CHARACTERIZATION OF HYDATID CYST ANTIGENS BY CROSSED IMMUNOELECTROPHORESIS AND ITS IMPLICATIONS FOR THE SERODIAGNOSIS OF HYDATID DISEASE IN LIVESTOCK

A thesis submitted in fulfilment for the degree of Doctor of Philosophy in the University of Nairobi.

1985
DECLARATION

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

M.M. KAGIKO

This thesis has been submitted for examination with our approval as University supervisors.


DR. J.M. GATHUMA, B.V.Sc., M.Sc., Ph.D
Hydatidosis is a disease of major worldwide public health importance and with considerable economic consequences for the livestock industry. It shows a variable geographic distribution but reaches its highest levels in certain areas of Kenya and in South America.

Meat inspection reports show that the disease is common in livestock in Kenya. A small survey carried out in this study showed a 14% prevalence of hydatid cysts in animals slaughtered in two abattoirs in Nairobi.

In an attempt to find a specific antigen that can be used in an in vivo diagnostic test for livestock hydatidosis, it was considered appropriate to study the antigenic composition of hydatid cyst fluid (HCF), the stage of the parasite which is found in livestock.

Hydatid cyst fluid was harvested from cysts which were collected from cattle, sheep and goats. A reference pool of concentrated HCF was made by mixing various preparations of HCF from these animals. In order to study the cross-reactivity of HCF antigens and other livestock parasites, saline homogenates of common parasites were also prepared.

Polyvalent antisera against HCF and various parasites were raised in calves, sheep.
goats and rabbits. Two monospecific antisera were raised against the two major HCF antigens A and B of Oriol et al., (1971); (Am. J. Trop. Med. Hyg. 20, 569-574). All sera were absorbed with erythrocytes and polymerised cattle, sheep and goat plasma to remove any heterologous host contaminants as well as P1 blood group substances.

Double immunodiffusion tests were performed to search for antigens A and B in different hydatid cysts originating from different animal species and to study cross reactivity between HCF antigens and antigens of other parasites. Crossed immunoelectrophoresis (CIEP) was used to characterize the HCF antigens and to study cross reactivity of these antigens and other parasites. The two major antigens in HCF (A and B) were precipitated out and jointly used in enzyme-linked immunosorbent assay (ELISA) to screen for hydatidosis in animals.

A search for antigens A and B (which correspond to our antigens 5 and 4) revealed that sheep or goat liver cysts fluid was the best source of the two antigens. Fertile cysts were a superior source of the antigens than sterile cysts. Antigen 4 occurred more frequently than antigen 5. Sterile cattle lung cyst fluid was found to contain mostly antigen 4.

Using CIEP, HCF was found to contain 13 antigens of parasite origin. These antigens were
given numbers 1 - 13 according to their electrophoretic mobility, starting with the most anodic component. Although antigen 6 showed a faster mobility than antigen 5, the numbers were interchanged so that antigen 5 would correspond to the "arc 5" described by Capron and co-workers. All the antigens showed an anodic migration. Some antigens, especially 6 and 7, usually gave faint arcs and were not reproducible in every run. Antigens 4 and 5 gave the most prominent arcs and could easily be visualised before staining.

In the double diffusion (DD) tests, HCF antigens were found to react with antigen extracts of Moniezia expansa, Cysticercus tenuicollis, Taenia saginata, Cysticercus bovis, Stilesia hepatica and Avitellina centripunctata. There were no reactions with antigens of Haemonchus contortus, Spirocerca lupi, Oesophagostomum radiatum, Fasciola gigantica, Paramphistomum microbothrium, Ascaridia galli, Trichuris vulpis, Ascaris suum and Bunostomum phlebotomum.

Using CIEP, all the 13 parasitic antigens, including Capron's "arc 5" were found to cross-react with other parasites that were tested as mentioned above.

Using antiserum specific for antigen 4 this antigen was detected in M. expansa, C. tenuicollis,
T. saginata, C. bovis, S. hepatica, A. centripunctata, H. contortus, S. lupi and O. radiatum. It was not found in F. gigantica, P. microbothrium, A. galli, T. vulpis, A. suum and B. phlebotomum. Antigen 5 was found in M. expansa, C. bovis, C. tenuicollis, T. saginata and S. lupi but not in the other parasites.

Crossed immunoelectrophoresis and DD methods were able to detect circulating antibodies in only a few animals naturally infected with hydatidosis.

Antigens 4 and 5 were highly immunogenic in experimental immunization schedules. An attempt was made to utilise these two antigens in a recently established and highly sensitive test, ELISA. Based on the results of 180 samples, the sensitivity of the test was 98% while the specificity was 70%. In spite of the relatively low specificity, ELISA using the two antigens was considered a useful test in in vitro diagnosis of hydatidosis.

In conclusion, it was found that HCF contained at least 13 antigens originating from the parasite, none of which was unique to the parasite. Antigens 4 and 5 were the main antigens present in HCF. The presence of the two antigens varied with the source of the cyst in that fertile sheep and goat hydatid cysts showed the most frequent occurrence of antigens 4 and 5 but sterile bovine lung hydatid cysts predominantly contained antigen 4.
Cross reactivity with HCF antigens was common in species of cestodes and, to a lesser extent, nematodes and P. microbothrium. It may therefore be difficult to establish a serodiagnostic test for livestock hydatidosis because of the observed cross reactions. It would appear that the major obstacle to immunodiagnosis of livestock hydatidosis was metacestodes of T. saginata, Taenia hydatigena and Taenia ovis since the metacestodes are tissue parasites which tend to stimulate a high immunological response in the host animal.
ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all those who contributed directly or indirectly towards the fulfilment of this goal. No words or deeds can express my thanks to the two supervisors, Prof. Lindqvist and Dr. Gathuma who continuously encouraged and guided me even at times when despair filled my heart and retreat seemed the only choice. Without their assistance and concern this work would have been impossible.

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I wish also to thank the Norwegian Agency for International Development (NORAD) and the Deans' Committee for providing the research funds. A major proportion of the funds was however, provided by NORAD and this is greatly acknowledged.

I would like to note with pleasure the endurance of my family during the course of this work and more so my wife who kept challenging me to push on and who finally very willingly typed the manuscript.
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<td>The precipitation pattern obtained when <em>A. centripunctata</em> antigens were run against SA-SHCFS while the intermediate gel contained HCF pool antigens.</td>
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1. INTRODUCTION

Hydatid disease, a cyclozoontotic infection, is recognised as being one of the major zoonoses, having a worldwide distribution but a variable incidence. It is normally associated with underdeveloped countries or those with a large rural population where public health measures are hard to enforce. The disease is caused by the larval cystic stage of mainly two species of *Echinococcus*, namely, *E. granulosus* which causes classical hydatid disease in which the cyst is typically spherical and unilocular, and *E. multilocularis* which produces the so-called alveolar hydatid cysts.

Man acquires the infection by ingesting eggs of the parasite passed in faeces of an infected carnivore. The majority of cases remain asymptomatic for long periods, and others are never recorded. This makes it difficult to give definite prevalence data for infection by the disease in man. Schantz and Schwabe (1969) have pointed out that the prevalence of the disease in man tends to be more common in regions of the world where the raising of livestock is a major industry. They listed such areas as Mediterranean littoral, New Zealand, southern part of Australia including Tasmania, and South America. Their exhaustive survey shows that hydatidosis exists on a very large scale.
In Africa, the disease is widespread in many countries. In their survey, Schantz and Schwabe (1969) listed 22 countries showing from moderate to widespread occurrence. Since that time, even countries that had not reported the disease have now been added to the list. In Nigeria, Dada et al. (1980) found 6.2% of the dogs to harbour *E. granulosus*. They also found the cysts in 14.7% of cattle, 11.4% of sheep, 26.5% of goats and 55.5% of camels.

Ginsberg (1958) reported that hydatidosis had reached alarming proportions in Kenya especially in sheep and goats, although human cases were few. More than 30% of cattle, sheep and goats slaughtered at the Kenya Meat Commission, Athi River Abattoir, contained hydatid cysts in the liver or lungs (Ginsberg, 1958). Froyd (1960) studied 1000 cattle and found that 25.2% had hydatid cysts. This clearly shows the magnitude of the economic loss due to condemnation of viscera.

Nelson and Rausch (1963) found adult *Echinococcus* in 27 out of 43 domestic dogs, 1 out of 9 jackals and 3 out of 19 hyenas.

Human hydatidosis is rarely seen outside two endemic foci in northern Turkana District. This area has the highest human hydatid cases in the world (Wray, 1958; O'Leary, 1976). Although an estimate of 40 cases per 100,000 per year
(Schwabe, 1969) appeared alarmingly high by then, O'Leary (1976) observed, in the same area, an average prevalence rate of 96 cases per 100,000 per year over a 5 year period; this is more than double the figure reported by Schwabe (1969). This disease is therefore a major problem in this area and could be spreading to other areas.

Hydatidosis in animals rarely exhibits overt signs of disease and yet animals are essential for the continuation of the parasite's life cycle. The study of the epidemiology of the disease and its control would benefit from the development of reliable technics for immunological diagnosis of hydatidosis in live intermediate hosts. Attempts to apply the technics used in the diagnosis of the condition in humans to animals have proved less satisfactory in the hands of many investigators (Schantz, 1973; Yong and Heat\, 1979). Schantz (1973) listed the following tests which have been employed in animal intermediate hosts; the intradermal test (IDT), the indirect haemagglutination test (IHA), the conglutinin test (CT), the bentonite flocculation test (BFT), the latex agglutination test (LAT), the indirect immunofluorescence test (IIF), immunoelectrophoresis (IEP) and scolex-precipitin reaction (SPR). No recent reports on real improvement of their sensitivity
and specificity are available.

Njeruh (1981) purified an antigen (antigen 4) from hydatid cyst fluid (HCF) and found it to be useful in IHA and enzyme immuno assays. He obtained better results when he used the antigen than when whole HCF was used. He also reported that the antigen was present in other parasites of livestock, an observation which would reduce the usefulness of the tests based on the use of that particular antigen.

Biguet et al. (1962) demonstrated common antigens in HCF and other helminth species using immunoelectrophoresis. Of 9 antigenic components observed from HCF, 2 were also found in Taenia saginata. Reactions of identity were also found with Schistosoma mansoni, Fasciola hepatica, Onchocerca volvulus, Ascaris lumbricoides and Trichinella spiralis. One antigen from HCF has also been found in T. solium (Yarzabal et al., 1977) and T. ovis (Yong and Heath, 1979). Hence, interpretation of positive results is confounded by frequent concurrent infection with helminth parasites which cross react antigenically with E. granulosus. As cross reactivity reduces the specificity of the methods used, there is a need to analyse the antigens present in HCF and purify those, if any, that are unique to E. granulosus for use
in diagnostic tests.

Using different technics such as double diffusion (DD) (Stanford, 1973), immunoelectrophoresis (IEP) (Janicki et al., 1971) and crossed immunoelectrophoresis (CIEP), (Closs et al., 1975) workers have been able to define a reference system for mycobacterial antigens. A similar approach was undertaken in this project to study the antigens of hydatid cyst fluid. After establishing the antigens present in HCF as a reference pattern, DD and CIEP were used to define the HCF antigens that are shared in common with other livestock parasites. To facilitate this exercise, an intermediate gel was incorporated between the first dimension electrophoresis agarose and the antibody containing agarose (Axelsen, 1973). By this procedure it was possible to study the effect of either an antigen preparation or antiserum on the reference pattern. In this way, cross reactivity could be detected even when the structural similarity was too small for a precipitate to form (Closs et al., 1975).

This was achieved by the addition of an antiserum to the intermediate gel. Structural similarity was tested for by addition of the test antigen in the intermediate gel (Closs et al., 1975).

Cross reactivity was also studied through absorption of the reference serum by antigenic preparations from the other test parasites.
One antigen "arc 5" of Capron et al. (1967) has been found to be important in the diagnosis of hydatid disease. Antigen 4 seems to be the one occurring in the greatest quantity in HCF. However, the presence of these two antigens seems to depend on the source of the cyst fluid. An effort was made to study the occurrence of antigens 4 and 5 in individual fertile and sterile cysts obtained from livers of sheep, goats and cattle.

The reference anti-serum used in this study was obtained from a laboratory sheep heavily immunized with concentrated HCF in adjuvant for over 2 years. An attempt was made to find out whether naturally infected animals produced the same antibodies and in same quantities as laboratory immunized animals. This was done by DD and CIEP on sera collected from slaughterhouses around Nairobi City.

Double diffusion was chosen for its ease of performance while CIEP was used due to its great resolution ability. The tests were performed both on neat and 5 times concentrated sera.

Out of 13 HCF antigens defined on the reference pattern, two of them, namely antigens 4 and 5, were found to occur most frequently and gave strong precipitin lines. The two antigens were purified from HCF by precipitation with phosphotungstic acid/magnesium chloride. These antigens were used in
enzyme-linked immunosorbent assay (ELISA) to screen 180 cattle sera from slaughter stock.
2. REVIEW OF LITERATURE

2.1 THE ANTIGENS OF HYDATID CYSTS

2.1.1 Source of HCF antigens

Variation in the quality of the antigens used in the diagnostic tests may account for the wide range of results obtained in different parts of the world with respect to the specificity of the tests for hydatid disease in common intermediate hosts (Matossian, 1977). Real improvement in specificity in the diagnosis of hydatid disease in lower animals must await the purification of any antigens peculiar to E. granulosus. The alternative would be a standardization of methods utilizing crude antigens so as to be able to distinguish specific from non-specific reactions. Antigenic variants of the parasite evolving in different host populations must be considered. This concept has been supported by Smyth (1977) who has been considering possible differences within E. granulosus.

Hydatid cyst fluid has been extensively used as the source of antigens either in crude or partially purified form (Kagan and Agosin, 1968). According to Capron et al. (1967) the best antigen for immunoelectrophoresis (IEP) was found to be HCF from fertile horse liver cyst. Quilici et al. (1971) reported that many samples of sheep hydatid cyst
fluid (SHCF) are inadequate for use in IEP test because of a deficiency in antigenic components. This, however, did not deter researchers from using SHCF; many of them, including Coltorti and Varela-Diaz (1972), Williams (1972), Lopez-Lemes and Varela Diaz (1975) and Ardehali et al. (1977), used SHCF in their diagnosis of human hydatidosis, while Schantz (1973), and recently Conder et al. (1980), used SHCF antigens to diagnose hydatidosis in sheep. Yarzabal et al. (1974; 1975) successfully used bovine liver cyst fluid in immunoelectrophoresis (IEP) and Casoni tests in diagnosing human hydatidosis. They found that the antigen responsible for the diagnostic arc 5 was present in hydatid fluid from both fertile and sterile bovine cysts. In an effort to sort out the differing views, Varela-Diaz et al. (1974) determined the frequency of arc 5 antigen in different HCF pools obtained from livers and lungs of naturally infected sheep for diagnosis of human hydatidosis. They detected arc 5 by IEP using rabbit anti-SHCF serum in 95% of the SHCF pools made up of 42 different sheep livers and lungs. This led to the conclusion that the observed high frequency was indicating that SHCF was a good source of antigens for diagnosis of hydatidosis by IEP if arc 5 was the criterion of positivity as suggested by Capron et al. (1967). Even Dottorini and Tassi (1977) suggested that sheep and human HCF
was the most reactive and should therefore be used for studying antigenic components.

Ardehali et al. (1977) used human and sheep HCF in an evaluation of 3 tests for the diagnosis of human hydatid disease. They found crossed electro-immunodiffusion and counter immunoelectrophoresis (CIE) to be more sensitive and specific tests for the diagnosis of hydatid disease when human hydatid fluid was used as the source of antigen. Dada et al. (1981) utilized camel hydatid fluid in an evaluation of 3 immunodiagnostic tests. All the 3 tests, namely indirect haemagglutination (IHA), double diffusion (DD) and CIE were always positive in camels with hydatid cysts infection in both livers and lungs. All this evidence indicates that there is no need to consider the cyst fertility, or host organ of origin of the cyst before the fluid is used as antigen source (Varela-Diaz et al., 1974; Ardehali et al., 1977).

Oriol et al. (1971) prepared purified HCF antigens by a series of dialysis of SHCF against phosphate buffer, acetate buffer and precipitation by ammonium sulphate. They obtained a purified fraction with two major parasite lipoprotein antigens named A and B, of which the latter occurred in several polymeric forms. Williams et al. (1971) evaluated the Oriol et al. (1971) antigens in the immunodiagnosis of 91 human cases using IHA, IEP,
and IDT. They found that certain parasite antigens that contribute to the positivity of the tests were eliminated in the purification procedure since seven of the 46 sera from hydatidosis patients that were negative in the tests using the purified fraction produced one to four bands with the whole cyst fluid, though all the sera that were positive with the purified fraction were also positive with whole cyst fluid. Even though this preparation was less reactive in IHA, and IEP, it was found to be very reactive (80%) in IDT using 1.5 µg protein antigen. Williams et al. (1971) found very little non-specific skin reactivity which led them to suggest that the absence of non-specific reactivity was probably due to elimination, during purification, of components of SHCF that provoke non-specific reaction, or that the amount of antigen used (1.5 µg protein) was too low to provoke non-specific reaction as non-specificity is associated with high protein content in the antigen used (Kagan, 1968).

A similar increase in the reactivity of the skin test using a fraction EG III was reported by Kent (1963). A major disadvantage of this purified antigen was the finding that it was unstable under field conditions. The author therefore did not recommend the routine use of the lipoprotein for diagnostic purposes.

Pozzuoli et al. (1974) attempted to purify antigens from crude SHCF by absorbing sheep serum
protein on a polymer of rabbit antisera against sheep serum, sheep serum albumin and sheep gamma globulin. In this way, the parasitic antigens in SHCF were separated from the serum components originating from the host. They found this method to be easier and more convenient than eluting bound parasitic antigens from specific polymerised antiserum as they had previously done (Pozzuoli et al., 1972). When the proteins so obtained were subjected to IEP and DD tests, five parasitic antigens were detected and no host serum contaminants were observed. However, no net gain was obtained when optimal amounts of the purified as compared to crude SHCF were utilized as antigens in passive haemagglutination and IEP as regards sensitivity, but a higher specificity was observed. Kent (1963) fractionated HCF by column chromatography on diethylamino ethyl cellulose and found that among the fractions isolated, fraction EG-111 represented a potent antigen which produced one precipitin band with sera obtained from proven human infections. This protein also elicited very strong skin reactivity on persons with the infection.

Dottorini and Tassi (1977) separated and studied hydatid fluid antigen by polyacrylamide gel filtration, IEP, DD, sucrose gradient separation, and SDS-polyacrylamide gel electrophoresis on slab gel with the major emphasis on the characterisation of
"arc 5". They used whole SHCF as the reference source of arc 5. In IEP, arc 5 antigen was found in SHCF with remarkable reproducibility and was easily recognised. On its purification on sucrose gradient it demonstrated a refractive index of $n=1.3737$ and on slab gel separation, it revealed a specific component of a M.W. of 69,000 daltons. Prior to SDS treatment, however, chromatographic analysis revealed a M.W. 100,000-300,000 which may indicate that the 69,000 M.W. component was a monomeric form. They found the antigen to be stable to heat up to 56°C at which temperature, the titre fell by 50%. It was also stable in a pH range of 4-7.

The molecular weight (M.W.) of sheep HCF components was studied by (Pozzuoli et al., 1972; 1974) who analysed the chromatographic behaviour of SHCF on Sephadex G200 and Sepharose 4 B column, thereby obtaining a rough estimate of the M.W. of Echinococcus antigens. They also used dodecylsulfate polyacrylamide gel electrophoresis to determine the molecular weight. They determined that the M.W. of a heavier component was 400,000 daltons and that of a light component to be 150,000. When the major antigen was radio-labelled and its immunoreactivity tested, it was found out that there was a gain in sensitivity. The labelled fractions showed that antigen 4 was the largest component of the first peak (Pozzuoli et al., 1972; 1974).
In a comparison of the radio-labelled crude SHCF and antigenic fractions eluted in the first and third peaks of G200 column, it was found that purified antigens were twice as effective as crude SHCF, and that the first peak containing the major antigens was more reactive than the third peak. Subsequent efforts by Pozzuoli et al. (1975) revealed that the two major antigens 4 and 5 of Chordi and Kagan (1965) could be obtained quickly by affinity chromatography on concanavalin-A-sepharose, a method which decreased markedly the amount of time and material necessary for the immunoadsorption step. Welch and Dobson (1978) also found that antigens from *Dirofilaria immitis*, *Toxocara canis* *Angiostrongylus cantonensis* and *Ascaris lumbricoides* prepared by affinity chromatography and linked to commercially prepared CNBr-Sepharose 4B beads gave greater specificity and sensitivity. Pozzuoli et al. (1975) found that when the two main SHCF antigens (4 and 5) were tested by IEP, CIE and IHA tests, best results were obtained with antigen 4. This antigen was the most immunoreactive parasitic antigen, for antibodies against it were found in all the sera of hydatidosis patients.

2.1.2 The number of antigenic components in HCF

The number of precipitin bands that have been reported from HCF shows a tremendous variation
ranging from 5 (Pozzuoli et al., 1974) to 13 (Varela-Diaz et al., 1974; Guisantes et al., 1975). Yarzabal et al. (1974) used bovine HCF lots, provided that such lots contained at least six parasite antigens, including the characteristic arc 5. Earlier on, by means of IEP, Chordi and Kagan (1965) had identified and characterised 10 bands of parasite antigens in SHCF. However the greatest number of precipitin bands has been reported by Varela-Diaz et al. (1974) who characterised a 10 litre pool of whole SHCF and revealed the presence of 13 parasitic antigens, among them being arc 5. A purified fraction from the 10 litre pool revealed the presence of antigens A and B of Oriol et al. (1971) and two other minor components and a host contaminant (Guisantes et al., 1975). Later on, Varela-Diaz et al. (1978) detected 8 bands, including arc 5, from the same SHCF pool which Varela-Diaz et al. (1974) had previously shown to contain 13 bands. Conder et al. (1980) observed 8 bands from SHCF collected from liver and lung cysts.

2.1.3 Antigenic cross reactivity

Blundell-Hasell (1969) reported that sera from sheep naturally infected with T. ovis gave a positive reaction in IHA test using antigens from either T. hydatigena or E. granulosus cyst fluid. Schantz et al. (1980) found cross reactions between
sera of pathologically confirmed *E. granulosus* hydatidosis and *T. solium* cysticercosis using *Echinococcus* and *Taenia* antigens. To try and differentiate infections with parasites that share common antigens, Yong *et al.* (1978) and later Schantz *et al.* (1980), used IHA titres observed when the cross reacting antigens were tested on the same serum. Both teams came to the same conclusion that it was not possible to distinguish infections by cross reacting parasites by comparing titres with the homologous or heterologous antigens.

Other parasites that have been reported to share antigens with HCF include *M. expansa, T. saginata, C. tenuicollis, A. centripunctata* and *O. radiatum* (Njeruh and Lindqvist, 1982).

Capron *et al.* (1967) reported that a specific antigen, which they called "arc 5", was diagnostic for hydatid disease. Following this finding, other reports (Capron *et al.*, 1970; Yarzabal *et al.*, 1974; Varela-Diaz *et al.*, 1975a) confirmed the diagnostic usefulness of the antigen. But subsequent research demonstrated that antigen 5 was also present in the larval stages of *E. multilocularis* (Rickard *et al.*, 1977) and *Taenia hydatigena* (Varela-Diaz *et al.*, 1977a). Furthermore, antibodies against this antigen were demonstrated in sera of patients suffering from
cysts of *E. multilocularis* (Varela-Diaz et al., 1977b), *E. vogeli* (Varela-Diaz et al., 1978) and *T. ovis* (Yong and Heath, 1979). These findings lead to a conclusion that the use of antigen 5 does not eliminate false positive results in areas where parasites which share antigens with HCF occur.
2.2 IMMUNODIAGNOSTIC METHODS

2.2.1 Intradermal test

Since its introduction by Casoni (1911), the intradermal test (IDT), an immediate type reaction (Type 1) which is less frequently followed by a reaction of delayed hypersensitivity (Type IV) (Barriga, 1981), has been extensively used in many parts of the world in the diagnosis of hydatid disease in humans as reported by many reviewers (Kagan, 1968; Matossian, 1977). Other than humans, the test has been used for diagnosis of hydatidosis in lower animals. Goddale and Krischner (1930) used fresh cow hydatid cyst fluid on 106 slaughter cattle to evaluate this test. On post-mortem examination of the organs, 44(41.5%) cows had hydatid cysts. Of these, 86.3% had given a positive Casoni test. The test, therefore, proved to be more sensitive than the complement fixation test (CFT). They, however, detected 11 false positive animals by this test. Hoghooghi et al. (1976) evaluated IDT and Latex agglutination (LAT) for echinococcosis. They found the tests to be well correlated. They observed an 80.4% specificity with IDT and 6.7% false positives.

However, other reports show that the use of the test is hindered by a high rate of false positives. Turner et al. (1937) used IDT on hydatid infected sheep and found that such animals usually, but not always, gave positive reactions. Never (1938)
found allergic reactions to be unreliable in cattle and sheep. Boko (1966) described reactions in 128 heads of cattle. Results showed that the IDT was unreliable. Todorov et al. (1979) compared this test with four serological tests in cases of pulmonary hydatidosis. They found that non-specific reactions were highest in the IDT although it had a significantly higher sensitivity than any of the other tests. The same problem limits its use in animals. Conder et al. (1980) evaluated the possibility of using DD, IEP, IHA and IDT in the immunodiagnosis of hydatid disease in sheep. They concluded that the results of IDT in diagnosing sheep hydatidosis should not be considered as conclusive since the antigen cross reacts with other parasitic antigens such as T. hydatigena.

The use of this test in epidemiological studies has given equivocal results especially since a high rate of false positives is constantly observed ranging from 6.7% (Hoghooghi et al., 1976) to 45% (Chordi, 1962). This observation is supported by Varela-Diaz et al. (1975a; 1976) who found the technic to be inadequate in epidemiological studies. Schantz et al. (1975) showed that not all patients develop skin reactions; some patients develop transient skin reactivity only. Schantz (1973) found that sheep showed a weaker skin hypersensitivity as
compared to humans tested with a similar type of antigen.

A major contribution towards reducing non-specificity was made by Kagan et al. (1966) who showed that there was an inverse correlation between the nitrogen content of the antigen (N \(100\mu g/ml\)) and the specificity of the IDT in the detection of humans free of hydatidosis. Schantz (1973) used three levels of different dilutions of hydatid cyst fluid in skin-testing of naturally infected ewes. The 75 and 42 \(\mu g\) protein per ml antigen lots showed little difference in reactivity, but the antigen lot containing 2 \(\mu g\) per ml had a much reduced sensitivity. Using an antigen containing 15 \(\mu g\) protein per ml, Williams (1972) obtained 86% specificity and considered the test highly satisfactory. However, Yarzabal et al. (1975) found that IDT still yielded false positive reactions even when performed with a relatively pure, boiled HCF antigen. They concluded that there would be no need to use IDT where more specific serological technics are available.

In conclusion, it would appear that IDT shows high sensitivity but low specificity which reduces the usefulness of the test (Matossian, 1977).

2.2.2. Complement fixation test

In the diagnosis of human hydatidosis, the complement fixation test (CFT) has been used for over
70 years (Barriga, 1981) but most of the available information tends to be controversial as the sensitivity reported fluctuated from a low of 36% to a high 93% with an average of 69% (Kagan, 1968), with a non-specificity that varied from 0.4% to 28% (Barriga, 1981).

Cross reactions with sera from cancer patients (Kagan, 1968) made the test even less useful. Matossian and Araj (1975), however, found it to be of value in detecting recurrent illness especially since CFT results revert to negative faster than agglutination tests following the removal of a hydatid cyst.

In a review of the diagnostic tests for animal hydatidosis, Kagan (1968) showed that there are conflicting reports regarding the usefulness of the CFT. Thus, 100% of pig sera were positive and 59% of cattle in another group were positive, yet non-specific positive reactions were obtained with normal rabbit serum tested with hydatid fluid. The test has been reported to detect antibodies to bovine cysticercosis only within a certain period, probably due to a change in the type of immunoglobulin elicited in the host at different stages of infection (Enyenihi, 1974).

2.2.3 Indirect haemagglutination test

Since its first use in the diagnosis of hydatid disease by Garabedian et al. (1957), indirect haemagglutination test (IHA) has found increasing use
in the diagnosis of the disease. Kagan (1968) reviewed the literature on serological tests for diagnosing hydatid disease and showed that the sensitivity of the test ranges from 66% to 97% with an average of approximately 84%. Giunchi et al. (1972) examined 1502 apparently healthy subjects using IHA test and found 9 positive reactors. On thorough clinical, radiological and scintigraphic investigations of 8 of the 9 reactors, only 6 presented masses and on operation on 4 of the 6, all 4 had at least one E. granulosus cyst. They concluded that IHA was highly specific and can be used both for epidemiological investigation and for the diagnosis of latent hydatid disease. Mahajan et al. (1973) compared the IHA, CFT, and IDT in diagnosing hydatid disease by using serum from 32 proven cases of hydatidosis in India. They found that IHA was the most sensitive and that the combination of IHA and IDT gave the highest sensitivity (30 out of 32 cases detected). Similar efficacy of IHA and IDT has been reported by Schantz et al. (1973) in Argentina and Spruance et al. (1974) in the United States of America.

The specificity of the test is influenced by the titre which is considered to be significant. Kagan (1968) suggested that a titre of 1:400 or above be considered positive. By considering this.
titre or above as positive, Schantz (1973) found a greater reactivity for IHA test as compared to the IDT. Conder et al. (1980), in an evaluation of DD, IEP, IHA and IDT, found that IHA test approached the specificity and sensitivity of DD and IEP if a titre of 1:1024 was considered positive. Njeruh (1981) used a purified HCF antigen in IHA test to screen 293 livestock serum samples for hydatidosis. He obtained a sensitivity of 92.7% when a titre of 1:128 or above was considered significant but the sensitivity dropped by 28% when a titre of 1:256 or above was considered significant.

The sensitivity of the test is dependent upon the type of chemicals (formaldehyde, glutaraldehyde or benzidine) used to sensitize the indicator erythrocytes, the type of red cells used (sheep or human) and the type of E. granulosus infecting the patient (Kagan, 1974). Williams and Prezioso (1971) found glutaraldehyde fixed cells to be the most satisfactory. In an evaluation of four technical variants of IHA for hydatid disease, Varela-Diaz et al. (1975b) found that the reactivity varied with the type of IHA test. Of the four variants, they found the one using tannic acid to be the best as compared to the use of glutaraldehyde, benzidine or formol. They concluded that a simple method was to use sheep red blood cells treated with tannic acid.
The usefulness of the test in post-operative followup is enhanced by the fact that antibodies could not be detected one year after surgery while immunofluorescence (IIF) and LAT detected antibodies many years after surgery (Lass et al., 1973). Indirect haemagglutination showed a strong cross reaction between sera of patients with schistosomiasis and echinococcosis but the same sera were negative on LAT.

2.2.4 Bentonite flocculation test

Though the bentonite flocculation test (BFT) was initially developed for the diagnosis of trichinosis, it has been adapted for the diagnosis of echinococcosis among other parasitic conditions in human (Kagan and Walls, 1981). Bentonite flocculation test was introduced in the diagnosis of human hydatidosis by Norman et al. (1959). Kagan et al. (1959) compared IHA with BFT and indicated that IHA test was slightly more sensitive. Gonzalez-Castro and Chordi (1960 cited in Kagan, 1968), however found BFT to be more sensitive than CFT but its specificity was poor.

Successful diagnosis with the BFT depends upon the ability to suppress auto-agglutination of the particles with Tween 80 and the reactivity of the antigen used to coat the particles (Kagan and Walls, 1981).
2.2.5 **Latex agglutination test**

Fischman (1960) introduced the latex agglutination in the diagnosis of hydatid disease. In this test, latex particles are coated with soluble hydatid antigen which, when exposed to the corresponding antibodies, agglutinate. Fischman (1960) reported good sensitivity with this test. Since then, the test has been found to be an easy, specific and sensitive test, useful in serological surveys of hydatid disease (Varela-Diaz et al., 1976). Castagnari and Pozzuoli (1969) showed that the latex particle size affected the specificity of the test. The concentration of the particles, the molarity and pH of the buffers, as well as the concentration of the antigen also affect the test (Kagan and Walls, 1981).

Varela-Diaz et al. (1976) used IHA and LAT in serological survey in a hydatidosis endemic area. They reported that LAT showed low non-specificity, greater simplicity and excellent correlation with IEP which meant that when LAT was used for screening, only a limited number of IEP tests need to be performed to confirm the diagnosis (Matossian, 1977).

Hoghooghi et al. (1976) compared LAT to IDT
test on 51 proven hydatidosis cases. Latex agglutination test was only slightly more sensitive (82.3%) than IDT (80.4%) and gave only 4.8% non-specific reactions. Like the other authors quoted, they found LAT to be simple, rapid, and therefore useful for diagnosis in hospitals and epidemiological surveys. Todorov et al. (1979) observed that LAT showed the lowest non-specific reactions as compared with 5 other tests. They proposed that LAT should be used together with the IDT in each case. However, like many other serological tests, LAT gives false positive results in case of liver cirrhosis and non-parasitic cysts in humans (Enyenihi, 1974).

2.2.6 Immunofluorescence

This technic has been used in the diagnosis of echinococcosis with encouraging results, and can be applied to human as well as animal echinococcosis (Feteanu, 1977). A major advantage of this technique is that the whole parasite which is easy to obtain as only a tiny amount of the parasite material is needed (Ambroise-Thomas, 1976) is used. Also the characteristics of the whole parasite antigen are less variable from batch to batch than those of soluble parasite antigens. Ambroise-Thomas (1976) noted this is a
great advantage especially in the case of hydatid infections where the great variability in hydatid fluid employed as antigen in serological tests is not observed in sections of the scolex of *E. granulosus* of whatever origin. However, in using the whole parasite, it becomes difficult to single out a particular antigen out of the many present in the worm and also one misses to study the secretory antigen that may be inciting major antibody production in the intermediate host (Ambroise-Thomas, 1976).

Yarzabal et al. (1976) found that by using indirect immunofluorescence (IIF), they were able to locate a specific antigen (antigen 5) in the inner portion of germinal membrane of a hydatid cyst as well as in the parenchyma of protoscolices. Mannweiler and co-workers (1977) compared the specificity and sensitivity of IIF and two other tests using antigens of *E. multilocularis* and *E. granulosus* protoscolices as well as an aqueous extract of *E. multilocularis*. They found that IIF with vital protoscolices was the most specific of the three tests whereas IHA using the soluble antigens (hydatid cyst fluid and aqueous extract of *E. multilocularis*) was most sensitive. Fluorescent antibody tests promise to be useful tests in future for the diagnosis of hydatid disease (Movsesijan et al., 1979) as they give a quick answer as compared to conventional methods (Howard, 1979).
Ambroise-Thomas (1976) concluded that the method is a valuable diagnostic tool and also in post-therapy follow up. He recorded 94% (279/296) specificity in serodiagnosis of hydatid disease by IIF.

2.2.7 Double immunodiffusion test

Various authors have compared the double immunodiffusion (DD) (Coltorti and Varela-Diaz, 1975, 1978; Schantz et al., 1980) on agar gel to other commonly used immunodiagnostic tests.

Coltorti and Varela-Diaz (1978) found the DD test based on arc 5 detection to be more sensitive than IEP based on the same arc 5 criterion, and was equally specific for the diagnosis of human hydatidosis. Due to its greater simplicity, they suggested that this test coupled with LAT are ideal for human hydatidosis in endemic areas. It also requires a lower amount of antigen and antiserum, less time to perform and simple equipment as compared with IEP (Coltorti and Varela-Diaz 1975, 1978). A similar view was expressed by Schantz et al. (1980) who used IEP and DD test to study cross reactions between hydatidosis and cysticercosis. In their experience DD test was more sensitive than IEP. Conder et al. (1980) evaluated four immunodiagnostic tests and found DD test to be more sensitive and at least as specific as IEP in detecting hydatidosis in sheep which led them to
conclude that the DD test (based on detecting arc 5) was reliable in sheep naturally infected with hydatidosis. Dada et al. (1981) evaluated DD test and two other tests on 36 hydatid positive camels' sera. Although DD test was almost as sensitive as IHA it was more specific than IHA and was as specific as counter-current immunoelectrophoresis.

However, some workers criticized the test. Kent (1963) fractionated hydatid cyst wall water extract and when a fraction (EG-VI-C) was tested in Ouchterlony plates, it failed to react but elicited a skin reaction identical to a fraction (EG-III) from hydatid fluid. Guisantes and Varela-Diaz (1975) however found DD test to be less specific than LAT or IEP although IEP was less sensitive. They subsequently recommended LAT to DD test when examining large numbers of sera in field studies. Likewise, Ardehali et al. (1977) used 3 tests for diagnosis of human hydatidosis. They found the sensitivity of simple DD test to be lower than that of counterimmunodiffusion. Torres Rodrigues and Wisnivesky (1978) found IEP to be more sensitive than DD test for echinococcosis in experimentally infected mice.

A major weakness of this test is the reported long period post infection before antibodies can be detected. Enyenihi (1970) was unable to detect precipitating antibodies in sera of mice infected with
Cysticercus longicollis before 30 days post infection (PI). Conder et al. (1980) could not detect any antibodies to arc 5 antigen by DD test and IEP prior to 120 days PI. They hypothesized that this delay suggests that either the immune response can only produce antibodies to antigen 5 to a recognisable level after a lag period or antigen 5 is not produced by the parasite in its early stages. The latter explanation is supported by Yarzabal et al. (1976) who demonstrated that antigen 5 was localised in the germinal membrane and protoscolices which take longer time to develop. However, sheep naturally infected in endemic areas may be subjected to constant worm egg challenge which was not the case in the laboratory challenge by Conder et al. (1980). This delay in detecting antibodies together with the existence of crossreacting parasite antigens may require that further evaluation of the DD test based on arc 5 in field conditions be carried out (Conder et al., 1980). Except for this disadvantage and the fact that it is slower than IEP, DD has the great advantage of reliability (Kent 1963), simplicity and economy (Kagan and Walls, 1981).

Immunoelectrophoresis (IEP)

A basic problem in immunological studies is the identification and quantitation of antibodies and antigens since a major proportion of the antisera
will have been raised against a large number of antigens. This need to identify and quantitate antigens and antibodies led to development of gel precipitin tests among them, the immunoelectrophoresis (IEP) (Grabar and Williams, 1953), the Ouchterlony (1958) DD technic as well as the radial diffusion technic by Mancini and co-workers (1965).

The most significant development in immunoelectrophoresis with regard to the immunodiagnosis of hydatid disease was the finding by Capron et al. (1967) that when sheep hydatid fluid was tested by IEP, there was an arc of precipitation that was distinct and specific for *E. granulosus*. Initially, this antigen was found by other workers to be *E. granulosus*-specific. Yarzabal et al. (1974) evaluated the diagnostic specificity of the antigen by using 100 normal persons, and 100 patients with tuberculosis, 40 with broncho-pulmonary cancer, 30 with hepatic cirrhosis, 20 with aspergillosis and 10 with acute pneumonias of diverse aetiology and found that by using the appearance of arc 5 as the diagnostic criterion, no "false positive" reaction was observed. Immunoelectrophoresis proved to be more sensitive than IHA in the preoperative diagnosis of pulmonary hydatidosis. Coltorti and Varela-Diaz (1978) could not detect "false positive" results in 335 sera from persons with diseases other than hydatidosis.
This antigen was given various names like antigen "four" (Chordi and Kagan, 1965) antigen "A" (Oriol et al., 1971) or antigen "five" (Bout et al., 1974). Further investigations revealed that the antigen-antibody precipitation arc cross reacts with other parasites as well as in a case of cysticercosis -multiple myeloma (Varela-Diaz et al., 1978). This proves clearly that arc 5 is not as specific as it was initially thought to be. Since this antigen cross reacts with three species of Echinococcus that cause hydatidosis, namely E. granulosus, E. multilocularis and E. vogeli, diagnosticians will have to consider the geographical origin of the patient to be able to assess the most likely species involved. In spite of the observed cross reactions, researchers have continued to use the test for diagnosis of the disease.

Coltorti and Varela-Diaz (1978) and Conder et al. (1980) found DD test to be as specific as IEP and IEP was less sensitive than DD test. In contrast, Torres Rodrigues and Wisnivesky (1978) reported that IEP was more sensitive than DD test.

2.2.9 Counterimmunoelectrophoresis (CIE)

In this test, an electrical current is passed through a gel containing antigens and immunoglobulins such that they are driven towards each other and form
a precipitin line where they meet. The method was found to be very sensitive, rapid and specific for the detection of viral infections such as hepatitis B virus (Prince and Burke, 1970; Gocke and Howe, 1970), dengue hemorrhagic fever virus (Churdboonchart et al., 1974) and antibody production in the case of California virus infection (Balfour and Edelman, 1974) as well as influenza (Berlin and Pirojboot, 1972). With regard to bacterial diseases, it has been found useful where organisms for staining may be missing. It has been used to detect Neisseria meningitidis, Diplococcus pneumoniae and Haemophilus influenzae (Edwards, 1971; Edwards et al., 1972; Coonrod and Rytel, 1972). Remington et al. (1972) used CIE to detect antibodies in systemic candidiasis. It has been used to detect antibodies in infection with Trypanosoma cruzi and Leishmania donovani (Desowitz et al., 1975) even though it was found to be less sensitive than immunofluorescence when crude antigens were used. Rezai et al. (1977) used IIF and CIE to diagnose Kala-Azar. In a review, Draper (1976) concluded that the test is rapid, easy to perform and of undoubted value in the diagnosis of some bacterial, viral and fungal diseases but it was usually less sensitive than other tests for protozoa and helminthic diseases. The test was adapted to the diagnosis of hydatidosis by Castagnari and Sorice (1971).
In comparison with IHA, Mahajan et al. (1976) found CIE to be both sensitive (82.2%) and specific for the diagnosis of hydatid disease and also compared well with IHA. The test was found to be more sensitive than IEP by Lopez-Lemes and Varela-Diaz (1975), but the same authors were unable to distinguish sera from hydatid and non-hydatid individuals by this technic. Kelkar and Kotwal (1975) examined six samples of sera from confirmed cases of hydatidosis and showed that CIE is more sensitive and rapid than DD. Ardehali et al. (1977) compared the use of CIE, crossed immunoelectrophoresis (CIEP) and DD test for the immunodiagnosis of hydatid disease and found out that the CIE was very specific and sensitive in that all 21 sera from confirmed cases of hydatidosis showed precipitin lines and none of the sera from healthy persons showed any precipitin lines. Subsequently, the authors recommended this test for the diagnosis of hydatid disease as well as epidemiological surveys. In their comparative studies on the sensitivity of IEP and CIE tests in the detection of hydatidosis cases, Torres et al. (1973) found IEP to be more sensitive but Castgnari and Sorice (1971) had found CIE to be more sensitive. These differences arise due to the use of varying antigen source and as Lopez-Lemes and Varela-Diaz (1975) stated, only the
isolation of a purified *E. granulosus* specific antigen would render the test useful for immunodiagnosis of hydatid disease.

2.2.10 **Crossed-immunoelectrophoresis**

As stated earlier, IEP was developed to aid in the identification and quantitation of antibodies and antigens. This test suffered from impaired electrophoretic resolution during the final immunodiffusion (Ganrot, 1972). However, there has been a modification of the technic to further ease the analysis of more and more complicated antigen-antibody systems. Thus, Ressler (1960a,b) and Laurell (1965) introduced the concept and operation of electrophoresis of antigens through antibody contained in buffered gel bed. This modification replaced the final immunodiffusion in IEP by introducing a second electrophoretic run at right angles to the first run (Laurell, 1965) so that the antigen was forced into a medium containing antibodies. At the appropriate pH, usually 8.6 (Axelsen and Bock, 1972) the immunoglobulins either do not migrate or migrate towards the cathode such that there is a precipitation line formed which defines an area proportional to the antigen-antibody ratio of the system (Axelsen and Bock, 1972). Given that the antigen concentration is fixed, it becomes easy to determine the antibody quantity and vice versa.
Laurell (1965) found this technic to be an extremely valuable tool for quantitating several individual proteins in one operation. At first this technic was called "antigen-antibody crossed electrophoresis" (Laurell, 1965) but since the technic was developed from conventional immunoelectrophoresis and the fact that the electrophoresis used is two dimensional, Ganrot (1972) suggested that it be known as crossed immunoelectrophoresis.

Subsequently, other workers introduced modifications. Svendsen and Axelsen (1972) incorporated an intermediate gel and developed a method which allowed them to identify and quantitate several human antibodies against Candida albicans in terms of those of a rabbit antibody pool in a single operation. Axelsen and Kirkpatrick (1973) used this modified technic in their studies on Candida and found horizontal precipitates present in the reference gel which were due to antigens which were in the intermediate gel, and which had subsequently migrated anodically during the second electrophoresis and formed precipitin lines with their corresponding rabbit antibodies. Axelsen and Kirkpatrick (1973) concluded that there are advantages in incorporating an intermediate gel in CIEP in that a higher resolution is achieved and at the same time quantitation is possible.
With these advantages, the method received wide acceptance in many immunochemical laboratories, especially in Scandinavia. Ekman et al. (1975) studied lipoproteins in normal and cholestatic human plasma using CIEP and electroimmunoassay. By crossed immunoassay, they were able to find a complex pattern of $\alpha$-lipoproteins. They were more satisfied with the electrophoretic distribution of proteins by CIEP than the picture obtained with plain IEP, although an abnormal distribution of plasma $\beta$-lipoproteins had been observed by IEP in cases of cholestatis. Ekman et al. (1975) were able to visualise the changes more distinctly by CIEP. Bhakdi et al. (1975) used the test to detect membrane-bound complement components C3 and C4. Bjerrum et al. (1975) used the test to show that there could be more than the usual lines of precipitation following proteolytic degradation of certain antigens in the antibody containing gel during the electrophoresis.

Even though the original method was gaining popularity, Grubb (1974 a,b) found that it was less useful in the study of immunoglobulins as they would show a similar electrophoretic migration. The study of immunoglobulins using CIEP therefore necessitated a slight modification which entailed running the test at a high pH (10.5) such that all native immunoglobulins migrated anodically while the antibodies remained
immobile due to a chemical change (Grubb, 1974 a,b). Grubb (1975) used this modified test to demonstrate circulating IgG-lactate dehydrogenase immune complexes in human serum. Groc and Jendrey (1974), Soderholm et al. (1975) and Loft (1975) introduced a thin layer of polyacrylamide gel in lieu of agarose for the first dimensional electrophoresis followed by the second electrophoresis in agarose. Loft (1975) considered it advantageous to use polyacrylamide gel in the first dimension electrophoresis because it gives better separation than agarose and the sample is further concentrated when discontinuous buffer systems are used.

Crossed IEP has been applied to the study of cross reactions between species of mycobacteria (Closs et al., 1975), Pseudomonas aeruginosa and several other bacterial species (Hoiby, 1975). In virology, Vestergaard (1973) characterised antigen types 1 and 2 of Herpesvirus hominis using this technic, and in 1975 the same author used the same technic to study presence of antigen 1 in herpes simplex virus (HSV) types 2 antigen preparations. Vestergaard and Bog-Hansen (1975) found four HSV glycoprotein antigens using this technic. In mycology, Axelsen et al. (1975) used crossed IEP with an intermediate gel to study the precipitins to C. albicans in human sera.
In spite of showing such great powers of resolution, CIEP was ignored for the diagnosis of hydatid disease. Ardehali et al. (1977) demonstrated that CIEP was as sensitive and as specific as CIE the only disadvantages of the former over the latter being that CIEP requires more operational time and more serum.

2.2.11 Radioimmunoassay

Before the introduction of radioimmunoassay (RIA) in the 1950's, substances present in small amounts in blood and other body fluids were very difficult to measure. Radioimmunoassay was developed initially to detect hormones in circulation (Yalow, 1978) and it turned out to be a very specific and sensitive test, able to detect as little as 0.05 picomole of gastrin (Yalow, 1978). This high sensitivity and specificity led to the utilization of RIA in immunological assays whereby minimal amounts of reactive antibody or antigen may be diagnostically significant (Yalow, 1978). Although this technic has been extensively used in endocrinology, pharmacology, toxicology and pharmacokinetics of new drugs (Egginger, 1981), it has not received much attention in the detection of antibodies to infectious diseases (Yalow, 1978). This test is superior when compared to other analytical procedures in veterinary research with regard to sensitivity,
reproducibility and simplicity.

Voller et al. (1977) compared the RIA with enzyme-immunoassay for the diagnosis of tropical parasitic diseases, namely, trypanosomiasis, schistosomiasis, malaria and entamoebic dysentry. They found that RIA was excellent for the measurement of antibodies of these parasites. It was sensitive and reproducible and compared well with enzyme linked immunosorbent assay. Musiani et al. (1974) described the application of a solid phase RIA in plastic tubes or caps coated with a purified hydatid antigen and incubated them with $^{125}$I-radio-iodinated human anti-hydatid antigen IgG and sera from hydatidosis cases. In a comparison of this test with radio-immunoelectrophoresis, passive haemagglutination and radio-immuno precipitin tests, they found that the competitive inhibitory RIA offered clear advantages such as high sensitivity and specificity over the other tests utilized. This is particularly interesting because most of the patients had pulmonary hydatidosis which is difficult to detect by IHA or LAT (Kagan, 1974). Musiani et al. (1974) proposed the test as the method of choice in human hydatidosis. Huldt et al. (1973) found that by using a radioallergosorbent test (RAST) they could detect high levels of IgE in a few cases of Kautokeino Lapps with hydatid disease who had otherwise shown no response to antibodies of other class.
In comparing a simplified RIA technic and IHA Matossian (1981) found greater sensitivity with RIA in sera from 72 patients with hydatid disease. Due to its advantages, this author commented that "RIA will serve as a useful addition to tests already available in hydatid serology".

2.2.12 Enzyme-linked immunosorbent assay (ELISA)

Miles and Hales (1968) conceived the idea of combining an unknown substance with a reagent to obtain a product which could be assayed. Based on this concept, Engvall and Perlmann (1971) in Sweden and Van Weeman and Schuurs (1971) in Holland independently developed enzyme immunoassays whereby enzymes or co-enzymes replaced radioactive labels in non-competitive radioactive assay. These enzyme immunoassays have successfully acted as alternatives to RIA which had previously been used to detect soluble antigens and antibodies in body fluids. Following their introduction several names were given to these systems, such as enzyme-, enzymic-, enzymatic-, and enzymo-immunoassay (generally shortened to E.I.A) enzyme-linked immunoassays, immuno enzymatic assay and enzyme-linked immunosorbent assay (Wisdom, 1976). Bullock and Walls (1977) suggested that since the last test has been altered from the original test which was designed in line with radio immunosorbent assay, it should now be referred to as enzyme-linked-immuno-specific assay (ELISA).
In ELISA, the test is conducted by coupling antigen to a solid surface which may be provided by polystyrene material. Engvall and Perlmann (1971) successfully used polystyrene tubes but these have subsequently been replaced by microtitre plates and cuvetteee racks (Gilford PR 50). Quantitation of specific antibodies is obtained by adding serum to the antigen already coupled on the solid surface. When the serum is incubated together with the antigen sensitized carrier, it forms an antigen-antibody complex. The amount of antibody is then assessed by adding enzyme-labelled anti-immunoglobulin. The enzyme remaining in the tubes, wells or cuvettes gives a measure of the amount of specific antibodies in the serum (Ruitenberg et al., 1974)

Current literature on enzymatic assay refers to the use of a number of enzyme conjugates and substrates. However, it seems like alkaline phosphatase, p-nitrophenyl phosphate at a concentration of 1mg/ml and a pH between 9.6-9.8 are used more often than others (Carlsson et al., 1972). Of the substrates used, 5 aminosalicylic acid (0.8 mg/ml) with 0.005% hydrogen peroxide pH 6.0 is the most common substrate and 0.1m-0.2m sodium hydroxide is normally used as the stop solution (Mills et al., 1978).

Based on this principle, several groups of scientists have used these technics to serodiagnose various diseases. This technic has also been used to
detect bacterial infections (Sippel et al., 1978).
In serodiagnosis of parasitic diseases, the technic has been extensively used against amoebiasis (Bos et al., 1975); toxoplasmosis (Voller et al., 1976); malaria (Voller et al., 1977); and visceral leishmaniasis (Hommel et al., 1978). The test has also been used to serodiagnose infectious viral diseases such as herpes simplex virus isolates (Vestergaard et al., 1977; Mills et al., 1978); hog cholera antibodies (Saunders, 1977); rotavirus infection in calves (Ellens and de Leeuw, 1977); hepatitis B surface antigens (Wolters et al., 1976); Coxsackievirus antibodies (Katze and Crowell, 1978); antirubella virus antibodies (Leinikki et al., 1978); and antibodies against chlamydia (Lewis et al., 1977).

Farag et al. (1975) applied the technic to the diagnosis of human hydatidosis and found the test to be simple, specific and sensitive. Arambullo III et al. (1978) found a remarkably good correlation between ELISA and IHA in serodiagnosis of human cysticercosis. Harrison and Sewell (1981) monitored the serological response of six calves dosed orally with T. saginata eggs using ELISA and IHA. They detected antibodies by both tests. Craig and Rickard (1980) used a crude antigen prepared from T. saginata on ELISA and found ELISA to be a useful test. Njeruh (1981) used a purified HCF antigen in ELISA and found the test, using an isolated antigen, to be
3. MATERIALS AND METHODS

3.1 PREPARATION OF THE VARIOUS ANTIGENS

3.1.1 Hydatid cyst fluid

3.1.1.1 Collection and harvesting

Hydatid cysts were collected intact in situ. Cysts from different intermediate host species were kept separate in plastic containers and delivered to the laboratory as soon as possible. When a large number of cysts were collected, the batch was kept in a cold room at about 4°C. All fluid was harvested on the same day that the cysts were collected to avoid possible leakage of contaminant host antigens into the cyst fluid during storage. When a cyst was superficial the surface of the cyst was cleaned with running tap water.

For the cysts which were deeply seated in tissues like the liver, lungs or spleen, the tissues surrounding the cyst were first cleared to expose the cyst wall. This was to avoid contamination with host tissues covering the cysts during fluid aspiration.

Suction of the cyst fluid was achieved by the use of a 14 gauge needle connected to a syringe or to a flask connected to a suction pump. Delicate massage was applied to the cyst wall to free any protoscoleces which may have been attached to the germinal membrane so that they would be aspirated together with other already loose protoscolices.
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The harvested fluid samples were pooled and centrifuged in a refrigerated centrifuge. The clear fluid was decanted and preserved frozen with 0.1% sodium azide. The frozen fluid was thawed at a room temperature or in a water bath set at 37°C. In the latter case, the container was constantly rotated and shaken periodically to avoid overheating of the thawed fluid. This was done to avoid denaturing any heat sensitive antigens that may be present in the fluid (Dottorini and Tassi, 1977).

The fluid was dialysed in Visking dialysis tubing, initially against saline containing sodium azide (0.1%) and subsequently against distilled water with sodium azide. It was finally dialysed against distilled water alone to remove sodium azide. This dialysis was carried out in a cold room at 4°C to suppress microbial growth in the cyst fluid.

3.1.1.2 Concentration of hydatid cyst fluid (HCF)

After dialysis, the HCF was concentrated by one or a combination of the following methods.

3.1.1.2.1 Pervaporation

A measured amount of the thawed fluid was poured into Visking dialysis tubing and tied on one end onto a stand. Air was circulated around the exposed hanging bag at a fast rate using an ordinary table top fan. This was carried out at 4°C for 2.
3 days. Using this method it was possible to concentrate the fluid about 20 times (Chordi and Kagan, 1965).

3.1.1.2.2 Polyethylene glycol

Flakes of polyethylene glycol (PEG) (Carbowax 200 M) were placed in a plastic tray. Measured hydatid cyst fluid was placed in a Visking dialysis tubing and buried within the crisps of PEG and left overnight (Ardehali et al., 1977). The concentrated fluid was dialysed against distilled water to remove any PEG molecules that may have entered the dialysis bag. Some precipitation was detected in the bag.

3.1.1.2.3 Membrane filtration

Centrifuged HCF was further clarified by filtration through a Whatman No. 1 qualitative filter paper to separate solid matter and floating lipids. Further clarification was achieved by filtration through a 0.3 micrometer Sartorius membrane filter at a pressure of about 50 pounds per square inch (PSI). The clarified fluid was concentrated by using Amicon PM 10 ultrafilter with an approximate exclusion point of 10,000 daltons under a pressure not exceeding 50 PSI. In this way, it was possible to achieve a 20 fold concentration of the fluid. This preparation was stored at -20°C and as concentration took only a few hours, no sodium azide preservative was added.
3.1.1.2.4 Lyophilization

An Edwards High Vacuum lyophilizer was used. The powder so obtained could be reconstituted to any required concentration and dialysed (Coltorti and Varela-Diaz, 1972; Conder et al., 1980).

3.1.2 Hydatid cyst membrane

Having removed HCF as previously described, the cyst was aseptically opened to expose its inner membrane. In some cysts, and in particular liver cysts, it was common to find daughter cysts and hence many membranes could be obtained from a single main cyst. Using a pair of forceps the whitish membrane found lying free in the cavity was removed and placed in saline in a beaker. They were thoroughly washed with many changes of physiological saline to remove any traces of the fluid. The inner surface was lightly scraped to dislodge protoscolices. The membranes were preserved with sodium azide and stored frozen or processed immediately for immunization, in which case no azide was added. Before use, the membranes were ground up in physiological saline. Grinding was carried out for 3 minutes at a maximum speed. The homogenate was transferred to a glass tissue grinder in an ice bath and ground to a viscous mass. This mass was finally ultrasonicated, with or without complete Freund's Adjuvant, using a Braunsonic 1510
Sonicator. Sonication was done in one minute bursts at 200 Watts. These bursts were repeated three times.

3.1.3 **Hydatid cyst protoscoleces**

Protoscoleces were obtained after centrifugation of the cyst fluid and also from scrapings of the cysts membranes. These protoscoleces were thoroughly washed in saline or phosphate buffered saline (PBS). In every case no less than 5 washings were carried out, with each washing lasting at least 15 minutes. The supernatant was discarded and the sediment agitated with a fresh wash solution before being centrifuged again. This was meant to clean off any remaining traces of cyst fluid antigens. Effort was made to separate any tiny fragments of the membranes that may still be present by picking them manually with a pair of forceps. The cleaned protoscolices were subjected to a freeze-thaw process and finally homogenised in a glass tissue grinder in an ice bath. After this grinding, the material was sonicated as previously described for membranes.

3.1.4 **E. granulosus**

The worms which were harvested from the intestines of dogs from Turkana District in N.W. Kenya were supplied by Dr. Macpherson. The worms were washed repeatedly with physiological saline after
which they were preserved with sodium azide and frozen. To reduce the lipid level, the worms were defatted using a protocol as described by Kent (1963) and adopted by Gathuma (1977). Briefly the procedure was as follows:

Washed fresh frozen worms
↓
Absolute ethanol
↓
Anhydrous ether
↓
Defatting at -70°C
↓
Crude extract

This antigen solution was then dialysed to remove sodium azide and used to immunize a goat. The worms were ground in a glass tissue grinder and ultrasonicated as described for protoscolices.

3.1.5 Preparation of the reference antigens

A pool of reference antigens was made by mixing all the various preparations of HCF either from cattle, sheep and goat hydatid cysts, both fertile and non-fertile. This pool was used with the reference serum to define the antigens present in HCF. Through the use of immunoelectrophoresis and double diffusion it was found out that sheep 822 had produced antibodies against the largest number of antigens in HCF and was therefore used as the reference antiserum in later tests. A phosphotungstic acid/magnesium chloride precipitate of $\beta$-lipoprotein
from HCF was prepared using a method described by Burstein (1963) and modified by Njeru and Lindqvist (1982).

3.1.6 Preparation of antigens from other parasites

Several parasites were collected from abattoirs in and around Nairobi. They were washed in several changes of physiological saline so as to reduce contaminants. Some parasites were donated by the Department of Pathology and Microbiology of the Faculty of Veterinary Medicine, Kabete.

All parasites were stored at -20° in saline containing 0.1% sodium azide until they were needed.

A portion of the frozen parasites was placed in a mortar containing an arbitrary amount of saline. Grinding with a pestle was carried on until no more homogenate could be obtained. The resultant extract was sonicated for 3 minutes using a Braunsonic 1510 Sonicator and stored at -20°C in the presence of 0.1% sodium azide.

3.2 SOURCES OF SERA

3.2.1 Immunization of laboratory animals

3.2.1.1 Calves

Calves aged about nine months and weighing about 80 kgs were treated for gastro-intestinal and external parasitic infections and infestations before a pre-immunization bleeding was carried out. The calves were immunized with the respective antigens as shown in Table 1. The antigen preparations were
mixed with complete Freund's Adjuvant (CFA) in a ratio of one part of antigen to two parts of the adjuvant and thoroughly mixed. The antigen-adjuvant mixture was administered intramuscularly in four different sites. A series of fortnightly bleedings was carried out.

3.2.1.2 Sheep and goats

Local breeds of adult sheep and goats were used. The pre-immunization, immunization and bleeding protocols were identical to those described for calves.

3.2.1.3 Rabbits

New Zealand large white rabbits were used. The antigen-adjuvant mixture was administered into two intramuscular sites and two subcutaneous sites.

3.2.1.4 Goat no 880

Sheep anti-HCF serum was obtained from the pool of sheep 822 antisera prepared as explained later in section 3.2.3. This antiserum was tested against hydatid cyst fluid in agar gel immunodiffusion (Ouchterlony, 1958). Of the resultant precipitin lines, the most prominent arc was carefully cut out, suspended in PBS at pH 7.2 and finally homogenized in CFA using Braunsonic 1510 ultrasonicator. The material was used to immunize goat 880 whose antiserum was absorbed against sheep γ-globulins, *F. gigantica* and *P. microbothrium* coupled to insoluble
Table 1. Immunization of calves, sheep, goats and rabbits with various antigens.

<table>
<thead>
<tr>
<th>Animal Species</th>
<th>Immunizing Agents Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>bovine calf 28</td>
<td>bovine HCF</td>
</tr>
<tr>
<td>bovine calf 806</td>
<td>bovine hydatid cyst protoscolices</td>
</tr>
<tr>
<td>bovine calf 900</td>
<td>Whole <em>T. saginata</em> extract</td>
</tr>
<tr>
<td>bovine calf 835</td>
<td><em>C. bovis</em> extract</td>
</tr>
<tr>
<td>sheep 822</td>
<td>Sheep HCF</td>
</tr>
<tr>
<td>sheep 805</td>
<td>Sheep hydatid cyst protoscolices</td>
</tr>
<tr>
<td>goat 804</td>
<td>goat hydatid cyst protoscolices</td>
</tr>
<tr>
<td>goat 814</td>
<td>whole <em>E. granulosus</em> extract</td>
</tr>
<tr>
<td>goat 880</td>
<td>isolated antigen 4 (see text)</td>
</tr>
<tr>
<td>goat 888</td>
<td><em>C. tenuicollis</em> fluid</td>
</tr>
<tr>
<td>goat 346</td>
<td>isolated antigen 5 (see text)</td>
</tr>
<tr>
<td>rabbit 200</td>
<td><em>E. granulosus</em> extract</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>E. granulosus</em> extract</td>
</tr>
<tr>
<td>&quot;</td>
<td>hydatid cyst protoscolices</td>
</tr>
<tr>
<td>&quot;</td>
<td>hydatid cyst membranes</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>M. expansa</em> extract</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>F. gigantica</em> extract</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>S. hepatica</em> extract</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>O. radiatum</em> extract</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>P. microbothrium</em> extract</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>T. vulpis</em> extract</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>H. contortus</em> extract</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>A. galli</em> extract</td>
</tr>
<tr>
<td>&quot;</td>
<td><em>A. suum</em> extract</td>
</tr>
</tbody>
</table>
immunosorbents prepared as described in section 3.2.4, in case there were natural infections with these parasites. Further absorptions removed blood group substances.

3.2.1.5 Goat no. 346

An arc suspected to be the Arc 5 of Capron et al. (1967) was identified on crossed immunoelectrophoresis using concentrated HCF of bovine origin as the antigen and serum of calf 28 immunized as described in section 3.2.1.1. The isolated arc was used to immunize goat 345 so as to produce a monospecific anti-serum against arc 5.

Eighty of these immunoprecipitin arcs were prepared and carefully cut out to avoid other possible immunoprecipitates. The arcs were washed severally in PBS over a number of days. They were then sonicated as described earlier in CFA and used to immunize a goat. However, after the 4th bleeding of the goat, two lines appeared when the antigen and antiserum were tested on immunodiffusion, indicating that contamination had occurred. Since antiserum from the third bleeding of goat 345 had shown a single precipitin line, this third bleeding antiserum was then used to prepare more precipitin lines which were sonicated and injected into a fresh goat 346. This goat was boosted and bled for almost one year to give hyperimmune serum, showing a strong arc both on immuno-
diffusion and immunoelectrophoresis. This arc was identified as arc 5 of Capron, using reference serum supplied by Dr. Capron.

3.2.2 Sera from naturally infected and non-infected slaughter animals

Blood was collected from animals at small slaughterhouses. Carcases were tagged before flaying was completed and the species of the animal noted. An investigation for hydatid cysts in the liver, lungs, spleen and heart was carried out by palpation and incision of these organs according to the Meat Control Act of Kenya (Anon., 1977).

The pre-immunization serum was tested for any activity against the antigenic preparation with which the animal would be immunized. The tests used were double diffusion in agar gel, straight immunoelectrophoresis and crossed immunoelectrophoresis. Once the animals were immunized, the antiserum obtained after every bleeding was tested against the respective antigenic preparation used for immunization to trace the production of antibodies in the various animals.

All the antisera obtained from the same animal were pooled so as to provide a standard reference pool with a constant immunological pattern. The pooled anti-serum was absorbed against possible host contaminants to ensure that all the antibodies present were actually produced against parasitic
antigens and not host contaminants. A detailed description of the absorption protocol is given in section 3.2.3.

3.2.3 Absorption of various antisera

3.2.3.1 Absorption of sheep anti-HCF serum with plasma

To remove the antibodies which would have been produced against heterologous host material contaminants and possible homologous host allotypes as well as $P_1$ blood group substances (Ben-Ismail et al., 1980), the sheep 822 serum pool was absorbed with insoluble bovine, goat and sheep plasma as follows:

Three pools of plasma were made, each one consisting of 20 animals each from cattle, goats and sheep. A gel of each plasma pool was formed by polymerising the proteins by the method of Avrameas and Ternynck (1969). Briefly, the method is as follows: To 20 ml of normal bovine (20 animals) plasma was added 20 ml of 0.2 M acetate buffer of pH 5.0. The pH was readjusted and maintained at 5.0 using 1N HCl. To this mixture was added 1.2 ml ethylchloroformate. The mixture was placed on a magnetic stirrer and stirred while maintaining the pH at between 4.5 - 5.0 with 1 N NaOH. Stirring was stopped when the mixture became cloudy and allowed to stand at room temperature for one hour to gel. The gel was cut up, homogenised with a tissue grinder and washed three times with 0.2 M glycine-HCl buffer.
(pH 2.5), twice with 0.2 M sodium carbonate buffer (pH 9.0) and once with saline. The homogenate was mixed with the pooled sheep anti-sheep HCF antiserum 822 or any other serum which was to be absorbed with these antigens and left on a shaker for 5 hours at room temperature. After this shaking, sodium azide was added and the mixture was left overnight in the cold before insoluble immunosorbent was removed by centrifugation in the cold. The antiserum was then absorbed with pooled goat plasma and finally with pooled sheep plasma prepared in the same manner. Crossed immunoelectrophoresis (Closs et al., 1975) was carried out after every absorption to check for antibodies that may have been absorbed out. To check for absorption, controls were done by double diffusion (Ouchterlony, 1958) and crossed immunoelectrophoresis procedures using varying dilutions of sheep, goat, or cattle serum as the antigen against the antiserum, as well as controls of HCF against absorbed and unabsorbed antiserum.

When the absorption was completed a reference pattern was established using the reference pool of HCF and anti-HCF from sheep 822. Optimum operational conditions were also established as shown in section 3.3, on "immunological tests".
3.2.3.2 Absorption of sheep anti-sheep HCF serum (822) with crude parasitic extracts in solution or coupled to insoluble immunosorbents

The antiserum was absorbed with five parasites in a series as shown below:

Sheep 822 antiserum

\[ \text{Sheep 822 antiserum} \]

\[ \xrightarrow{\text{P. microbothrium}} \]

\[ \text{Aliquot} \xrightarrow{} \text{Test} \]

\[ \xrightarrow{\text{S. hepatica}} \]

\[ \text{Aliquot} \xrightarrow{} \text{Test} \]

\[ \xrightarrow{\text{M. expansa}} \]

\[ \text{Aliquot} \xrightarrow{} \text{Test} \]

\[ \xrightarrow{\text{C. tenuicollis}} \]

\[ \text{Aliquot} \xrightarrow{} \text{Test} \]

\[ \xrightarrow{\text{T. saginata}} \]

\[ \text{Aliquot} \xrightarrow{} \text{Test} \]

The tests carried out were double diffusion and crossed immunoelectrophoresis as described in section 3.3.1 and 3.3.3. respectively.
3.2.3.3. Absorption of sheep anti-sheep HCF serum (822) with a pool of crude parasitic extracts

A 0.25 ml aliquot of saline extract of each of the five parasites (totalling 1.25 ml) was mixed with 3 ml of antiserum 822 and left rotating in the cold for 48 hours after which it was centrifuged in a refrigerated centrifuge. The supernatant was freeze-dried and reconstituted to the original volume of the antiserum 822.

3.2.3.4 Preparation of immunosorbents

These were prepared according to the manufacturer's instructions. Briefly, antigens were dissolved in a bicarbonate buffer and mixed with reswelled sepharose 4B gel (Pharmacia Fine Chemicals) and placed on a rotator for 24 hours. The gel was washed with bicarbonate buffer followed by acetate buffer containing sodium chloride and finally distilled water.

3.3 IMMUNOLOGICAL TESTS

3.3.1 Immunodiffusion

The double diffusion in agar gel tests of Ouchterlony (1958) was performed as follows: A 1% buffered agar was prepared using PBS. Three ml of the molten agar were poured on a microscope slide to form a gel 3 mm thick. A set of wells was punched out of the gel and the wells filled to capacity with
various reagents.

The slides were placed in a humid chamber at room temperature for 18-48 hours after which the slides were pressed, dried and stained with Coumassie Blue following the procedure of Weeke (1973). The dry, stained slide was viewed on an X-ray viewer and the precipitin arcs traced on paper using a photographic enlarger.

This procedure was followed when processing slides from immunoelectrophoresis and crossed immunoelectrophoresis tests.

3.3.2 Immunoelectrophoresis.

The immunoelectrophoresis technic developed by Grabar and Williams (1953) was applied as follows: Fifteen mls of a 1% buffered agarose gel was layered on glass plates using the LKB electrophoresis system measuring 92x85 mm. Wells were made in the agar gel and filled with the antigen. One well was filled with bovine serum albumin (BSA) mixed with bromothymol blue dye to monitor the progress of the electrophoresis. Electrophoresis was carried on for 60 minutes on a water -cooled surface. Agarose was connected to the buffer using spongy wicks. Electrophoresis was carried out at 10V/cm measured immediately and adjusted if necessary 10 minutes after the commencement of the electrophoresis.
After the completion of the run, troughs were made in the gel between the antigen wells and antiserum filled in the troughs. The slide was further processed as described in 3.3.1.

3.3.3 Crossed immunoelectrophoresis

This test involved electrophoresis in two dimensions (Laurell, 1965; Soderholm et al., 1975). In the first dimension, the test was run as explained for straight IEP except that it was run for 45 minutes.

On completing the first dimensional run, the plate with separated antigens in agarose, was placed in a slide holder and sliced parallel to the direction of the first run. On average, the slices were about 13 mm wide and 50 mm long. As no HCF antigen migrates cathodically (this had been tested for previously through the use of a large plate and puncturing the wells way away from the wick-end)(see section 3.3.5), the cut behind the well was made close to the well. The slice was transferred on to the crossed immunoelectrophoresis glass plate.

All plates prepared for this test contained an intermediate gel (Axelsen et al., 1973) which was either plain agarose or contained antigens or antiserum (Closs et al., 1975). To restrict the extent of flow of agarose while pouring in the intermediate gel, a thin slice of agarose from the unused side-ends of the agarose used in the first run was cut and
placed about 18 mm from the top edge of the already placed slab containing the antigens and the intermediate gel poured on. The plate was transferred to the slide holder and the agarose-barrier slab cut off together with a thin slice of the intermediate agarose measuring about 2 mm wide. Top gel was poured on to the remaining part of the plate.

When test antigen or antiserum was to be incorporated in the intermediate gel, 0.2 ml of antiserum or antigen was added to the liquid agarose in a water bath at 50-60°C. Should a different proportion of the material in the intermediate gel be required the amount of agarose was varied too, such that the sum of the agarose and the test substance always added up to 2 ml to avoid overflowing of the agarose while pouring.

The top gel always contained an antiserum. The sum of the antiserum and agarose added to 2 ml. The rest of the protocol was similar to that used for the intermediate gel.

The plates were transferred into the same electrophoresis chambers as used for the first run. Electrophoresis was carried out at 2V/cm for over 18 hours on a water cooled surface.
3.3.4 Determination of the optimum time for the first dimensional electrophoresis

Electrophoresis of the standard HCF antigen was carried out over different time intervals as follows: 30, 40, 50, 60 and 90 minutes. A BSA Bromothymol Blue marker was placed in one of the wells to help monitor the progress of the electrophoresis. Current was applied at 10V/cm and the same buffer and gel used in section 3.3.2 were used. The second dimensional electrophoresis was carried out on plates of the same size.

3.3.5 Test for any cathodic antigens

A glass plate measuring about 90 mm x 80 mm was covered with buffered agarose. A well was punched out about 20 mm from the left edge of the plate and the reference antigen added. Electrophoresis was carried out with the anode to the right. The standard operating conditions were maintained.

3.3.6 Modification of crossed immunoelectrophoresis to use paper strips impregnated with antiserum

The first dimensional electrophoresis as well as the layering of the intermediate gel was performed as described in section 3.3.3. However, plain agarose was poured on the top part of the glass plate. A strip of absorbent paper holding a known amount of soaked antiserum was placed at the centre of the top
gel such that the antiserum would diffuse evenly into the top gel.

3.3.7 **Indirect enzyme-linked immunosorbent assay**

Indirect ELISA was performed according to Engvall and Perlmann (1971). The procedure involved adsorption of a purified HCF antigenic preparation containing two antigens (4 and 5) precipitated out of HCF (Section 3.1.5). The antigen was diluted in coating buffer* and 100 μl placed in each microtitre plate well. After an overnight incubation in a humid chamber kept at room temperature, the plates were washed* 5 times at 5 minutes intervals. Diluted serum was then added followed by incubation at 37°C for 30 minutes and washing. Goat antibovine globulin conjugated to horse radish peroxidase* was added and incubated for 30 minutes at 37°C, and washed. Orthophenylene diamine* (OPD) at a concentration of 0.1% was added and incubated at room temperature in the dark for about 10 minutes. The reaction was stopped with 2 N Sulphuric acid and the absorbance read on Dynatech microelisa minireader* at 490 nm.

The concentration of the various reactants was based on titration. Controls for positive serum, negative serum, antigen, serum, conjugate and substrate were included on each plate. All test sera were run in duplicate.

* See appendix for materials
4. RESULTS

4.1 ANTIGENS PRESENT IN HCF

Using crossed immunoelectrophoresis (CIEP) sheep hydatid cyst fluid was found to contain 13 antigens: each of the antigens gave rise to a precipitin line. Some precipitin lines were visible before staining but some weak arcs were only visible after staining.

Based on their electrophoretic mobility the arcs were assigned numbers beginning with the fastest anodic arc with others following serially as shown in Fig. 1. However, not all the precipitin lines were always reproducible. The most prominent antigen was number 4. It extended over most of the agarose and occasionally masked other arcs, especially arcs 2, 3 and the left "foot" of arc 1. At times it would split into two bands on the left and then merge into one before it formed the peak. This antigen was among the antigens that elicited early antibody production in laboratory animals.

Another major antigen was 5 (Capron et al., 1967) which was always found below arc 4.

Table 2 shows the electrophoretic mobility of the various antigens. Also shown is the relative electrophoretic mobility of the antigens using arc 5 as the reference point. Arcs 6 and 7 were inconsistent and were not studied. Arcs 11, 12 and 13 did not show a definite peak and therefore no measurements
Fig. 1 Reference pattern (see text) observed when the standard HCF pool was electrophoresed against the standard anti-HCF serum.

A- Antigens from pooled HCF. (-) and (+) indicate position of electrodes during the first (1) and second (2) dimensional electrophoreses.

B- Plain buffered agarose.

C- Sheep anti-sheep hydatid cyst fluid serum (SA-SHCF-S) absorbed for possible antibodies to host contaminants.
Table 2. The electrophoretic mobility and standard deviations (s.d.) of some HCF antigens.

<table>
<thead>
<tr>
<th>Antigen Number</th>
<th>Mean Electrophoretic Mobility (mm)</th>
<th>Electrophoretic Mobility Relative to Antigen 5.</th>
<th>S.D</th>
<th>Number of Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>1.6</td>
<td>4.7</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>1.4</td>
<td>4.3</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>1.1</td>
<td>4.3</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>1.2</td>
<td>4.5</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>1.0</td>
<td>4.4</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>0.7</td>
<td>3.2</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>0.6</td>
<td>2.7</td>
<td>13</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>0.5</td>
<td>1.5</td>
<td>13</td>
</tr>
</tbody>
</table>

Notes: 

a) Antigens 6 and 7 frequently missed from the reference pattern and were therefore left out of the analysis.

b) Measurements were taken from the centre of the well to the centre of the peak in question.

c) Antigens 11 to 13 inclusive tended to be flat and had no definite peaks from where measurements could be taken. These antigens were also left out of the analysis.
could be taken since measurements were taken from the centre of the well to the centre of the peak. The fast migrating antigens showed a higher standard deviation (s.d.) than the slow migrating antigens. The s.d. varied from 4.7 for antigen 1 to 1.5 for antigen 10.

4.2 THE PREVALENCE OF HYDATIDOSIS IN SLAUGHTER SHEEP AND GOATS

During the collection of hydatid cysts to provide HCF, protoscoleces and membranes, a note was made of the prevalence of hydatidosis in slaughter sheep and goats in two small abattoirs. Table 3 summarises the results. In total, 14.2% of all sheep and goats slaughtered in both abattoirs had hydatid cysts in liver and/or lungs. No great significance was attached to these results.
Table 3. The Prevalence of Hydatidosis at two abattoirs.

<table>
<thead>
<tr>
<th>Abattoir</th>
<th>Species</th>
<th>Number examined</th>
<th>Number Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dagoretti</strong></td>
<td>Sheep</td>
<td>76</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>144</td>
<td>14</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>Spp Unidentified</td>
<td>46</td>
<td>6</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>266</td>
<td>39</td>
<td>14.7</td>
</tr>
<tr>
<td><strong>Ongata Rongai</strong></td>
<td>Sheep</td>
<td>120</td>
<td>21</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>239</td>
<td>29</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>359</td>
<td>50</td>
<td>13.9</td>
</tr>
<tr>
<td><strong>Both Abattoirs</strong></td>
<td>Sheep</td>
<td>196</td>
<td>40</td>
<td>20.4</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>383</td>
<td>43</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>Spp Unidentified</td>
<td>46</td>
<td>6</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>Grand Total</td>
<td>625</td>
<td>89</td>
<td>14.2</td>
</tr>
</tbody>
</table>
4.3 **TEST FOR CIRCULATING ANTIBODIES TO HCF**

This test was carried out on serum from animals which on post mortem (P.M.) examination had been found to harbour at least one hydatid cyst. Out of 82 serum samples from animals which had been naturally infected with the disease only 13 (16%) sera contained antibodies which could be detected by DD test (Fig. 2).

4.4 **THE FREQUENCY OF OCCURRENCE OF ANTIGENS 4 AND 5 IN HCF**

At a 15 times concentration of the HCF, all cyst fluid originating from sheep or goat liver showed the presence of both antigens 4 and 5 (Table 4). None of the cysts fluid from sheep or goat liver showed either of antigen 4 or 5 alone. But when HCF originating from the lung cysts of the two animal species was tested, 8% of the sources did not contain either antigen 4 or 5. Antigen 4 was present in 13% of the lung cyst HCF but antigen 5 did not occur alone in any sheep or goat lung cyst fluid. Both antigens 4 and 5 occurred together in 79% of the sheep and goat lung hydatid cysts fluid (Table 4).

For cattle, 54% of liver hydatid cysts fluid showed the presence of both antigens 4 and 5. Antigen 4 alone occurred in 46% of the liver cysts but antigen 5 alone was not found in any cyst. All the cyst fluid
Fig. 2. Demonstration of circulating antibodies to HCF in naturally infected animals, using DD test.

**KEY:**

<table>
<thead>
<tr>
<th>Left pattern</th>
<th>Right pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Well</strong></td>
<td><strong>content</strong></td>
</tr>
<tr>
<td>A</td>
<td>HCF pool</td>
</tr>
<tr>
<td>1</td>
<td>Serum No. 321</td>
</tr>
<tr>
<td>2</td>
<td>&quot; &quot; 337</td>
</tr>
<tr>
<td>3</td>
<td>&quot; &quot; 556</td>
</tr>
<tr>
<td>4</td>
<td>&quot; &quot; 613</td>
</tr>
<tr>
<td>5</td>
<td>&quot; &quot; 627</td>
</tr>
<tr>
<td>6</td>
<td>&quot; &quot; 629</td>
</tr>
</tbody>
</table>

**NB:** An odd precipitin line was observed between wells 5 and 6 on the right pattern.
Table 4. The frequency of occurrence of antigens 4 and/or 5 in organs of sheep/goat and cattle.

<table>
<thead>
<tr>
<th>Animal Spp</th>
<th>Cyst Location</th>
<th>Arcs seen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>None</td>
<td>Arc 4</td>
</tr>
<tr>
<td>Goat</td>
<td>Liver</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>2(8%)</td>
<td>3(13%)</td>
</tr>
<tr>
<td>Bovine</td>
<td>Liver</td>
<td>0(0%)</td>
<td>6(46%)</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>0(0%)</td>
<td>28(50%)</td>
</tr>
</tbody>
</table>
contained at least either antigen 4 alone or antigens 4 and 5 together.

Forty-five percent of all cattle lung cysts fluid contained both antigens 4 and 5. Antigen 4 alone was found in 50% of the lung cysts fluid while antigen 5 alone occurred in 5% of the samples. All lung cysts contained at least one or both antigens (Table 4).

Table 5 shows that fertile cysts from cattle, sheep or goat had a higher occurrence of both antigens 4 and 5 together. Non-fertile cysts from cattle showed a greater occurrence of antigen 4 alone than for both antigens. Non fertile cysts from sheep or goats showed a higher occurrence of both antigens than antigen 4 alone.

4.5 OPTIMUM RUNNING TIME FOR THE FIRST DIMENSIONAL ELECTROPHORESIS

It was found that as the electrophoresis running time was extended from 40 towards 90 minutes, the precipitin arcs were well separated. However, the arcs extended beyond 50 mm, the maximum length of the micro-technic crossed immunoelectrophoresis plate available. A running time of 45-50 minutes at a voltage drop of 10V/cm was found best and adopted. The coloured marker had migrated about 25 mm after this electrophoresis period.
Table 5. The frequency of occurrence of antigens 4 and 5 in fertile and non-fertile hydatid cysts. (Fertility was based on the presence of protoscoleces).

<table>
<thead>
<tr>
<th>Source of Hydatid cysts</th>
<th>Fertile cysts</th>
<th>Non-fertile cysts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antigens 4 &amp; 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>missing</td>
<td>present</td>
</tr>
<tr>
<td>Cattle lung</td>
<td>3 (9%)</td>
<td>25 (73%)</td>
</tr>
<tr>
<td>Cattle liver</td>
<td>0</td>
<td>6 (84%)</td>
</tr>
<tr>
<td>Sheep/goat lung</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Sheep/goat liver</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Cattle lung</td>
<td>1 (2%)</td>
<td>12 (27%)</td>
</tr>
<tr>
<td>Cattle liver</td>
<td>1 (17%)</td>
<td>1 (17%)</td>
</tr>
<tr>
<td>Sheep/goat lung</td>
<td>2 (15%)</td>
<td>8 (62%)</td>
</tr>
<tr>
<td>Sheep/goat liver</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>
4.6 TEST FOR CATHODIC ANTIGENS

It was found that there were no cathodically migrating antigens in the reference HCF pool.

4.7. CROSS REACTIVITY OF HCF ANTIGENS WITH ANTIGENS FROM OTHER PARASITES USING DOUBLE DIFFUSION TEST

By the use of crude saline extracts of various parasites or concentrated larval cyst fluid, cross-reactions with at least one HCF antigen was observed with the following six parasite extracts when tested in the Ouchterlony double diffusion (DD) test; twenty per cent (w/v) concentrated _C. tenuicollis_ cyst fluid showed four antigens to cross-react with HCF antigens (Fig. 3; Table 6); whole _C. bovis_ bladder homogenate showed three common antigens (Fig. 4); _T. saginata_ homogenate showed two common antigens (Figs. 3 and 4); _A. centripunctata_ homogenate showed two common antigens (Fig. 4) and _S. hepatica_ homogenate showed one common antigen (Fig. 4).

The following 10 saline parasitic extracts did not show cross-reactivity with any HCF antigens as demonstrated in Fig. 4: _H. contortus, B. phlebotomum, O. ostertagi, O. radiatum, T. vulpis, S. lupi, A. galli, A. suum, F. gigantica_ and _P. microbothrium_.

Fig. 3 Immunodiffusion test for cross reactivity between HCF and other parasites.

**KEY:**

**Left pattern**

<table>
<thead>
<tr>
<th>Well</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sheep anti-sheep HCF serum</td>
</tr>
<tr>
<td>1</td>
<td>Sheep HCF conc 10x</td>
</tr>
<tr>
<td>2</td>
<td><em>C. tenuicollis</em> conc 20% w/v</td>
</tr>
<tr>
<td>3</td>
<td><em>M. expansa</em></td>
</tr>
<tr>
<td>4</td>
<td><em>S. hepatica</em></td>
</tr>
<tr>
<td>5</td>
<td><em>C. tenuicollis</em> conc 20% w/v</td>
</tr>
<tr>
<td>6</td>
<td><em>M. expansa</em></td>
</tr>
</tbody>
</table>

**Right pattern**

<table>
<thead>
<tr>
<th>Well</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sheep anti-sheep HCF serum</td>
</tr>
<tr>
<td>1</td>
<td><em>T. saginata</em></td>
</tr>
<tr>
<td>2</td>
<td><em>C. tenuicollis</em></td>
</tr>
<tr>
<td>3</td>
<td><em>M. expansa</em></td>
</tr>
<tr>
<td>4</td>
<td><em>T. saginata</em></td>
</tr>
<tr>
<td>5</td>
<td><em>M. expansa</em></td>
</tr>
<tr>
<td>6</td>
<td><em>C. tenuicollis</em></td>
</tr>
</tbody>
</table>

**RESULTS**

*C. tenuicollis, M. expansa and T. saginata* show cross reactivity.
Fig. 4 Immunodiffusion test for cross reactivity between HCF and other parasites.

KEY:

**Left pattern**

<table>
<thead>
<tr>
<th>Well</th>
<th>content</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sheep anti-sheep HCF serum</td>
</tr>
<tr>
<td>1</td>
<td>Pool of HCF</td>
</tr>
<tr>
<td>2</td>
<td>S. hepatica</td>
</tr>
<tr>
<td>3</td>
<td>A. galli</td>
</tr>
<tr>
<td>4</td>
<td>A. centripunctata</td>
</tr>
<tr>
<td>5</td>
<td>O. ostertagi</td>
</tr>
<tr>
<td>6</td>
<td>T. vulpis</td>
</tr>
<tr>
<td>7</td>
<td>A. suum</td>
</tr>
<tr>
<td>8</td>
<td>T. saginata</td>
</tr>
</tbody>
</table>

**Right pattern**

<table>
<thead>
<tr>
<th>Well</th>
<th>content</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sheep anti-sheep HCF serum</td>
</tr>
<tr>
<td>1</td>
<td>C. tenuicollis</td>
</tr>
<tr>
<td>2</td>
<td>B. phlebotomum</td>
</tr>
<tr>
<td>3</td>
<td>P. microbothrium</td>
</tr>
<tr>
<td>4</td>
<td>F. gigantica</td>
</tr>
<tr>
<td>5</td>
<td>H. contortus</td>
</tr>
<tr>
<td>6</td>
<td>C. bovis</td>
</tr>
<tr>
<td>7</td>
<td>O. radiatum</td>
</tr>
<tr>
<td>8</td>
<td>S. lupi</td>
</tr>
</tbody>
</table>

RESULTS:

S. hepatica, A. centripunctata, T. saginata, C. tenuicollis and C. bovis show cross-reactivity.
Table 6. Results of Double Diffusion test for cross reactions between HCF antigens and other parasites.

<table>
<thead>
<tr>
<th>Source of Antigens</th>
<th>Sheep anti HCF serum</th>
<th>No. of antigens in common</th>
<th>Ref. Figures</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. ouicollis</em> fluid</td>
<td>+</td>
<td>4</td>
<td>Fig. 2, Fig. 3</td>
</tr>
<tr>
<td><em>C. bovis</em> bladder</td>
<td>+</td>
<td>3</td>
<td>Fig. 4</td>
</tr>
<tr>
<td><em>M. expansa</em></td>
<td>+</td>
<td>2</td>
<td>Fig. 2</td>
</tr>
<tr>
<td><em>T. saginata</em></td>
<td>+</td>
<td>2</td>
<td>Fig. 2</td>
</tr>
<tr>
<td><em>A. centripunctata</em></td>
<td>+</td>
<td>2</td>
<td>Fig. 3</td>
</tr>
<tr>
<td><em>S. hepatica</em></td>
<td>+</td>
<td>1</td>
<td>Fig. 3</td>
</tr>
</tbody>
</table>
RESULTS OBTAINED AFTER ABSORPTION OF ANTI-HCF SERUM WITH CRUDE PARASITE HOMOGENATES USING DOUBLE DIFFUSION TEST

Figure 5 shows that when sheep anti-sheep HCF serum was absorbed with a saline homogenate of S. hepatica and T. saginata, one antibody against HCF antigens remained. The resulting arc (Fig. 6) corresponded to antigen 4 of HCF. M. expansa showed an arc which was not related to arc 4 as shown in Fig. 6. C. tenuicollis showed four precipitin lines (Fig. 5).

In Fig. 7, M. expansa absorbed other antibodies from sheep anti-HCF serum except antibody to antigen 4 (Fig. 8). There was no arc formed against S. hepatica, T. saginata or P. microbothrium. An arc was formed against C. tenuicollis but the antigen was not related to antigen 4 (Fig. 8).

Figure 7 shows that on absorbing the reference anti-HCF serum (822) with concentrated (20% w/v) C. tenuicollis fluid, at least one antibody remained and this was shown to be anti-antigen 4 (Fig. 8).

There was no precipitin line against S. hepatica, T. saginata and P. microbothrium, but two arcs were seen against C. tenuicollis indicating that absorption was incomplete.
Fig. 5 Immunodiffusion test for the effect of absorbing sheep anti-SHCF serum with *T. saginata* and *S. hepatica* homogenates.

**KEY:**

<table>
<thead>
<tr>
<th>Well</th>
<th>Left pattern content</th>
<th>Right pattern content</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sheep anti-SHCF serum absorbed with <em>T. saginata</em></td>
<td>A Sheep anti-SHCF serum absorbed with <em>S. hepatica</em></td>
</tr>
<tr>
<td>1</td>
<td>SHCF conc 10x</td>
<td>1 SHCF conc 10x</td>
</tr>
<tr>
<td>2</td>
<td><em>M. expansa</em></td>
<td>2 <em>M. expansa</em></td>
</tr>
<tr>
<td>3</td>
<td><em>P. microbothrium</em></td>
<td>3 <em>P. microbothrium</em></td>
</tr>
<tr>
<td>4</td>
<td><em>T. saginata</em></td>
<td>4 <em>T. saginata</em></td>
</tr>
<tr>
<td>5</td>
<td><em>S. hepatica</em></td>
<td>5 <em>S. hepatica</em></td>
</tr>
<tr>
<td>6</td>
<td><em>C. tenuicollis</em> 20% w/v</td>
<td>6 <em>C. tenuicollis</em> 20% w/v</td>
</tr>
</tbody>
</table>

**RESULTS:**

Absorption with *T. saginata* and *S. hepatica* was complete.

An antigen of HCF was not absorbed out in both cases.
Fig. 6 Immunodiffusion test for the effect of absorbing sheep anti-SHCF serum with T. saginata and S. hepatica homogenate

KEY:

Left pattern

<table>
<thead>
<tr>
<th>Well content</th>
<th>Right pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Sheep anti-SHCFs absorbed with T. saginata</td>
<td>A Sheep anti-SHCFs absorbed with S. hepatica</td>
</tr>
<tr>
<td>1. SHCF conc 10x</td>
<td>1 SHCF conc 10x</td>
</tr>
<tr>
<td>2 M. expansa</td>
<td>2 M. expansa</td>
</tr>
<tr>
<td>3 P. microbothrium</td>
<td>3 P. microbothrium</td>
</tr>
<tr>
<td>4 C. tenuicollis 20% w/v</td>
<td>4 C. tenuicollis fluid</td>
</tr>
<tr>
<td>5 S. hepatica</td>
<td>5 T. saginata</td>
</tr>
<tr>
<td>6 Phosphotungstic acid/magnesium chloride precipitate from HCF (880)</td>
<td>6 Phosphotungstic acid/magnesium chloride precipitate from HCF (880)</td>
</tr>
</tbody>
</table>

RESULTS:

The sheep anti-SHCFs retained activity against M. expansa, C. tenuicollis, and HCF. The remaining antibody against HCF was anti-antigen 4. M. expansa had an antigen which was not recognized by antigen 4.
Fig. 7  Immunodiffusion test for the effect of absorbing sheep anti-SHCFS with C. tenuicollis or M. expansa

**KEY:**

<table>
<thead>
<tr>
<th>Left pattern</th>
<th>Right pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Well</strong></td>
<td><strong>content</strong></td>
</tr>
<tr>
<td>A</td>
<td>Sheep anti-SHCFS absorbed with C. tenuicollis</td>
</tr>
<tr>
<td>1</td>
<td>Sheep HCF conc 10x</td>
</tr>
<tr>
<td>2</td>
<td>M. expansa</td>
</tr>
<tr>
<td>3</td>
<td>P. microbothrium</td>
</tr>
<tr>
<td>4</td>
<td>T. saginata</td>
</tr>
<tr>
<td>5</td>
<td>S. hepatica</td>
</tr>
<tr>
<td>6</td>
<td>C. tenuicollis</td>
</tr>
</tbody>
</table>

**RESULTS:**

Absorption with both parasite antigens was incomplete. One strong anti-HCF antibody was not absorbed out. The arcs remaining against C. tenuicollis and M. expansa were not recognized by the remaining HCF arc.
Fig. 8 Immunodiffusion test for the effect of absorbing sheep anti-SHCFS with *C. tenuicollis* or *M. expansa*

**KEY:**

**Left pattern**

<table>
<thead>
<tr>
<th>Well</th>
<th>content</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sheep anti-SHCFS absorbed with <em>C. tenuicollis</em></td>
</tr>
<tr>
<td>1</td>
<td>Sheep HCF conc 10x</td>
</tr>
<tr>
<td>2</td>
<td><em>M. expansa</em></td>
</tr>
<tr>
<td>3</td>
<td><em>P. microbothrium</em></td>
</tr>
<tr>
<td>4</td>
<td><em>T. saginata</em></td>
</tr>
<tr>
<td>5</td>
<td><em>S. hepatica</em></td>
</tr>
<tr>
<td>6</td>
<td>&quot;880&quot;</td>
</tr>
</tbody>
</table>

**Right pattern**

<table>
<thead>
<tr>
<th>Well</th>
<th>content</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sheep anti-SHCFS absorbed with <em>M. expansa</em></td>
</tr>
<tr>
<td>1</td>
<td>Sheep HCF conc 10x</td>
</tr>
<tr>
<td>2</td>
<td><em>C. tenuicollis</em></td>
</tr>
<tr>
<td>3</td>
<td><em>P. microbothrium</em></td>
</tr>
<tr>
<td>4</td>
<td><em>T. saginata</em></td>
</tr>
<tr>
<td>5</td>
<td><em>S. hepatica</em></td>
</tr>
<tr>
<td>6</td>
<td>&quot;880&quot;</td>
</tr>
</tbody>
</table>

**RESULTS:**

Only activity against one antigen (880) remained in the antiserum. *C. tenuicollis* showed an arc (right hand pattern) which was not recognized by antigen "880".
Table 7: The results obtained after serial absorption of SA-SHCF with five common parasitic extracts.

<table>
<thead>
<tr>
<th>SA-SHCF Absorbed with the following parasitic extracts serially</th>
<th>Effects on Arcs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13</td>
</tr>
<tr>
<td>1. P. microbothrium</td>
<td>+ + - + + + + - - + + + +</td>
</tr>
<tr>
<td>2. S. hepatica</td>
<td>- - - + + - - - + - - -</td>
</tr>
<tr>
<td>3. M. expansa</td>
<td>- - - - + - - - + - - -</td>
</tr>
<tr>
<td>4. C. tenuicollis</td>
<td>- - - - - + - - + (+) - -</td>
</tr>
<tr>
<td>5. T. saginata</td>
<td>- - - + - - - - + - - -</td>
</tr>
<tr>
<td>After concentration of aliquots</td>
<td>- - - + + - - - + + - -</td>
</tr>
<tr>
<td>After absorbing with a pool of the parasites</td>
<td>- - - + + - - - + + - -</td>
</tr>
</tbody>
</table>

Notes:  
- = Absent  
+ = Present  
(+) = present but weak

Results: Antibodies to antigens 4, 5, 9 and 10 were not absorbed out.
Fig. 9 The precipitation pattern obtained when HCF pool antigens were run against SA-SHCFS which had been absorbed step by step in a series with *P. microbothrium* and *S. henatica*.

**KEY**

A - HCF pool antigens were electrophoresed in the first dimension run at 10V/cm for 45 minutes on agarose bed.

B - Blank agarose

C - Absorbed SA-SHCFS mixed with agarose.

**RESULTS**: Arc 4 was significantly elevated. Arcs 4, 5, 9 and 10 were not absorbed out.
The precipitation pattern obtained when HCF pool antigens were run against SA-SHCFS.

**KEY:**

A - HCF pool antigens were electrophoresed in the first direction run.

B - Blank agarose.

C - Absorbed SA-SHCFS mixed with agarose.

**RESULTS:**

Antibodies against antigens 5, 9 and 10 were still not absorbed out. Arcs 5 and 10 had been elevated.
Fig. 11  The precipitation pattern obtained when HCF pool antigens were run against SA-SHCFs which had been absorbed with five parasites.

KEY:
A - HCF pool antigens were electrophoresed in the first direction run.
B - Blank agarose.
C - SA-SHCFs absorbed with the five parasites as explained above.

RESULTS:
Arrows 5 and 10 still remained although their staining intensity was very weak.
Fig. 11 The precipitation pattern obtained when HCF pool antigens were run against SA-HCFS which had been absorbed with five parasites.
Fig. 12 The precipitation pattern obtained when the HCF pool antigens were run against concentrated SA-SHDFS which had been absorbed with five parasites in a series.
The precipitation pattern obtained when the HCF pool antigens were run against concentrated SA-SHCFS which had been absorbed with five parasites in a series.

**KEY:**

A - HCF pool antigens were electrophoresed in the first direction run.

B - Blank agarose.

C - Absorbed SA-SHCFS mixed with agarose.

**RESULTS:**

It was observed that only four arcs, numbers 4, 5, 9 and 10 remained. The four arcs were elevated, especially arc 4.
Fig. 13 The precipitation pattern obtained when HCF pool antigens were run against SA-SHCFS which had been absorbed with a pooled mixture of five parasites.
The precipitation pattern obtained when HCF pool antigens were run against SA-SHCFS which had been absorbed with a pooled mixture of five parasites.

**KEY:**

A - HCF pool antigens were electrophoresed in the first direction run.

B - Blank agarose.

C - SA-SHCFS mixed with agarose.

**RESULTS:**

Arcs 4, 5, 9 and 10 were observed. Arc 4 was elevated.
Table 8: The distribution of HCF antigens in other common parasites.

<table>
<thead>
<tr>
<th>HCF Antigen Numbers</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. expansa</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. tenuicollis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. hepatica</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>T. saginata</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. bovis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>H. contortus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>F. gigantica</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>O. radiatum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T. vulpis</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>A. suum</td>
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<tr>
<td>P. microbothrium</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>A. centripunctata</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A. galli</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>S. phlebotomum</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>S. lupi</td>
<td>+</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>
Fig. 14 The precipitation pattern obtained when HCF antigens were run against rabbit anti-M. expansa serum.
Fig. 14 The precipitation pattern obtained when HCF antigens were run against rabbit anti-\textit{M. expansa} serum.

**KEY:**

A - HCF pool antigens were electrophoresed in the first dimension run.

B - Blank agarose.

C - Agarose mixed with anti-\textit{M. expansa} serum.

**RESULTS:**

A total of 9 HCF antigens were recognised by anti-\textit{M. expansa} serum
Fig. 15 The precipitation pattern obtained when concentrated HCF pool antigens were added to the intermediate gel.
The precipitation pattern obtained when concentrated HCF pool antigens were added to the intermediate gel.

**KEY:**

A - *M. expansa* saline extract was electrophoresed in the first dimension.

B - HCF reference pool was incorporated into the intermediate gel.

C - Agarose mixed with anti-*M. expansa* serum.

**RESULTS**

A total of 11 arcs were elevated.
Fig. 16 The precipitation pattern obtained when *M. expansa* antigens were run against anti *M. expansa* serum without HCF antigens in the intermediate gel.

**KEY:**

A - *M. expansa* saline extract electrophoresed in the first dimension.

B - Blank agarose in the intermediate gel.

C - Anti *M. expansa* serum mixed with agarose.

**RESULTS:**

When compared to Fig. 15, it was observed that 11 arcs were elevated in Fig. 15.
Fig. 17 The precipitation pattern obtained when anti-M.expana serum was added to the intermediate gel between HCF antigens and the homologous antiserum.
Fig. 17 The precipitation pattern obtained when anti-M. expansa serum was added to the intermediate gel between HCF antigens and the homologous antiserum.

KEY:

A - HCF pool electrophoresed in the first dimension.

B - Anti-M. expansa serum mixed with agarose.

C - SA-SHCF mixed with agarose.

RESULTS:

Arcs 2, 5, 7, 8, 9, and 10 were pulled down. Arc 11 was also slightly pulled down. The right leg of arc 4 was shifted to the left.
4.10.2 *C. tenuicollis*

Electrophoresis of HCF against anti-*C. tenuicollis* showed that at least five antigens were recognised by anti-*C. tenuicollis* serum. When *C. tenuicollis* antigens were incorporated into the intermediate gel (Fig. 18), partial identity was observed with antigen 1 and complete identity with antigen 5. Arcs 4, 8, 9 and 10 were shifted upwards while arc 6 is completely missing. There were horizontal bands running across the slide just above the interface of the intermediate gel and the top gel. Figure 19 shows that the anti-*C. tenuicollis* serum lowered the positions of arcs 1, 2, 5, 6, 7 and 8 while the legs of arcs 3 and 13 were extended downwards. Antigens 11 and 12 have been absorbed out. By the use of a higher concentration of anti-*C. tenuicollis* serum in the intermediate gel, arcs 9 and 10 were lowered. The presence of anti-antigen 4 serum in the intermediate gel lowered one arc and similarly antigen 5 was found to be present. When *C. tenuicollis* antigens were run against the reference sheep anti-sheep HCF serum (822) a total of eight arcs were seen.

4.10.3 *S. hepatica*

When HCF was run against anti-*S. hepatica* serum, two arcs were seen. On incorporating *S. hepatica*
Fig. 18 The precipitation pattern obtained when 20% w/v *C. tenuicollis* was added to the intermediate gel between HCF and SA-SHCFS.
The precipitation pattern obtained when 20% w/v C. tenuicollis was added to the intermediate gel between HCF and SA-SHCFS.

**KEY:**

A - HCF pool electrophoresed in the first direction.

B - Twenty percent w/v C. tenuicollis fluid was mixed with agarose.

C - SA-SHCFS mixed with agarose.

**RESULTS:**

A spur of partial identity was observed with antigen 1, and complete identity with antigen 5. Arcs 4, 8, 9 and 10 were elevated and horizontal lines were observed in the top gel.
Fig. 19 The precipitation pattern obtained when goat anti-\textit{C. tenuicollis} was added to the intermediate gel between HCF and SA-SHCFS.
The precipitation pattern obtained when goat anti-C. tenuicollis was added to the intermediate gel between HCF and SA-SHCF.

**KEY:**

A - HCF pool electrophoresed in the first dimension run.

B - Agarose containing anti-C. tenuicollis.

C - Agarose containing anti serum to SHCF.

**RESULTS:**

Arrows 1, 2, 3, 5, 6, 7 and 8 were lowered and the legs of arcs 3 and 13 extended further down. Antigens 11 and 12 seemed to have been absorbed out.
saline homogenate into the intermediate gel (Fig. 20) other arcs, except arcs 4, 5 and 10, disappeared from the pattern. The peak of arc 4 was shifted upwards. When anti-\textit{S. hepatica} serum was incorporated into the intermediate gel (Fig. 21) arcs 1 and 4 showed no change. The positions of arcs 2, 5 and 10 were slightly lowered. The monospecific anti-antigen 5 did not recognise that antigen but antigen 4 was recognised by its monospecific serum. Figure 22 shows that \textit{S. hepatica} saline extract had at least five antigens which could be recognised by the reference 822 serum.

4.10.4 \textit{T. saginata}

On incorporation of a \textit{T. saginata} homogenate into the intermediate gel (Fig. 23) arcs 2, 3, 7, 11, 12 and 13 disappeared. An arc suspected to be arc 6 showed cross reaction and the precipitin line was prominently stained. On using hyperimmune anti-\textit{T. saginata} serum in the intermediate gel, positions of arcs 2, 3, 8, to 13 were lowered. The only arcs that seemed not to have been influenced were arcs 1, 4 and 5. Arcs 6 and 7 might have been superimposed on other arcs. When the monospecific antisera against antigens 4 and 5 were incorporated into the intermediate gel both antigens were found to be present. Figure 24 in which \textit{T. saginata} was run against anti-HCF shows that the serum used against \textit{T. saginata} was not specific.
Fig. 20 The precipitation pattern obtained when a *S. hepatica* saline extract was added to the intermediate gel between HCF and SA-SHCFS.
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Fig. 20 The precipitation pattern obtained when a S. hepatica saline extract was added to the intermediate gel between HCF and SA-SHCFS.

KEY:

A - HCF pool was electrophoresed in the first dimension run.

B - Agarose mixed with a saline extract of S. hepatica.

C - Agarose mixed with SA-SHCFS.

RESULTS:

Aracs 4, 5 and 10 remained.
Fig. 21 The precipitation pattern obtained when anti-\textit{S. hepatica} serum was added to the intermediate gel between HCF and SA-SHCFs.
Fig. 21  The precipitation pattern obtained when anti-
\textit{S. hepatica} serum was added to the intermediate gel between HCF and SA-SHCFS.

\textbf{KEY:}

A - HCF pool electrophoresed in the first dimension run.

B - Agarose mixed with anti-\textit{S. hepatica} serum.

C - Agarose mixed with SA-SHCFS.

\textbf{RESULTS:}

Arcs 2, 5 and 10 were pulled down.
Fig. 22 The precipitation pattern obtained when a *S. hepatica* saline extract was electrophoresed against SA-SHCFS with a blank intermediate gel.
Fig. 22 The precipitation pattern obtained when a *S. hepatica* saline extract was electrophoresed against SA-SHCFS with a blank intermediate gel.

**KEY:**

A - *S. hepatica* saline extract was electrophoresed in the first direction.

B - Blank agarose.

C - Agarose mixed with SA-SHCFS.

**RESULTS:**

Five antigens from *S. hepatica* were recognised by SA-SHCFS.
Fig. 23 The precipitation pattern obtained when a *T. saginata* saline extract was added to the intermediate gel between HCF and SA-SHCFS.
The precipitation pattern obtained when a *T. saginata* saline extract was added to the intermediate gel between HCF and SA-SHCFS.

**KEY:**

A - HCF pool electrophoresed in the first direction run.

B - *T. saginata* saline extract was added to agarose.

C - SA-SHCFS added to agarose.

**RESULTS:**

Arc 6 formed a peak on an otherwise horizontal line.
Fig. 24 The precipitation pattern obtained when a *T. saginata* saline extract was run against HCF antibodies.
Fig. 24 The precipitation pattern obtained when a *T. saginata* saline extract was run against HCF antibodies.

**KEY:**

A - *T. saginata* saline extract electrophoresed in the first direction run.

B - Blank agarose.

C - SA-SHCFS mixed with agarose.

**RESULTS:**

Twelve precipitin bands were observed, indicating that 12 *T. saginata* antigens were recognised by SA-SHCFS. These antigens could not be matched with reference HCF antigens.
4.10.5 **C. bovis**

When HCF antigens were run against anti-**C. bovis** serum, three weak arcs were seen. On incorporation of a whole extract of **C. bovis** into the intermediate gel 10 arcs disappeared leaving only arcs 4, 5 and 10 (Fig. 25). However, the positions of these remaining three arcs had been shifted upwards. When a hyperimmune anti-**C. bovis** serum was incorporated into the intermediate gel (Fig. 26), the positions of eight arcs were lowered. These were arc numbers 1, 2, 3, 7, 8, 9, 10 and 13. The right "feet" of arcs 4 and 5 were pushed to the left. By the use of the monospecific sera in the intermediate gels, antigens 4 and 5 were found to be present. When **C. bovis** antigens were run against anti-HCF, four arcs were seen.

4.10.6 **H. contortus**

When HCF was electrophoresed against rabbit anti-**H. contortus**, no arc was observed, but when a crude **H. contortus** saline extract was run against sheep anti-HCF, one broad band appeared in the top gel but no other arcs were detected (Fig. 27). On incorporating the **H. contortus** extract to the intermediate gel, the position of arc 4 was shifted upwards (Fig. 28). When anti-**H. contortus** serum was added to the intermediate gel, arc 4 was retained. Monospecific
Fig. 25  The precipitation pattern obtained when a *C. bovis* saline extract was added to the intermediate gel between HCF and SA-SHCF.
Fig. 25  The precipitation pattern obtained when a C. bovis saline extract was added to the intermediate gel between HCF and SA-SHCFS.

**KEY:**

A - HCF pool.

B - Whole C. bovis homogenate.

C - SA-SHCFS mixed with agarose.

**RESULTS:**

Ten HCF reference arcs had disappeared completely leaving arcs 4, 5 and 10 only. The positions of these arcs were elevated.
Fig. 26 The precipitation pattern obtained when anti-\textit{C. bovis} was added to the intermediate gel between HCF pool and SA-SHCFS.
Fig. 26  The precipitation pattern obtained when anti-
C. bovis was added to the intermediate gel
between HCF pool and SA-SHCFS.

KEY:

A - HCF pool was electrophoresed in the
first direction.

B - Anti-C. bovis serum was added to the
intermediate gel.

C - SA-SHCFS mixed with agarose.

RESULTS:

Anti-C. bovis lowered the position of arcs
1, 2, 3, 7, 8, 9, 10 and 13. The right legs
of arcs 4 and 5 were slightly pushed to the
left.
Fig. 27 The precipitation band obtained when a *H. contortus* saline extract was run against SA-SHCF5 and a blank intermediate gel.
Fig. 27  The precipitation band obtained when a 
H. contortus saline extract was run against 
SA-SHCFS and a blank intermediate gel.

KEY:
A - H. contortus saline extract was electrophoresed in the first direction run.
B - Blank agarose.
C - SA-SHCFS mixed with agarose.

RESULTS:
A soluble H. contortus antigen was recognised by anti-HCF serum.
Fig. 28 The precipitation pattern obtained when a saline extract of *H. contortus* was added to the intermediate gel between HCF antigens and SA-SHCFS.
Fig. 28 The precipitation pattern obtained when a saline extract of H. contortus was added to the intermediate gel between HCF antigens and SA-SHCS.

KEY:

A - HCF pool was electrophoresed in the first direction.

B - A H. contortus saline extract was added to the intermediate gel.

C - SA-SHCS mixed with agarose.

RESULTS:

The position of arc 4 was elevated but only slightly. Arcs 6, 7 and 12 disappeared.
anti-antigen 4 serum was able to retard a thick arc, but antigen 5 serum did not pull down any arc.

4.10.7 *F. gigantica*

Anti-*F. gigantica* antibodies were not able to detect any HCF antigens. On incorporating a *F. gigantica* saline extract into the intermediate gel, the positions of the arcs were not raised. On incorporating anti-*F. gigantica* antiserum into the intermediate gel (Fig. 29) there was no change in the position of the arcs. Also *F. gigantica* antigens were not detected by anti-HCF serum. When HCF antigen were incorporated into the intermediate gel between *F. gigantica* antigens and anti HCF serum, horizontal bands appeared but they did not form any peaks.

4.10.8 *O. radiatum*

When HCF antigens were run against anti *O. radiatum* serum, five arcs were seen. Of these arcs, one arc formed a broad vertical band whose peak had run out of the gel. On incorporating *O. radiatum* antigens into the intermediate gel (Fig. 30), arc 4 was significantly shifted upwards. Arcs 7 and 8 were missing. When anti-*O. radiatum* serum was placed in the intermediate gel, arc 6 was lowered. The "feet" of arc 4 extended deep down into the intermediate gel (Fig. 31). Monospecific antiserum against antigen 4 detected the presence of weak antigen 4;
Fig. 29 The precipitation pattern obtained when anti-\textit{F. gigantica} serum was added to the intermediate gel between HCF pool antigens and SA-SHCFS.
The precipitation pattern obtained when anti-F. gigantica serum was added to the intermediate gel between HCF pool antigens and SA-SHCFS.

**KEY:**

A - HCF pool was electrophoresed in the first direction run.

B - Anti-F. gigantica serum was added to the intermediate gel.

C - SA-SHCFS mixed with agarose.

**RESULTS:**

None of the HCF was recognised by the anti-F. gigantica serum.
Fig. 30  The precipitation pattern obtained when a saline extract of _O. radiatum_ was added to the intermediate gel between HCF pool antigens and SA-SHCFS.
Fig. 30 The precipitation pattern obtained when a saline extract of *O. radiatum* was added to the intermediate gel between HCF pool antigens and SA-SHCFS.

**KEY:**

A - HCF pool antigens were electrophoresed in the first dimension run.

B - A saline extract of *O. radiatum* was added to the intermediate gel.

C - SA-SHCFS mixed with agarose.

**RESULTS:**

Arc 4 was significantly elevated.
Fig. 31 The precipitation pattern obtained when rabbit anti- *O. radiatum* was added to the intermediate gel between HCF antigens and SA-SHCFs.

**KEY:**

A - HCF pool antigens were electrophoresed in the first dimension run.

B - Rabbit anti-*O. radiatum* was added to the immediate gel.

C - SA-SHCFs mixed with agarose.

**RESULTS**

Arcs 6 and 7 were pulled down and the legs of arc 4 extended deep into the intermediate gel.
however antigen 5 was not detected by the monospecific antiserum to it. When *O. radiatum* antigens were run against the reference anti HCF serum, no arcs were seen. When HCF was incorporated between *O. radiatum* and anti-HCF serum, one antigen from HCF formed a bump (peak) on an otherwise horizontal band.

### 4.10.9  *T. vulpis*

When HCF antigens were run against anti-*T. vulpis* serum, no arcs were observed. On incorporating *T. vulpis* extract into the intermediate gel, arcs 1, 4 and 9 were shifted upwards (Fig. 32). When anti-*T. vulpis* serum was incorporated between HCF and anti-HCF, the feet of arc 4 extended deep into the intermediate gel (Fig. 33). Monospecific antiserum against antigens 4 did not detect the presence of the antigens. When *T. vulpis* was electrophoresed against anti-HCF serum no arcs were observed. When *T. vulpis* extract was electrophoresed against anti-HCF serum with HCF in the intermediate gel, two bumps were seen. It was difficult to tell whether these were artefacts or genuine arcs.

### 4.10.10  *A. suum*

When HCF was electrophoresed against anti-*A. suum*, three arcs were seen (Fig. 34). The incorporation of an extract of *A. suum* into the intermediate
The precipitation pattern obtained when a saline extract of *T. vulpis* was added to the intermediate gel between HCF pool antigens and SA-SHCFS.

Fig. 32
The precipitation pattern obtained when a saline extract of T. vulpis was added to the intermediate gel between HCF pool antigens and SA-SHCFS.

**KEY:**

A - HCF pool antigens were electrophoresed in the first direction run.

B - Agarose mixed with T. vulpis saline extract.

C - SA-SHCFS mixed with agarose.

**RESULTS:**

Arcs 1, 4 and 9 were elevated.
Fig. 33 The precipitation pattern obtained when rabbit anti-\textit{T-vulpis} serum was added to the intermediate gel between HCF pool antigens and SA-SHCFS.
Fig. 33 The precipitation pattern obtained when rabbit anti-\textit{T. vulpis} serum was added to the intermediate gel between HCF pool antigens and SA-SHCFS.

\textbf{KEY:}

A - HCF pool antigens were electrophoresed in the first direction run.

B - Rabbit anti-\textit{T. vulpis} serum was added to the intermediate gel.

C - SA-SHCFS mixed with agarose.

\textbf{RESULTS:}

The legs of arc 4 extended deep into the intermediate gel and did not spread out.
Fig. 34 The precipitation pattern obtained when HCF pool antigens were run against rabbit anti-A. suum.
Fig. 34 The precipitation pattern obtained when HCF pool antigens were run against rabbit anti-\textit{A. suum}.

**KEY:**

A - HCF pool antigens were electrophoresed in the first dimension run.

B - Blank agarose.

C - Rabbit anti-\textit{A. suum} serum mixed with agarose.

**RESULTS**

Three precipitin lines were seen.
gel did not raise any of the arcs (Fig. 35) and neither did the presence of the anti- _A. suum_ serum in the intermediate gel lower any arcs. Both antigens 4 and 5 were not present. _A. suum_ antigens were not detected by anti-HCF serum. When HCF antigens were incorporated into the intermediate gel, no bump was seen in the horizontal bands formed by HCF antigens.

**4.10.11 P. microbothrium**

When HCF was electrophoresed against anti- _P. microbothrium_ serum, two arcs were seen. The incorporation of a _P. microbothrium_ saline extract into the intermediate gel left only arcs 1, 4, 5, 9 and 10 untouched (Fig. 36). When HCF antigens were incorporated into the intermediate gel, (Fig. 37) two arcs showed bumps.

**4.10.12 A. centripunctata**

In Fig. 38, arc 7 shows that there is cross-reaction between antigen 7 of HCF with another antigen originating from _A. centripunctata_. In the same figure arc 2 has shifted its position upwards while arcs 6, 8 to 13 have all disappeared. The positions of arcs 1, 4 and 5 seem not to have been changed. Figure 39 shows two arcs depicting typical cross-reacting antigens number 2 and 7. Another horizontal line does not show the peaks seen with arcs 2 and 7. Anti-antigen 5 serum did form an arc with _A. centripunctata_. 
Fig. 35  The precipitation pattern obtained when a saline extract of *A. suum* was added to the intermediate gel between HCF pool antigens and SA-SHCFs.
Fig. 35  The precipitation pattern obtained when a saline extract of *A. suum* was added to the intermediate gel between HCF pool antigens and SA-SHCFS.

**KEY:**

A - HCF pool antigens were electrophoresed in the first dimension run.

B - A saline extract of *A. suum* added to the intermediate gel.

C - SA-SHCFS mixed with agarose.

**RESULTS**

Six arcs disappeared. The remaining 7 arcs were not elevated.
The precipitation pattern obtained when a saline extract of *P. microbothrium* was added to the intermediate gel between HCF pool antigens and SA-SHCFs.
Fig. 36  The precipitation pattern obtained when a saline extract of *P. microbothrium* was added to the intermediate gel between HCF pool antigens and SA-SHCFS.

**KEY:**

A - HCF pool antigens were electrophoresed in the first dimension run.

B - A saline extract of *P. microbothrium* was added to the intermediate gel.

C - SA-SHCFS was mixed with agarose.

**RESULTS**

Arcs 1, 4, 5, 9 and 10 were not elevated. Other reference arcs disappeared.
Fig. 37 The precipitation pattern obtained when *P. microbothrium* antigens were run against SA-SHCFS with HCF pool antigens in the intermediate gel.
The precipitation pattern obtained when P. microbothrium antigens were run against SA-SHCFS with HCF pool antigens in the intermediate gel.

**KEY:**

A - A saline extract of P. microbothrium was electrophoresed in the first dimension run.

B - HCF pool antigens were mixed with agarose in the intermediate gel.

C - SA-SHCFS was mixed with agarose.

**RESULT:**

Two horizontal lines showed definite peaks.
Fig. 38 The precipitation pattern obtained when HCF pool antigens were electrophoresed against SA-SHCFS.
The precipitation pattern obtained when HCF pool antigens were electrophoresed against SA-SHCFS.

**KEY:**

A - HCF pool antigens were electrophoresed in the first direction run.

B - A saline extract of *A. centripunctata* was mixed with agarose.

C - SA-SHCFS mixed with agarose.

**RESULTS:**

Arc 7 formed a peak on a horizontal line. Arc 2 was elevated but arcs 1, 4 and 5 were not affected.
The precipitation pattern obtained when A. centripunctata antigens were SA-SHCFS while the intermediate HCF pool antigens.

**KEY:**

A - A saline extract of A. centripunctata was electrophoresed in the intermediate gel agarose.

B - HCF pool antigens were added to intermediate gel agarose.

C - SA-SHCFS mixed with agarose.

**RESULTS:**

Arcs 2 and 7 showed peaks on horizontal line. Another horizontal line did not
antigens. An antiserum against this parasite was not available.

4.10.13 *A. galli*

A crude saline extract of *A. galli* incorporated into the intermediate gel did not raise the positions of any of the HCF arcs. When this extract was electrophoresed against polyvalent sheep anti-HCF serum, no arcs were seen. The monospecific antisera against antigens 4 and 5 of HCF did not detect the presence of these antigens. An antiserum against this parasite was not available.

4.10.14 *B. phlebotomum*

A crude saline extract of *B. phlebotomum* incorporated into the intermediate gel did not raise the positions of any arcs. When this extract was run against polyvalent anti-HCF serum, no arcs were seen too. The monospecific antisera against antigens 4 and 5 of HCF did not detect the presence of these antigens.

An antiserum against this parasite was not available.

4.10.15 *S. lupi*

The incorporation of *S. lupi* antigens into the intermediate gel shifted upwards the position of arc 4 only (Fig. 40). When a *S. lupi* extract was
Fig. 40 The precipitation pattern obtained when HCF pool antigens were run against SA-SHCFS while the intermediate gel contained a saline extract of \textit{S. lupi}. 
The precipitation pattern obtained when HCF pool antigens were run against SA-SHCFS while the intermediate gel contained a saline extract of *S. lupi*.

**KEY:**

A - HCF pool was electrophoresed in the first dimension run.

B - A *S. lupi* saline extract was added to the intermediate gel.

C - SA-SHCFS was mixed with agarose.

**RESULTS:**

Arc 4 was elevated significantly. Arcs 3 and 8 were elevated only slightly.
run against anti-HCF serum, no arcs were observed. When monospecific antisera were incorporated into the intermediate gel between *S. lupi* and anti-HCF serum, both antigens 4 and 5 were found to be present. An antiserum against this parasite was not available.

4.11 DETECTION OF ANTIBODIES BY CIEP IN SERA OF NATURALLY INFECTED ANIMALS

Figure 41 shows that CIEP was able to detect two antibodies produced by a goat naturally infected with hydatid disease. The two precipitin lines shown could not be identified with any of the lines on the reference pattern. Of 44 known hydatidosis cases, only 3 cases showed a precipitin line on CIEP.
Fig. 41 The precipitation pattern obtained when HCF pool antigens were run against serum from a goat naturally infected with hydatidosis.
The precipitation pattern obtained when HCF pool antigens were run against serum from a goat naturally infected with hydatidosis.

**KEY:**

A - HCF pool antigens were electrophoresed in the first direction run.

B - Blank agarose.

C - Serum from a goat naturally infected with hydatidosis.

**RESULTS:**

Two precipitin lines were observed. However, the lines could not be identified with any arcs of the reference pattern.
4.12 RESULTS OF ELISA

A total of 180 serum samples from cattle with or without hydatid disease were examined by ELISA using two purified antigens. The results are shown in Table 9.

Table 9: Four-fold classification table to analyse the sensitivity, specificity and predictive value of ELISA using Antigens 4 and 5.

<table>
<thead>
<tr>
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<th>+</th>
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<th>Total</th>
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<tr>
<td>+</td>
<td>127</td>
<td>15</td>
<td>142</td>
</tr>
<tr>
<td>-</td>
<td>3</td>
<td>35</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>50</td>
<td>180</td>
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</table>

Sensitivity = \( \frac{127}{130} \times 100 = 97.7\% \ (98\% \)

Specificity = \( \frac{35}{50} \times 100 = 70\% \)

Predictive value = \( \frac{127}{142} \times 100 = 89.4\% \)

Using both antigens 4 and 5, the sensitivity was 98% and the specificity was 70% giving 30% false positive and 2% false negatives. In one case of false positive, the animal had fascioliasis. The predictive value of the test was 89%.
Control of hydatid disease is aimed at prevention of dogs from gaining access to raw offal and the reduction of the parasite biomass either by reducing the dog population or by mass dog-treatment programs (Gemmell, 1979). This approach is faced with problems as some dog owners may not co-operate.

Probably a more effective approach to control would be to eliminate the source of infection to dogs. Well managed abattoirs can play an important positive role in the control and eradication of hydatidosis by disposing of the larval cysts, thereby interrupting the life cycle of the disease. However palpation and incision cannot expose the tiny cysts which, if fertile, might be consumed by a carnivore to perpetuate the disease. If an intermediate host could be diagnosed as having fertile cysts before slaughter, it would be possible to treat such selected animals and dispose the organs of predilection so that such organs would have no chance of being eaten by a carnivore. This way the disease could be eradicated. Detection of such animals has been hampered by the lack of sensitive and specific methods of diagnosing hydatidosis in animals prior to their slaughter (Schantz, 1973).

Advances in the immunodiagnosis of hydatid disease in man (Farag et al., 1975) suggest that hydatidosis in livestock too can be detected by immunodiagnostic technics. A major drawback to immunodiagnostic tests in hydatidosis of livestock is the presence of
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other parasites that possess antigens that cross react with hydatidosis. This would lead to a high rate of false positives with an accompanying wastage in drugs during treatment (when such a drug becomes available) or waste of time in the subsequently unjustified search for non-existent hydatid cysts.

Lopez-Lemes and Varela-Diaz (1975) postulated that only the isolation of a purified *E. granulosus* specific antigen would render tests useful for immunodiagnosis of hydatid disease. Farag et al. (1975) utilised ELISA, a fairly sensitive test, in the diagnosis of hydatid disease in man. They found that when whole HCF was used there was cross reactivity with sera from patients infected with fascioliasis. They suggested that the cross reactivity was due to the existence of a lipoprotein antigen common to many helminths. When a purified antigen 5 was used, Farag et al. (1975) found ELISA to be highly sensitive and specific. This shows that there is a need to study the exact antigenic composition of hydatid cyst fluid and find out which particular antigens do or do not cross react with common livestock parasites.

Gel-diffusion technics have been used greatly to analyse the antigens in parasites. These methods can give precise and reliable information regarding the antigens being analysed. To analyse the HCF antigens, crossed immunoelectrophoresis was found to be an effective tool. Weeke (1973), commenting on
other parasites that possess antigens that cross react with hydatidosis. This would lead to a high rate of false positives with an accompanying wastage in drugs during treatment (when such a drug becomes available) or waste of time in the subsequently unjustified search for non-existent hydatid cysts.

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the technic, said that it enables one to study the correlation between the protein changes, to discover new protein changes and to re-examine the protein pattern later on. Closs et al. (1975) used CIEP with intermediate gel to study antigenic cross reactivity between mycobacteria species. Mycobacterial (BCG) antigen and its corresponding antibody were added separately to the intermediate gel of M. lepraeurium reference system. They found the method to be well suited for studying cross reactions between mycobacteria.

Crossed immunoelectrophoresis performed as described in section 3.3.3 was used to characterise the soluble antigens present in HCF. Crude HCF was used as the source of antigens based on previous reports by Williams et al. (1971) and Pozzuoli et al. (1974) who demonstrated that the sensitivity of their tests was considerably decreased if fractionated HCF was used. This technic was used to study cross reactions between HCF antigens and 15 other parasites.

A total of 13 bands as shown in Fig. 1 were observed in our test. Analysis of sheep, goat and cattle HCF on CIEP has not been reported. Ardehali et al. (1977), who compared CIEP, counter immunoelectrophoresis and double diffusion tests in diagnosis of human hydatid disease, found that there were at most four bands. Other reports on the number of arcs seen refer to analysis using immunoelectrophoresis method.
(Chordi and Kagan, 1965; Varela-Diaz et al., 1974; Conder et al., 1980). Even then the results have been very inconsistent inspite of using the same antigen pool (Varela-Diaz et al., 1977b; 1978). The same variation in the number of bands seen was observed in our tests. An effort was always made to use a pool which gave the maximum number of bands but still some bands were not always reproducible. Closs et al. (1975) suggested that the sensitivity of CIEP could be enhanced by using more concentrated reagents but there is a limit as to how many bands could be conveniently studied and 13 was considered a fair number in view of the size of the plate used. Perhaps with refinement of technics, more bands might be identified.

In our experimental conditions, all bands of HCF antigen detected by CIEP were in the anodic side of the antigen well. Similar results were observed by Varela-Diaz et al. (1975a) who found that all bands revealed by both hydatid and non-hydatid sera were in the anodic portion of the antigen well. This gave us the freedom to slice the strip from the first dimensional run close to the antigen well thereby increasing the length of the strip so that all the antigens were included in the microtechnic plate.

The use of CIEP by clinicians has its own limitations. Ardehali et al. (1977) found that there were differences in the pattern of arcs seen when two
different human hydatidosis patients sera were tested against the same source of antigen. They also demonstrated differences between precipitation patterns of a single patient's serum with human or sheep HCF. Also the widespread application of CIEP would be limited by the requirement of a concentrated hydatid cyst fluid antigen and relatively costly and sophisticated equipments which may not be within the reach of particularly rural hospitals or veterinary investigation laboratories where hydatidosis is endemic.

Another obstacle in this test is the need to concentrate test serum. Whereas a higher proportion of the serum could be incorporated into the top gel before it was poured on the plate there is a limit as to how much serum can be added otherwise the agarose might not gel unless a high percentage of agarose was used.

To overcome the problem of concentrating serum in IEP, Coltorti and Varela-Diaz (1975) repeatedly filled the troughs with the serum for three times during the development of the slides. This approach was impracticable in CIEP since the gel had already set.

In our experience we found CIEP to be an effective method for studying complex antibody-antigen systems but it was too complicated for clinical routine use as it required experience in the analysis of the
results. Despite changes in area and position, the individual precipitates as well as the overall pattern must be learnt for each test antiserum. Ardehali et al. (1977) examined 19 sera and each of the sera examined on CIEP showed a different precipitin pattern. In our experience, none of the naturally infected animal sera (Fig. 41) showed a precipitin pattern similar to the reference pattern.

In our test on sera from naturally infected animals, IEP was unable to detect any positive sera. Crossed immunoelectrophoresis and double diffusion showed very poor sensitivity. The sensitivity of immunodiagnostic tests for hydatid disease has been reported to vary with the location and physical condition of the cysts (Capron et al., 1970). Recently ruptured cysts induce intense immunological response (Yarzabal et al., 1974) while patients with hyaline or calcified cysts show no or very little antibody production (Williams et al., 1971). The animals sent for slaughter which provided the sera that we tested were generally less than 3 years old and the cysts tended to be small and intact. This might explain the poor sensitivity observed with these tests on the slaughter animal sera.

Variations in test sensitivity and specificity have been associated with variations in the source of the antigen used. Pauluzzi et al. (1971) suggested
the use of sheep liver hydatid cyst fluid. But as shown in the literature review other workers have used HCF from other sources and found it satisfactory (Yarzabal et al., 1975; Ardehali et al., 1977; Dada et al., 1980). As the main HCF antigens were found to be antigens 4 and 5, an effort was made to determine whether there was a variation as regards source of the fluid in relation to the presence of antigens 4 and/or 5. The different HCF from separate cysts was concentrated 15 times to ensure that even small traces of any of the antigens would form a precipitin arc.

This HCF was tested against sheep anti-sheep HCF serum. The results (Table 4) showed that it would be better to use HCF originating from sheep and goat liver for all such sources showed the presence of these two antigens and none contained either of the antigens 4 or 5 alone. Sheep and goat lung cysts were also satisfactory sources of antigens 4 and 5. Whereas antigen 5 was absent in 13% of the lung cysts, antigen 4 was always present when antigen 5 was present. Cattle liver and lung HCF was of poorer quality. Table 4 shows that although sheep liver cyst was the best source of HCF when studying the full spectrum of antigens, should the interest be for antigen 4 alone, cattle lung HCF would be a better source since 50% of the cysts of cattle lung contained antigen 4 alone as compared to sheep or goat liver where antigen 4 did not occur alone.
Depending on whether protoscoleces were or were not found in HCF, the cysts were classified as fertile or sterile. Table 5 shows that fertile cysts from cattle, sheep and goats had a higher rate of occurrence of both antigens 4 and 5 together. It was also observed that cattle non-fertile cysts often contained antigen 4 alone than for both antigens unlike sheep and goat non-fertile cysts which continued to show a higher occurrence of both antigens. This observation becomes significant when one wants to isolate antigen 4 alone in which case cattle sterile cysts fluid would be a better source of the antigen since chances of contamination with antigen 5 or other antigens are small. It would also mean that if in immunodiagnosis only arc 4 was found in the patients serum, such an animal might be harbouring a sterile cyst or that the infection is recent since other results showed that laboratory animals immunized intramuscularly responded to antigen 4 soon after immunization. However, in experimental oral infection, it took 120 days for the first tests to be positive (Conder et al., 1980) and even then the authors did not state whether arc 4 had appeared before or after arc 5 since the test positivity was based on arc 5 alone.

A test for the optimum duration of the first dimensional run was attempted. There was an association observed between the duration of electrophoresis in the
first dimension and the shape of the precipitin arcs formed. The optimal time for the first electrophoresis was found to be 45-50 minutes at which time all the 13 arcs were separated out well but were still within the 50mm x 50 mm microtechnic slides. These results are similar to those observed by Guisantes et al. (1975) who found the appearance of arc 5 to be most characteristic after 90 minutes of electrophoresis. However by that time their dye had migrated 33 mm. In our experiments we found that after 45 minutes the dye had migrated 23 mm anodically. Guisantes et al. (1975) suggested that the migration of antigen 5 may also be standardized in terms of distance migrated by the dye.

However, in a complex mixture of antigens it would be difficult to tell which antigen was migrating with the dye. A relative electrophoretic migration relationship between the antigens in HCF was calculated based on the more reliable antigen 5. The average electrophoretic migration of this antigen was found to be 20 mm which was only 5 mm slower than the BSA marker. This was useful in that all we had to do to follow the progress of the electrophoresis was to track the marker and stop the run when the marker was halfway through the plate.

Having analysed the antigens present in HCF, agar gel diffusion methods were then used to study the cross reactions between HCF antigens and
antigens originating from other common animal parasites. When an animal is injected with a mixture of antigens, it produces distinct antibodies to each of the constituent antigens and such antibodies generally react only with the homologous antigen except if cross reactions occur with substances other than the homologous antigen (Kabat and Mayer, 1961). Such cross reacting antigens are assumed to possess similar structural groupings within their molecule. Thus cross reactivities pose a problem in immunodiagnosis.

On double diffusion test, it was possible to detect cross reactions between at least one HCF antigen and antigens originating from *C. tenuicollis*, *C. bovis*, *M. expansa*, *T. saginata*, *A. centripunctata*, and *S. hepatica*. Cross reactivity was considered to be present when an arc was observed. To a large extent, these results agree with those of Njeruh (1981) except for two parasites, namely *O. radiatum* and *S. hepatica*. Whereas Njeruh (1981) found *O. radiatum* to contain antigen 4, our double diffusion test could not detect antigen 4 in the parasite. On the other hand, our test showed *S. hepatica* to contain antigen 4 but Njeruh (1981) had not been able to detect the antigen in this parasite.
When soluble or particulate antigens and their homologous immune sera react, all the antibody may be removed by precipitation or agglutination with the antigen (Kabat and Mayer, 1961). On exhaustively absorbing an antiserum with heterologous antigen it is usually found that only part of the antibody reacts and that a portion left in the supernatant is only capable of reacting with the homologous antigen (Kabat and Mayer, 1961).

Absorption of anti-HCF serum with crude parasite homogenates either in solution or on activated CNBR-Sepharose 4B to remove any antibodies which might be present in the anti HCF serum and happen to be recognised by other parasite antigens was attempted. Generally, although some antibodies were absorbed out antibodies to antigen 4 tended to remain even after the absorption with a parasitic extract which had been shown to contain the antigen. This may be due to the presence of a lower concentration of the antigen in the parasite as compared to the level of the antibody in the hyperimmune serum so that even after all the antigen had been used up, some antibody activity still remained.

The use of immunoelectrophoresis in the diagnosis of hydatid disease was initiated by Capron et al. (1967) who found what they considered to be a
specific arc indicative of hydatidosis in humans. Further work by Capron et al. (1970), Yarzabal et al. (1974, 1975) and Varela-Diaz et al. (1975b) supported the earlier claim to specificity of the arc.

However, this antigen was found in E. multilocularis (Rickard et al., 1977), T. hydatigena (Varela-Diaz et al., 1977a), T. solium (Yarzabal et al., 1977; Schantz et al., 1980) and T. Ovis (Yong and Heath, 1979). This reduced the usefulness of arc 5 in the diagnosis of hydatidosis.

This research was aimed at recognising all the parasite antigens present in HCF and finding if any of those antigens was specific to hydatid disease or one that will not cross react with a large number of the parasites found in livestock in Kenya. To achieve this, crossed immunoelectrophoresis was found to be the appropriate method. The use of antigens and antisera in the intermediate gel as postulated by Axelsen et al. (1973) and applied by Closs et al. (1975) to study cross reactions between mycobacteria was adopted as the test method so that each arc could be identified. The diagrams in Fig. 42 reproduced from Closs et al. (1975) were used to assist in the analysis of the effect of adding cross reacting antigen or the corresponding antibody to the intermediate gel in CIEP method.
Analysis of the effect of adding cross-reacting antigen (AgX) or the corresponding antibody (AbX) to the intermediate gel in crossed immunoelectrophoresis.

I. The reference antibody (AbA) is directed against many determinants on the reference antigen (AgA). A. The reference system. B & C. AgA and AgX are immunologically identical; AbA cannot differentiate between them and AbX contains antibody against AgA in high concentrations. D & E. Partial identity between AgA and AgX; AbX contains antibody against AgA in low concentrations. F & G. Partial identity between AgA and AgX, but only AbX contains antibody against the common determinants. H & I. AgA and AgX have only one determinant in common.

II. The reference antibody is directed against only two determinants on the reference antigen. A. The reference system. J & K. The patterns of cross-reaction. Symbols: AgA = \( a_1, a_2, \ldots, a_n \) - the reference antigen contains the determinants \( a_1, a_2, \ldots, a_n \). AbA = \( a_1, a_2, \ldots, a_{m-1} \) - the reference antibody is directed against the determinants \( a_1, a_2, \ldots, a_{m-1} \).
With this method it was possible to study the effect of a test antigen or antiserum on the reference pattern. If a test antigen's structure was not close enough to the reference antigen to form a precipitate, its cross reactivity was tested for by the use of the test antigen in the intermediate gel (Closs et al., 1975) whereby the cross reacting antigen's arc would be elevated. In some cases, the antisera and antigen incorporated into the intermediate gel showed cross reactivity but in some other cases only one of the two reagents showed cross reactivity which necessitated the use of both test antigen and antiserum in the intermediate gel to supplement one another. However, antisera to four parasites was not available and their results were based on the use of the antigen and two monospecific antisera. Since antisera to antigens 4 and 5 were available, the test for these two antigens was conclusive in all parasites.

To supplement the use of these two reagents as recommended by Closs et al. (1975) the test antigen was run into a gel bed containing sheep anti-sheep hydatid cyst fluid serum while the intermediate gel was left blank. In this way it was possible to tell if any relationship exists between the test parasite antigens and HCF although it was not possible to identify the particular antigens that cross reacted,
if at all there was cross reactivity.

HCF was also run against antiserum produced against the test parasite while leaving the intermediate gel blank. In some cases it was possible to see a relationship, but the specific cross reacting antigen could not be identified with certainty. Hydatid cyst fluid was also incorporated into the intermediate gel between test antigens and test homologous antiserum. Again, it would help to see a relationship between the test antigens and HCF antigens without being able to pinpoint specifically the cross reacting antigen.

When an antiserum was used in the intermediate gel, cross reactivity was seen as depression of the cross reacting antigen. The peak's depression was dependent upon the concentration of the antibody added to the intermediate gel. By this technic it was possible to tell the number of antigens which were cross reacting even if cross reactivity was relatively weak.

The larval stage of cestodes has been known to stimulate active immunological responses as it penetrates the intermediate host's tissues (Weinmann, 1966; 1970) which is not the case with the adult worm as it is weakly immunogenic. For those cestodes found in the gastro-intestinal tract e.g. *M. expansa*, *A. centripunctata*, it would be expected that the animal will not be adequately exposed to the parasite...
to produce antibodies in high enough levels to confuse with HCF antibodies. But for *C. bovis*, *C. tenuicollis*, *S. hepatica* and *C. ovis* (Yong and Heath, 1979) this would be a problem because the parasites are in host tissues and would be well exposed to the host's immune system. The level of antibodies to these parasites could be higher than for HCF since it has been shown that the physical status of the cyst membranes affects the level of antigen stimulation of host immune system and thereby influences the success or failure of immuno-diagnostic tests (Yarzabal et al., 1974).

Our results confirm some of the reported cross reactions and add more parasites to the list. The greatest cross reactivity was observed with cestodes. This was not surprising since *E. granulosus* is also a cestode. During the evolution of parasites genera, it was possible that some key proteins in parasites within a group were spared of major changes so as to facilitate their vital function. Such proteins would be expected to show cross reactivity in several parasites. Farag et al. (1975) were of the opinion that cross reactions seen between hydatid disease and other helminthic diseases are due to the presence of a lipoprotein antigen which they suspected to probably be antigen B as it was common to many helminths. Work by Njeruh (1981) showed that the major antigen in HCF was a \( \beta \)-lipoprotein which corresponds to arc 4 in
our reference pattern.

Cross reactions with HCF antigen 4 were observed on CIEP with M. expansa, C. tenuicollis, T. saginata, C. bovis, S. hepatica, A. centripunctata, H. contortus, S. lupi and O. radiatum. This antigen was the most common of all the 13 HCF antigens as it was detected in nine out of 15 parasites being absent in F. gigantica, P. microbothrium, A. galli, T. vulpis, A. suum and B. phlebotomum. These results disagree with those of double diffusion in that H. contortus, S. lupi and O. radiatum did not show cross reactivity with HCF on double diffusion test yet they had shown the presence of arc 4 on CIEP. Njeruh (1981) did not find antigen 4 in S. hepatica, H. contortus or S. lupi using double diffusion. This may indicate that CIEP was superior to DDT in detecting antigen 4.

The next major antigen in HCF was found to be antigen 5. This antigen was present in M. expansa, C. bovis, C. tenuicollis, T. saginata and S. lupi. Its occurrence in HCF was less frequent than for antigen 4 as shown in Table 4. Also due to its presence in other parasites it is not suited for specific immunodiagnosis of this disease either. Varela-Diaz et al. (1975b) postulated that the number of bands other than arc 5 may be of value in cases where the diagnostic arc 5 was absent. In our view, what is needed is a thorough search for another specific arc
as arc 5 can no longer be considered specific for hydatid disease.

Of the nematodes tested, none showed more than two common antigens with certainty. Only *S. lupi* showed the presence of antigen 5 while *H. contortus*, *S. lupi* and *O. radiatum* showed the presence of antigen 4. For this group of helminths, it appears as if the use of antigen 5 would be of value in immunodiagnosis as it was detected in *S. lupi* only which does not exist in livestock. For those with a tissue migratory stage, the antibody level to the migratory larvae may wane as the adult form is reached and since the adult intestinal worms are only weakly immunogenic (Weinmann, 1966), their antibody titres are bound to be low and not be a serious obstacle for the immunodiagnosis of animal hydatidosis. Thus, if livestock in a certain locality are only exposed to nematodes, then CIEP using HCF as the source of antigens is of value in detecting hydatidosis.

Two trematodes were tested. Cross reactivity was not detected with *F. gigantica* but it was detected against *P. microbothrium*. The lack of cross reactivity was similar to that reported by Gathuma et al. (1978) who were unable to detect cross reactivity between *Fasciola* spp and *T. saginata* activated onchospheres.
A general observation can be made that so far no specific HCF antigen can be used for immunodiagnosis of hydatid disease in animals that might be concomitantly infected with *C. bovis*, *C. tenuicollis* *C. ovis* or *S. hepatica*. Although cross reactivity with *C. cellulosae* was not tested for, it would be expected that its antigens would cross react with HCF antigens. However, porcine cysticercosis is not a problem in this country which means that this test might be of value in detecting porcine hydatidosis. Although *T. saginata* showed heavy cross reactivity with HCF, it does not exist in livestock and hence these cross reactivities are of interest with regard to human hydatidosis only.

Antigens 4 and 5 of HCF were highly immunogenic in experimental immunization schedules but in naturally infected animals this was not the case. A more sensitive test, ELISA, was used to detect antibodies against these two antigens. The two antigens were precipitated by phosphotungstic acid and magnesium chloride following a procedure by Burstein (1963) as modified by Lindqvist (1979) and applied by Njeruh (1981). The HCF had been freed of host contaminants by an immunosorbent specific for bovine serum. This preparation was used to coat microtitre ELISA plates.
Enzyme linked immunosorbent assay is a sensitive and simple method for the qualitative test for antibodies or antigens. This test can be conveniently used in epidemiological studies as it can be automated. It has been used in serological diagnosis of hydatid disease in humans (Farag et al., 1975; Iacona et al., 1980). Farag et al. (1975) preferred a purified antigen 5 as it increased the sensitivity and specificity of the test. Njeruh (1981) used antigen 4 alone in ELISA and reported absolute specificity and 91% sensitivity. The present study using both antigens 4 and 5 showed a sensitivity of 98% and a 70% specificity. Iacona et al. (1980), using two antigens (A and B) which correspond to our antigens 5 and 4, respectively, reported a lower sensitivity (86%).

In this study, the use of both antigens 4 and 5 in ELISA was found to be satisfactory in view of the high sensitivity obtained although the specificity was lower than reported by Njeruh (1981) using one of the antigens. This relatively low specificity might be due to incomplete post mortem search for hydatid cysts, or the presence of other cross reacting parasites like C. bovis. In one case of false positive, the animal had fascioliasis as well. Although no cross reactivity was observed with Fasciola spp using CIEP, it is possible that ELISA would be able to detect cross reactions where CIEP could not.
In spite of the observed relatively low specificity, ELISA, using the two major antigens in HCF, remains a useful test in in vitro diagnosis of livestock hydatidosis. However, as Iacona et al. (1980) reported, ELISA in immunodiagnosis of hydatid disease is not an alternative but an addition to serological tests.
CONCLUSIONS

Based on the results obtained in this study the following observations and conclusions were made:

1. Hydatidosis is a problem in Kenya as shown by the post mortem survey carried out in the course of this study in combination with other reports.

2. Using a technic involving electrophoresis in two dimensions, a total of 13 parasitic HCF antigens were defined and given numbers based on their electrophoretic mobility. This means that HCF has at least 13 antigens of parasite origin.

3. Two antigens, numbers 4 and 5, were found to dominate over the other antigens both in the area covered on the glass plate and the intensity of staining with Coumassie Blue dye. Some antigens, especially antigens 6 and 7, were very weak and not always reproducible. The stronger antigens were visible even before the preparation was stained.

4. A search for the major antigens 4 and 5 in different hydatid cysts of three different animal species revealed that goat or sheep liver hydatid cysts were the best source of these antigens. Cattle lung cysts were the poorest sources.

5. A comparison of the frequency of occurrence of antigens 4 and 5 in fertile or sterile cysts revealed that fertile liver and lung cysts from sheep and goats were the best source of the two
antigens. However sterile cattle lung cysts were the best source of antigen 4 if it was the only antigen needed.

Immunelectrophoresis did not detect any circulating antibodies but DD and CIEP showed some positive results, although the sensitivity was very low. The bands seen on CIEP in cases of natural infection did not correspond to any of the bands seen in laboratory raised sera.

At least one of the 13 HCF antigens cross reacted with a cestode and to a lesser extent also with nematodes and *P. microbothrium* but not with *F. gigantica*.

Although some cross reacting adult parasites reside in the body lumen and therefore are weakly immunogenic (Weinmann, 1966; 1970), the test cannot discriminate against tissue larval stages which stimulate a high level of antibodies (Weinmann, 1966; 1970). This test's value is therefore greatly reduced where these cross reacting tissue parasites are present. Unfortunately they are present in this country.

Enzyme-linked immunosorbent assay using antigens 4 and 5 was found to be highly sensitive (98%) and also fairly specific (70%). The predictive value of the test was 89%. It would therefore be of some value in diagnosis of hydatid disease in livestock.
10. In spite of extensive immunization schedules using hydatid cyst constituents, only 13 antigens of parasite origin were detected. None of these was found to be unique to hydatid cysts. It is unlikely that any additional antigen(s) with exclusive specificity can be found which could provide higher specificity in serological tests. Antigens 4 and 5 which were shown to possess excellent immunogenicity in experimental immunization and were restricted to a small number of other parasites may after all represent the best choice in the serodiagnosis of hydatidosis since ELISA using these two antigens showed specificity and sensitivity that were within acceptable ranges for serological tests.


Ben-I:smail, B.C., G. Niel and M. Gentilini. 1980. Non-specific serological reactions with *Echino-


Bos, H.J., A.A. van Den Eijk, P.A. Steeren Lerg


Farag H., D. Bout and A. Capron. 1975. Specific immunodiagnosis of human hydatidosis by the enzyme-linked immunosorbent assay (ELISA).
Biomedicine 23:276-278.


Giunchi, G., S. Pauluzzi and F. De Rosa. 1972. Specificity of the indirect hemagglutination
test for the diagnosis of human hydatid disease.

Estratto Dal Boll. 1st Sieroter, Milanese 51: 145-151.


Rickard, M.D., C. Davis, D.T. Bout and J.D. Smyth. 1977. Immunohistological localisation of two hydatid antigens (Antigen 5 and antigen B) in the cyst wall, brood capsules and protoscolecites.
of *Echinococcus granulosus* (ovine and equine) and *E. multilocularis* using immunoperoxidase methods. *J. Helminthol.* 51:359-364.


Varela-Diaz, V.M., E.A. Coltorti, M.D. Rickard and J.M. Torres. 1977a. Comparative antigenic characterization of Echinococcus granulosus and


Buffers and solutions used in the preparation of insoluble immunosorbents.

1.1 1µM hydrochloric acid (HCL) solution (for washing the freeze-dried cyanogen bromide-activated sepharose 4B). 1µM Hcl solution was made from the 36% concentrated acid of Sp. gr 1.18 by the formula for diluting solutions, i.e. \( R \times V \) where

\[
R = \text{required concentration} \\
V = \text{total volume needed} \\
O = \text{original concentration}
\]

\[ R \times V - O = \text{amount of O to be mixed with distilled water to make V.} \]

1.2 0.1M sodium bicarbonate (NaHCO\(_3\)) pH 8.3 buffer containing 0.5M sodium chloride (for coupling protein).

- Sodium chloride 29.22g
- Sodium bicarbonate 8.40g

Sodium bicarbonate was dissolved in 200 mls of distilled water and the pH adjusted to 8.3 using 1 M sodium hydroxide solution under magnetic stirring. Sodium chloride was added and dissolved and the pH re-checked. The solution was made to one litre.

1.3 Diethanolamine pure pH 9.0 (blocking agent).

Pure diethanolamine 105.14
1.4 0.1M acetate buffer pH 4 containing 0.5M sodium chloride (for the removal of excess blocking agent).

Acetic acid 5.77 ml
Sodium acetate 8.20 g
Sodium chloride 29.22 g

The above compounds were dissolved in one litre of distilled water.
Buffers and solutions used in immunodiffusion and immunoelectrophoresis.

2.1 Phosphate buffered saline (for preparation of agar gel for immunodiffusion).

2.1.1 Phosphate buffer 0.15 M pH 7.4. Anhydrous di-sodium hydrogen phosphate 21.2g. The salt was dissolved in 800 mls of distilled water and the pH adjusted to 7.4 using IN hydrochloric acid. The solution was made to one litre.

2.1.2 Saline solution. Nine grams of sodium chloride were dissolved in 1 litre of distilled water.

2.1.3 Phosphate buffered saline (PBS) pH 7.4. One volume of 0.15M phosphate buffer pH 7.4 was added to 9 volumes of saline.

2.2 Barbital calcium lactate buffer (for immunoelectrophoresis)

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

The above salts were dissolved in 10 l of distilled water and the pH adjusted to 8.4 to 8.6.
2.2.1 For electrophoresis chambers.
   The above buffer was used.

2.2.2 For preparation of agarose.
   The above buffer was diluted with distilled water in the ratio 2 parts buffer and 1 part water (V/V).
APPENDIX 3.

The Gels used in immunodiffusion and immunoelectrophoresis.

3.1 1% agarose in barbital calcium lactate buffer
(for immunoelectrophoresis and crossed immunoelectrophoresis).

Litex agarose (type HSA) 2g
Barbital lactate buffer 50 ml
Distilled water 150 ml

The mixture was heated till all the agarose beads had melted.

3.2 1% agar in phosphate buffered saline (PBS) pH 7.4 (for immunodiffusion).

Purified oxoid agar 2 g
PBS 50 ml
Distilled water 150 ml
Sodium azide (NaN₃) Preservative 0.2 g
Protein staining and destaining solutions.

4.1 Ponceau S staining solution

- Ponceau S 2 g
- 1M glacial acetic acid 1000 ml
- 0.1M sodium acetate 1000 ml

4.2 Destaining solution for ponceau S stain.

3% (V/V) glacial acetic acid diluted in distilled water.

4.3 Coumassie Brilliant Blue 250 R solution

- Coumassie Brilliant 250R 10 g
- Ethanol 900 ml
- Glacial acetic acid 200 ml
- Distilled water 900 ml

4.4 Destaining solution for Coumassie Blue 250 R stain.

- Ethanol 900 ml
- Glacial acetic acid 200 ml
- Distilled water 900 ml
Preparation of buffers and diluents used in ELISA.

5.1 Preparation of stock 0.15M phosphate buffer pH 7.4
Solution A - 0.15 M NaHPO₄ : 21.3g dissolved in 1000mls of distilled water.
Solution B - 0.15 M NaH₂PO₄ · 2H₂O : 23.4g dissolved in 1000 ml of distilled water.
The pH of solution A was adjusted to 7.4 using solution B.

5.2 Preparation of stock 0.5 M phosphate buffer pH 8.0
Solution A - 0.5M NaH₂PO₄ · 2H₂O : 78.0g dissolved in 1000 ml of distilled water.
Solution B - 0.5 M Na₂HPO₄ : 70.98 g dissolved in 1000 ml of distilled water.
The pH of solution B was adjusted to 8.0 using solution A.

5.3 Preparation of citrate/ammonium acetate buffer pH 5.0. (used to dilute the substrate).
Citric acid 10.5 g
Benzoic acid 1.0 g
Glacial acetic acid 3.0 ml
The pH was adjusted to 5.0 using concentrated ammonia. Distilled water was added to make 1000 ml.
5.4 Diluent for the HCF antigen used to coat the ELISA plates.

O.015M phosphate Buffer pH 7.4 made from a 0.15 M stock) 1000 mls
Sodium chloride 23.38 g
Polyethylene glycol 20.0 g
Sodium azide 0.2 g

5.5 Diluent for the test serum.

O.05M phosphate buffer pH 8.0 (made from a 0.5 M stock). 1000 mls
Potassium chloride 75.0 g
EDTA 1.0 g
Benzoic acid 2.5 g
Tween 80 5.0 ml

Adjusted to pH 7.5 with 4 M sodium hydroxide.
Rabbit serum 5 %

5.6 Diluent for HRPO conjugate

O.05M phosphate buffer pH 8.0 1000 mls
Potassium chloride 75.0 g
EDTA 1.0 g
Benzoic acid 2.5 g
Tween 80 5.0 ml

pH adjusted to 7.5 with 4M sodium hydroxide
Goat plasma or serum 5 %
5.7 Preparation of the substrate

0.05M citrate/ammonium acetate buffer pH 5.0 10 mls
0-phenylene-diamine-dihCl (OPD) 10 mg
1% hydrogen peroxide 0.1 ml
The solution was stored in the dark.

5.8 Preparation of the plate washing buffer

PBS 1000 ml
Tween 80 5 mls
Distilled water 10,000 ml
Sodium azide 2 g