wld. Hlth. Org. 1968., 39: 13-24.

Chemtai, A.K. Bowry, T.R. and Ahmad, Z. (1981).

Evaluation of five immunodiagnostic techniques
in echinococcosis patients.

Bull. Wld. Hlth. Org. 59:767-772.

Chordi, A. and Kagan, I.G. (1965).

Identification and characterization of antigenic
components of sheep hydatid cyst fluid by
immuno-electrophoresis.

J. Parasitol. 51: 63-71.

Emelik, E. (1952).

Ein antigens polysacchrid aus den Echinococcusystem.
Biochemische Zeitschrift 322: 456-462.

Conder, G.A., Andersen, F.L. and Schantz, P.M. (1980).

Immunodiagnostic tests for hydatidosis in sheep:
An evaluation of Double diffusion, Immuno-
electrophoresis, Indirect haemagglutination and
Intradermal tests.

J. Parasitol. 66: 577-584.

Coombs, R.R.A. and Gell, P.G.H. (1957).

Classification of allergic reactions responsible
for clinical hypersensitivity and disease.

In: Clinical Aspects of Immunology (Eds. Gell,
P.G.H., Coombs R.R.A. and Lachmann P.J.)

Blackwell Scientific Publications pp 761-781.

Daubney, R. (1926).

Annual Report, 1925. Department of Agriculture,

Kenya 111-123.

Dennis, E.W. (1957).

Stable, concentrated, purified antigen for immunological study of hydatid disease.

J. Parasitol. 23: 62-67.

Dessaint, J.P., Bout, ., Wattre, P. and Capron, A. (1975).

Quantitative determination of specific IgE antibodies to Echinococcus granulosus and IgE levels in sera from patients with hydatid disease.

Immunology 29: 813 - 823.

Dottorini, S. and Carmelo, T. (1977).

E. granulosus characterisation of the main antigenic components (Arc 5) of hydatid fluid.

Exp. Parasitol. 43: 307-314.

Dunn, M.A. (1978).

Veterinary Helminthology.

William Heinemann Medical Books Ltd. London.

Dvorak, H.F. and Dvorak, A.M. (1974).

"Cutaneous basophil hypersensitivity"

Progress in Immunology II Vol. 3.

(Eds. L. Brent and J. Holbrorow) North Holland Publishing House.

Engvall, E. and Perlmann, P. (1971).

Enzyme-linked immunosorbent assay (ELISA).

Quantative assay of immunoglobulins.

Immunochem. 8: 871-874.

- Eugester, R.O. (1978). A contribution to the epidemiology of Echinococcosis/hydatidosis in Kenya (East Africa) with special reference to Kajiado District.
Thesis for Degree of Doctor of Vet. Med.
University of Zurich.
- Farag, H., Bout, D. and Capron, A. (1975).
Specific immunodiagnosis of hydatidosis by the enzyme-linked immunosorbent assay (ELISA).
Biomedicine 23: 276-278.
- Froyd, G. (1960). Cysticercosis and hydatid disease of cattle in Kenya.
J. Parasitol. 46: 491-496.
- Garabedian, G.A., Matossian, R.M. and Djanian, A.Y. (1957).
An indirect haemagglutination test for hydatid disease.
J. Immunol. 78: 269-272.
- Gershwin, L.J. (1981). Heterologous Passive Cutaneous Anaphylaxis with Bovine Immunoglobulin E.
Amer. J. of Vet. Med. 42: 1184-1187.
- Ginsberg, A. (1956). Incidence of hydatidosis in Kenya's slaughter animals.
E. Afr. Med. J. 33: 297-300.
- Gleich, G.J., Averbick, A.K.; and Swedlund, H.A. (1971).
Measurement of IgE in normal serum and allergic

serum by radioimmunoassay.

J. Lab. Clin. Med. 77: 690-694.

Gonzalez-Molina and Spiegelberg, H.L. (1977).

A subpopulation of normal human peripheral blood B lymphocytes that bind IgE.

J. Clin. Invest. 59: 616 - 624.

Hamilton R.G., Hussain, R., Ottensen, E.A. and

Adkinson, N.F. (1971). The quantitation of parasite specific IgG and IgE in sera: Evaluation of solid phase radioallergosorbent and Enzyme-Linked immunosorbent assay.

J. Immunol. Meth. 44: 101-105.

Hammerbeg, B., Musoke, A.J. and Williams, J.F. (1977).

Activation of complement by hydatid cyst fluid of Echinococcus granulosus.

J. Parasitol. 63: 327 -331.

Heath, D.D. (1970). The development biology of

larval cyclophillidean cestodes in mammals.

Ph.D thesis, Australian National University.

Heath, D.D. (1971). The migration of oncospheres of

T. pisiformis, T. serialis and E. granulosus within intermediate host.

Int. J. Parasitol. 1: 145-152.

- Heidelberger, M., Aisenberg, A.C. and Hassidi W.Z. (1954).
Quoted in Echinococcus antigens.
Bull. Wld. Hlth. Org. 1968., 39: 13-24.
- Hudson, L. and Hay, F.C. (1980).
Practical Immunology.
Blackwell Scientific Publications pp. 161-163.
- Huldt, G., Johansson, G.O. and Lantto S. (1973).
Echinococcosis in Northern Scandinavia,
Immune reactions to Echinococcus granulosus
in Kautekeino Lapps.
Arch. Environmental Health. 26: 36-40.
- Iacona, A., Pini, C., and Vicari, G. (1980).
Enzyme-linked immunosorbent assay (ELISA) in
the serodiagnosis of hydatid disease.
Amer. J. Trop. Med. Hyg. 29: 95-102.
- Ishizaka, K. (1970).
Human reaginic antibodies.
Ann. Rev. 21: 187.
- Jarrett, E.E.E. (1972). Helminth infection and
the biosynthesis of IgE antibodies.
Trans. Roy. Soc. Trop. Med. Hyg. 66: 546.
- Jarrett, E.E.E. (1973).
Reaginic antibodies and helminth infection.
Vet. Rec. 93: 480-483.

- Jercic, J., Ehrlich, I., Huber, D. and Huber, I. (1979). Dijagnosticka vriednost alrgijske reakcije za utvrdivanje larvalne ehinokokoze u zivotinja razlicitiharsta. Vet. Glasnik 33: 351.
- Kagan, I.G. and Norman, L. (1961). Antigenic analysis of Echinococcus antigens by agar diffusion techniques. Amer. J. Trop. Med. Hyg. 10: 727-734.
- Kagan, I.G., Osimani, J.J., Varela, J.C. and Allian, D.S. (1966). Evaluation of intradermal and serological tests for the diagnosis of hydatid disease. Amer. J. Trop. Med. Hyg. 15: 172-179.
- Kagan, I.G. (1968). A review of serologic tests for the diagnosis of hydatid disease. Bull. Wld. Hlth. Org. 39: 25-37.
- Kilejian, A., Sauer, K. and Schwabe, C.W. (1962). Host-Parasite relationships in Echinococcosis VIII. Infrared spectra and chemical composition of the hydatid cyst. Exp. Parasitol. 12: 377-392.
- Lascano, F.F., Coltorti, E.A. and Varela-Diaz, V.M. (1975). Fine structure of the germinal layer of E. granulosus cyst. J. Parasitol. 61: 853-860.

Lass, N., Laver, A. and Lengy, J. (1973). The immunodiagnosis of hydatid disease: post-operative evaluation of the skin test and four serological tests.

Ann. Allergy, 31: 430-436.

Leese, A.A. (1915). Annual Report 1913-1914.

Dept. Agr. Brit. E. Afr. 161.

Limaire, G. and Ribere, R. (1935).

Quoted in Echinococcus antigens.

Bull. Wld. Hlth. Org. 1968., 39: 13-24.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and

Randall, R.J. (1951).

Protein measurement with the folin phenol reagent.

J. Bio. Chem. 193: 265-275.

Macpherson, C. (1980). Epidemiology and strain

differentiation of E. granulosus in Kenya.

Ph.D. Thesis. University of London.

Mango, A.M. (1971). Helminthiasis and the East

African economy with special emphasis on hydatidosis and taeniasis.

E. Afr. Med. J. 48: 606-608.

Matossian, R.M. (1977). The immunological diagnosis of human hydatid disease.

Trans. Roy. Soc. Trop. Med. Hyg. 71: 101-104.

Matossian, R.M., Alami, S.Y. and Sarti, I. (1978).

Quoted by Rickard (1979). The immunological diagnosis of hydatid disease.

Aust. Vet. J. 55: 99-104.

McGuire, T.C., Musoke, A.J. and Kurtii, T. (1979).

Functional properties of bovine IgG₁ and IgG₂ interaction with complement, macrophages, neutrophils and skin.

Immunology 38: 249-256.

McMannus, D.P. (1981). A biochemical study of adult and cystic stages of E. granulosus of human and animal origin from Kenya.

J. Helminthol. 55: 21-27.

Melewicz and Spiegelberg, H.L. (1980). Fc receptors of IgE on a subpopulation of human peripheral blood monocytes.

J. Immunol. 125: 1026-1031.

Merrit, M. and Hardy, J. (1955). Quoted in Echinococcus antigens.

Bull. Wld. Hlth. Org. 1968., 39: 13-24.

- Hicusan, V.V. and Bordua, A. G. (1977).
Biological properties of goat immunoglobulins G.
Immunology, 32: 373-381.
- Mitchell, P., Mickleson, M.M. and Crimm, J.A. (1982).
Cutaneous Basophil Hypersensitivity.
J. Exp. Med. 135: 235-240.
- Moreira, S.W. Santos, A.F. and Oliveira, Q.C. (1978).
Casoni's intradermal test for detecting
hydatid infection in cattle.
Revista do centro de ciencias Burais 8 (2):
97-103.
- Musiani, P., Piantelli, M., Lauriola, L., Arru, E.
and Puzzuoli, R. (1978).
E. granulosus: Specific quantification of the
two most immunoreactive antigens in hydatid fluid.
J. Clin. Path. 31: 475-478.
- Nelson, G.S., and Rausch, R.L. (1963).
Echinococcus infections in man and animals
in Kenya.
Ann. Trop. Med. Parasitol. 57: 136-149.
- Ng'ang'a J.M. (1974). The incidence of cysticercosis
and hydatidosis in Kenya's livestock. In:
Parasitoses of Man and Animals in E. Africa.
(Eds. Anderson, C. and Kilawa, W.L.) E. Africa
Literature Bureau, 315-322.

Njeruh, F.M. and Lindqvist, K.J. (1982).

Serodiagnosis of hydatidosis using a defined antigen.

Proceedings of the Third Annual Medical (KEMRI/KETRI) Scientific Conference.

O'leary, P. (1976).

A five-year review of human hydatidosis in Turkana District, Kenya.

E. Afr. Med. J. 53(9): 540-544.

Orihara, M. (1970).

Studies on Echinococcosis XIX: Heat stable antigen in cyst fluid of E. multilocularis.

Japanese J. of Vet. Research 15: 86-91.

Oriol, R., Williams, J.F., Miguela, Perez, E. and Oriol, C. (1971).

Purification of lipoprotein antigens of E. granulosus from sheep hydatid fluid.

Amer. J. Trop. Med. Hyg. 20: 569-574.

Ovary, Z. (1958). Immediate reactions in the skin of experimental animals provoked by antibody-antigen interaction.

Prog. Allergy. 5: 459-508.

Parish, W.E. (1970). Short-term anaphylactic IgG antibodies in human sera.

Lancet 2: 591-592.

Puzzuoli, P., Musiani, P., Arru, E., Patrcno, C. and Piantelli, M. (1974).

Echiconoccus granulosis: Evaluation of purified antigens immunoreactivity.

Exp. Parasitol. 35: 52-60.

Rausch, R.L. (1967). A consideration of intra-specific categories in the genus E. Rudolphi, 1801 (Cestoda: Taeniidae).

J. Parasitol. 53: 484-491.

Rickard, M.D. (1979). The immunological diagnosis of hydatid disease.

Aust. Vet. J. 55(3): 99-104.

Rowe, D.A. (1969). Immunoglobulins. In: Clinical Aspects of Immunology. (Eds. Gell, P.G.H., Coombs R.R.A. and Lachmann P.J.). Blackwell Scientific Publications. pp. 761-781.

Roy, S.C., Chakravartty, M., Das, M.M. (1970).

World incidence of hydatid disease in general and pulmonary hydatid disease in particular with reference to India.

J. Indian Med. Assoc. 55: 212-217.

Russi, S., Alessandra, S. and Giuseppe, V. (1974).

Isolation and characterization of a blood P₁ active carbohydrate antigen of E. granulosis cyst membrane.

J. Immunol. 112(3): 1061-1069.

Schantz, P.M. and Schwabe, C.W. (1969). Worldwide status of hydatid disease control.

J. Am. Vet. Med. Assoc. 155: 2104-2121.

Schantz, P.M. (1973). Immunodiagnostic tests with Echinococcus antigens in sheep with homologous and heterologous larval cestode infections.

Rev. Inst. Med. Trop. (Sao Paulo) 15: 173-194.

Schantz, P.M., Ortiz-Valqui, R.E. and Lumbreras, H. (1975).

Non-specific reactions with the intradermal test for hydatidosis in persons with other helminth infections.

Amer. J. Trop. Med. Hyg. 24: 849-852.

Schantz, P.M., Shanks, D. Wilson, M. (1980).

Serologic cross-reactions with sera from patients with echinococcosis and cysticercosis.

Amer. J. Trop. Med. Hyg. 29(4): 609-612.

Schantz, P.M. (1982).

Echinococcosis

In: CRC Handbook Series in Zoonoses.

Section C: Parasitic zoonosis, Volume I (Eds.

Jacobs, L and Arambulo P). CRC Press pp 231-277.

Schwabe, C.W. (1969).

Veterinary Medicine and human health.

Baillere, Tindall, Cassell, London. Page 256.

Thompson, R.C.A. (1978). Aspects in speciation of

E. granulosus. Vet. Parasitol. 4: 121-125.

Thompson, R.C.A. (1979). Biology and speciation of
E. granulosus.

Austr. Vet. J. 55: 93-98.

Tizard, I.R. (1977).

An Introduction to Veterinary Immunology.

W.B. Saunders Company.

Todorov, T., Dakov, I. and Dimitrov, A. (1970).

Le diagnostic immunologique de l'hydatidose.

Medicine Tropicale 3: 207-213.

Van Weeman, B.K. and Schuurs, A.H.W.M. (1971).

Immunoassay using antigen-enzyme conjugates.

Febbs. Lett. 15: 232-236.

Varela-Diaz, V.M. and Coltorti, E.A. (1974).

Limitations of the Casoni intradermal test in
the immunodiagnosis of human hydatidosis.

Boletin de la Oficina Sanitaria Panamericana.

Vol. LXXVI No. 5, 400-405.

Varela-Diaz, V.M., Lopez-Lemes, M.H., Prezioso, U.,

Coltorti, E.A. and Yarzabal, L.A. (1975).

Evaluation of four variants of the indirect
haemagglutination test of human hydatidosis.

Amer. J. Tr. Med. Hyg. 24: 304-311.

Varela-Diaz, V.M. and Coltorti, E.A. (1978). Detection of
antibodies against E. granulosus arc 5 antigens by
double diffusion.

Trans. Roy. Soc. Trop. Med and Hyg. 72: 226-229.

Veterinary Services Division (Kenya).

Annual Report (1980).

Vibe, P. (1958).

Quoted In: Evaluation of economic loss due to echinococcosis and methods of echinococcosis control. (Ramanov, V.T. 1981) USSR/UNEP course on zoonotic diseases. Kazakh Research Veterinary Institute (Alma -Ata).

Voller, A., Bidwell, D.E. and Bartlett, A. (1979).

The Enzyme-Linked Immunosorbent Assay (ELISA). A guide with abstract of microplate applications. Flowline Publications, Guernsey. pp. 1-125.

Walker, J. (1925).

Annual Report, 1924. Department of Agriculture, Kenya, 84-85.

Weeke, B. (1973). Crossed Immuno-electrophoresis In:

A Manual of quantitative immuno-electrophoresis methods and application (Eds. Axelsen, N.H., Kroll, J. and Weeke, B.).

Universitets forlaget Oslo, Bergen, Tromsø.
pp. 47-56.

Weiszer, I. (1972).

"Allergic emergencies"

In: Allergic diseases. Diagnosis and management.

(Ed. Patterson, R.).

J.B. Lippincott Company, Philadelphia. pp. 327-340.

Williams, R.J. and Sweatman, G.K. (1963).

Transmission, biology and morphology of E. granulosus equinus, a new sub-species of Hydatid tapeworm in horses in Great Britain.

Parasitol. 53: 391-407.

Williams, J.F., Miguela, V., Perez, E. and Oriol, R.

(1971). Evaluation of purified antigens of E. granulosus in the immunodiagnosis of human infection.

Amer. J. Trop. Med. Hyg. 20: 575-579.

Williams, J.F. (1972).

The evaluation of the Casoni test in human hydatidosis using an antigen solution of low nitrogen concentration.

Trans. Roy. Soc. Trop. Med. Hyg. 66: 160-164.

Williams, J.F. (1982).

Cestode infections In: Immunology of Parasitic Infections (Eds. Cohen, S. and Warren, K.S.).

Blackwell Scientific Publications pp 679-696.

Wilson, M.B. and Nakane, P.K. (1978). Recent developments in the periodate method of conjugating horseradish peroxidase (HRPO) to antibodies.

In: Immunofluorescence and Related staining Techniques.

(Eds. W. Knapp, K. Holubar and G. Wick) Elsevier/North Holland Press pp. 215-224.

Yerzabal, L.A., Schantz, P.M. and Lopez-Lemes, M.H.
(1975). Comparative sensitivity and specificity
of the Casoni intradermal and immunoelectrophoresis
tests for the diagnosis of hydatid disease.
Amer. J. Trop. Med. Hyg. 24: 843-847.

Yusuf, J.N.; Hague, A. and Capron, A. (1982).
Detection of IgE antibodies in onchocerciasis.
Clin. Exp. Imm. 50: 541-548.

APPENDIX I: Barbital Calcium lactate buffer for immunoelectrophoresis

Sodium barbital (Sodium 5.5 - diethylbarbiturate)	105.1g
Barbital (5.5 diethylbarbituric acid)	16.6g
Calcium lactate	15.2g

This was made into 10 litres of distilled water.

The pH was adjusted to 8.4 or 8.6

APPENDIX II: Enzyme immunoassay wash solution

Phosphate buffered saline pH 7.4	1000ml
Tween 80	5ml
Chicken plasma	50ml
Sodium azide	2.0g
Distilled water	9000ml

The ingredients were mixed thoroughly by shaking and made up to 10 litres.

APPENDIX III: Diluent for serum used in the enzyme immunoassay

0.05M Phosphate pH 8.0	1000ml
Potassium chloride	75.0g
Benzoic acid	2.0g
Di-sodium ethylenediamitetra acetate (Na_2 EDTA)	1.0g

These were mixed well and the pH adjusted to 7.5 with 4M sodium hydroxide.

APPENDIX IV: Preparation of horseradish peroxidase substrate

For one microtitre plate with 96 wells

0.05M citrate/ammonium acetate buffer	11.0ml
ortho-phenylenediamine stock containing 100mg/ml	0.1ml
1% Hydrogen peroxide	0.1ml

APPENDIX V: Glucose oxidase substrate

For one microtitre plate with 96 wells.

0.05M citrate/ammonium acetate buffer pH 5.0	10.0ml
20% Glucose solution	1.0ml
Horseradish peroxidase solution (1000 ug/ml)	0.1ml
ABTS solution (25mg/ml)	0.1ml