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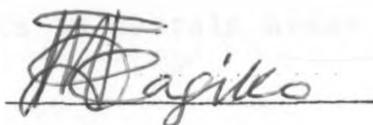SERODIAGNOSIS OF HYDATID DISEASE IN LIVESTOCK //

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the degree of Doctor of Philosophy in
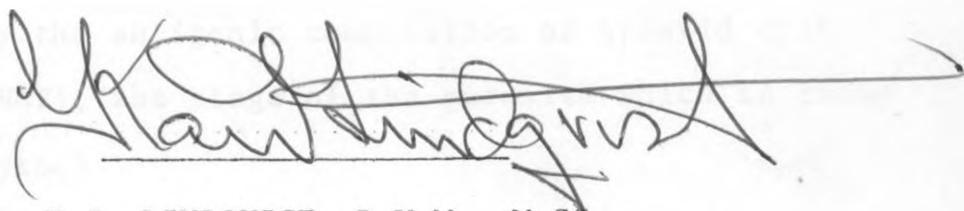the University of Nairobi.

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DECLARATION

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

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

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ABSTRACT

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 electropho-
retic mobility, starting with the most anodic component.
Although antigen 6 showed a faster mobility than
antigen 5, the numbers were interchanged so that
antign 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

Content   Page

Content   <u>Page</u>

Content		Page

(xiv)

LIST OF TABLES

(xv)

LIST OF FIGURES

1. <u>INTRODUCTION</u>

Hydatid disease, a cyclozoonotic infection, is recognised as being one of the major zoonoses, having a worldwide distribution but a variable incidence. It is normally associated with under-developed 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 <u>Echinococcus</u>, namely, <u>E. granulosus</u> which causes classical hydatid disease in which the cyst is typically spherical and unilocular, and <u>E. multilocularis</u> which produces the so-called alveolar hydatid cysts.

Man acquires the infection by ingesting eggs of the parasite passed in faeces of an infected carni-vore. 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 Heath, 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 immuno-fluorescence 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 immunoelect-
rophoresis (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 immuno-electrophoresis (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 diganosis 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 in-
direct 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 puri-
fied 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 $56^{O}C$ 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 concanvalin-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, inclu-
ding 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   100µg/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 µg
protein per ml antigen lots showed little difference
in reactivity, but the antigen lot containing 2 µg
per ml had a much reduced sensitivity. Using an antigen
containing 15 µg 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 sensi-
tivity 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 investi-
gations 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, glutaral-
dehyde 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 correspond-
ing 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 concentra-
tion 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 agglu-
tination 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 use-
ful 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 character-
istics 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 critisized 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 anti-
bodies 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 anti-
bodies 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 proto-
scolices 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).

2.2.8   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 immuno-
electrophoresis 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 diag-
nostic 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. Immunoelectro-
phoresis 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 immunofluore-
scence 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 immuno-
diffusion (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 α-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 β-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 precipitat-
ion 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 (10.3) such that all native immunoglobu-
lins 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 radioimmunoassy
(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 pharmaco-
kinetics 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, schistos-
miasis, 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 1gG and sera from hydatidosis cases.
In a comparison of this test with radio-immunoelectro-
phoresis, 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 Kautokcino 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 sub-
sequently been replaced by microtitre plates and
cuvettee 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 cuvettees 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 subst-
rates. 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 amino-
salicylic 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 $4^{o}C$. 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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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 $4^{o}C$. 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.

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 cut 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 .to

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 centri-
fugation 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 membr-
anes 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
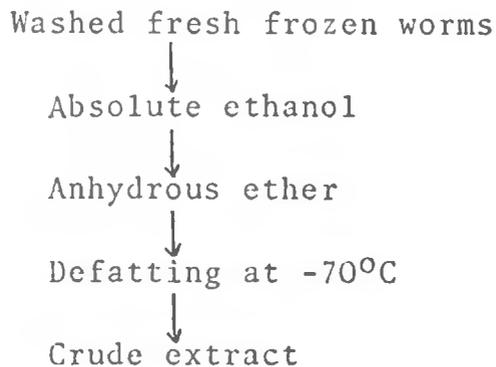
This antigen solution was then dialysed to remove sodium azide and used to immunize a goat. The worms were ground in a glass tissue grinder and ultra-sonicated as described for protoscolices.

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 β-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 adminstered 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.

Animal Species	Immunizing Agents Used
bovine calf 28	bovine HCF
bovine calf 806	bovine hydatid cyst protoscolices
bovine calf 900	Whole T. saginata extract
bovine calf 835	C. bovis extract
sheep 822	Sheep HCF
sheep 805	Sheep hydatid cyst protoscolices
goat 804	goat hydatid cyst protoscolices
goat 814	whole E. granulosus extract
goat 880	isolated antigen 4 (see text)
goat 888	C. tenuicollis fluid
goat 346	isolated antigen 5 (see text)
rabbit 200	E. granulosus extract
" 202	E. granulosus extract
" 201	hydatid cyst protoscolices
" 163	hydatid cyst membranes
" 169	M. expansa extract
" 184	F. gigantica extract
" 701	S. hepatica extract
" 702	O. radiatum extract
" 706	P. microbothrium extract
" 707	T. vulpis extract
" 708	H. contortus extract
" 709	A. galli extract
" 710	A. suum extract

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 immuno-electrophoresis 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 hyper-immune 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 immunoele-
ctrophoresis 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 mls of normal bovine (20 animals)
plasma was added 20 mls of 0.2 M acetate buffer of
pH 5.0. The pH was readjusted and maintained at 5.0
using 1N HC1. 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 immunoel-ectrophoresis 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 immuno-
          sorbents

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

    Sheep 822 antiserum
                |
                ↓
    P. microbothrium

              |
              | Aliquot ─────────→ Test
              ↓
    S. hepatica
              | Aliquot ────────→ Test
              ↓
    M. expansa
              | Aliquot ────────→ Test
              ↓
    C. tenuicollis
              | Aliquot ────────→ Test
              ↓
    T. saginata
              | Aliquot ────────→ Test
              ↓
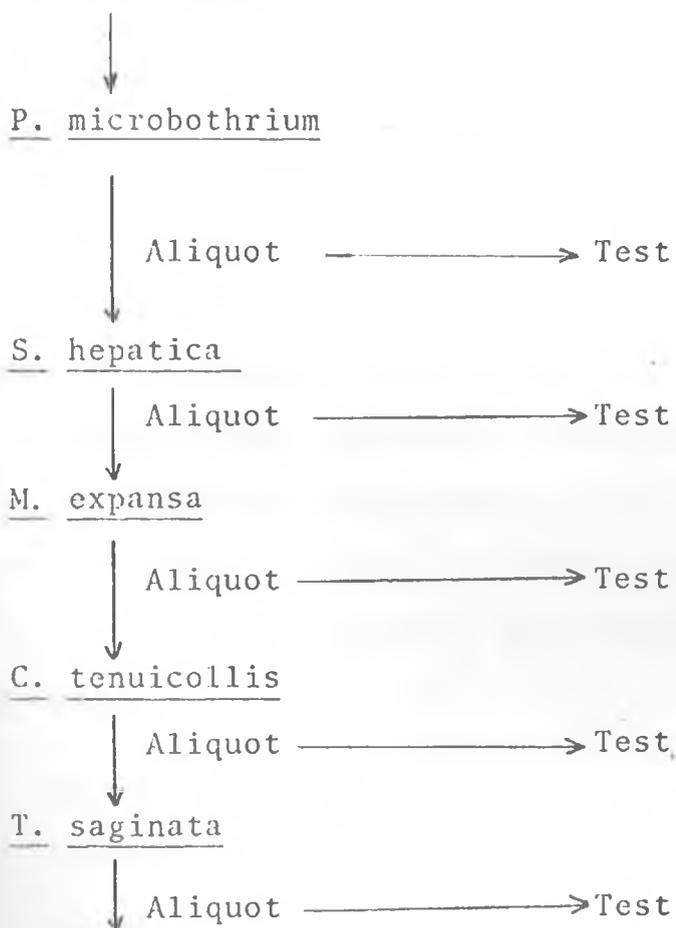
        The tests carried out were double diffusion
and crossed immunoelectrophoresis as described in
section 3.3.1 and 3.3.3. respectively.

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 mls) 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 manu-
facturer's instructions.   Briefly, antigens were
dissolved in a bicarbonate buffer and mixed with re-
swelled 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 mls 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 immuno-
electrophoresis 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 immuno-
electrophoresis 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. Electro phoresis 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
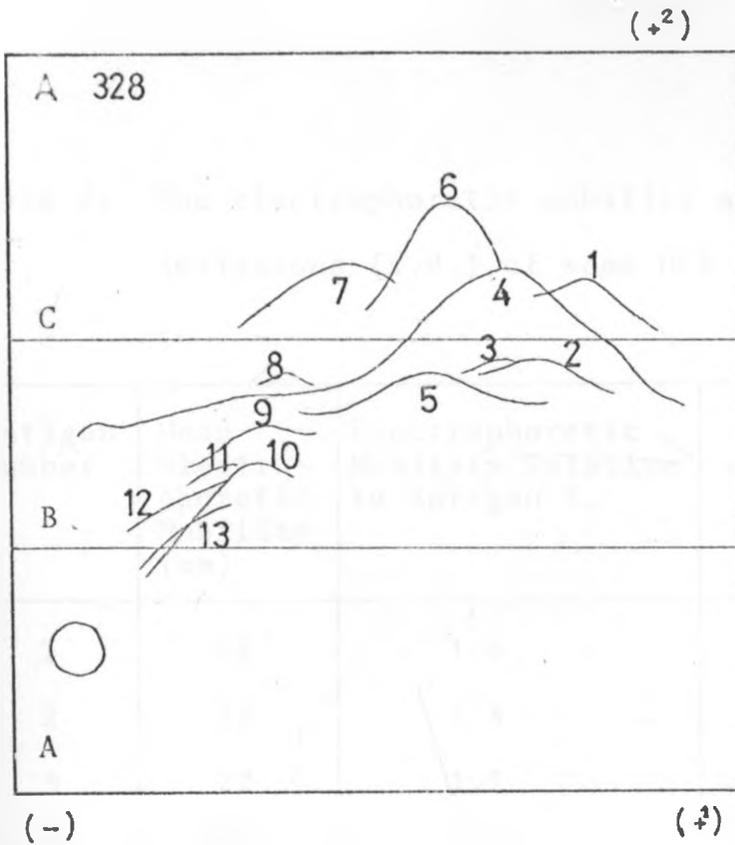
Fig. 1   Reference pattern (see text) observed when
          the standard HCF pool was electrophoresed
          against the standard anti-HCF serum.

    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-SHCFS) absorbed for possible antibodies to
       host contaminants.

Table 2.   The electrophoretic mobility and standard
deviations (s.d.) of some HCF antigens.

Antigen Number	Mean Electro- phoretic Mobility (mm)	Electrophoretic Mobility Relative to Antigen 5.	S.D	Number of Experiments
1	29	1.6	4.7	13
2	27	1.4	4.3	13
3	22	1.1	4.3	11
4	24	1.2	4.5	13
5	20	1.0	4.4	13
8	13	0.7	3.2	7
9	12	0.6	2.7	13
10	10	0.5	1.5	13

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 analy-
sis.

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 signficance was attached to these results.

Table 3. The Prevalence of Hydatidosis at two
abattoirs.

Abattoir	Species	Number examined	Number Positive	% Positive
Dagoretti	Sheep	76	19	25
	Goats	144	14	9.7
	Spp Unidentified	46	6	13.0
	Total	266	39	14.7
Ongata Rongai	Sheep	120	21	17.5
	Goats	239	29	12.1
	Total	359	50	13.9
Both Abattoirs	Sheep	196	40	20.4
	Goats	383	43	11.2
	Spp Unidentified	46	6	13.0
	Grand Total	625	89	14.2

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 origin-
ating 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
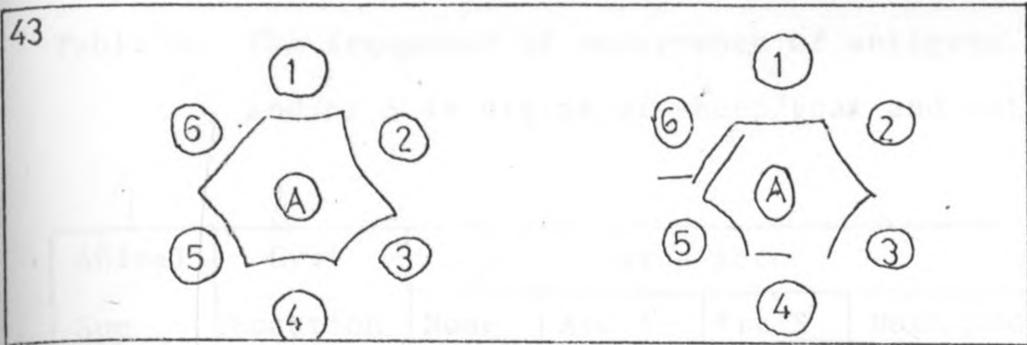
Fig. 2. Demonstration of circulating antibodies to HCF
in naturally infected animals, using DD test.

KEY:

Left pattern   Right pattern

Well   content   Well   content

A   HCF pool   A   HCF pool

1   Serum No. 321   1   Serum No. 919

2   "   "   337   2   "   "   923

3   "   "   556   3   "   "   971

4   "   "   613   4   "   " 1044

5   "   "   627   5   "   " 1124

6   "   "   629   6   "   " 1151

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.

Animal Spp	Cyst Location	Arcs seen				Total
		None	Arc 4	Arc 5	Both Arcs	
p/ Goat	Liver	0(0%)	0(0%)	0(0%)	8(100%)	8
	Lung	2(8%)	3(13%)	0(0%)	19(79%)	24
Bovine	Liver	0(0%)	6(46%)	0(0%)	7(54%)	13
	Lung	0(0%)	28(50%)	3(5%)	25(45%)	56

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 proto-
scoleces).

Source of Hydatid cysts	Fertile cysts			
	Antigens 4 & 5 missing	Antigens 4 & 5 present	Antigen 4 alone present	Total
Cattle lung	3 (9%)	25 (73%)	6 (18%)	34
Cattle liver	0	6 (84%)	1 (14%)	7
Sheep/goat lung	0	11	0	11
Sheep/goat liver	0	4	0	4
	Non-fertile cysts			
Cattle lung	1 (2%)	12 (27%)	32 (71%)	45
Cattle liver	1 (17%)	1 (17%)	4 (67%)	6
Sheep/goat lung	2 (15%)	8 (62%)	3 (23%)	13
Sheep/goat liver	0	4	0	4

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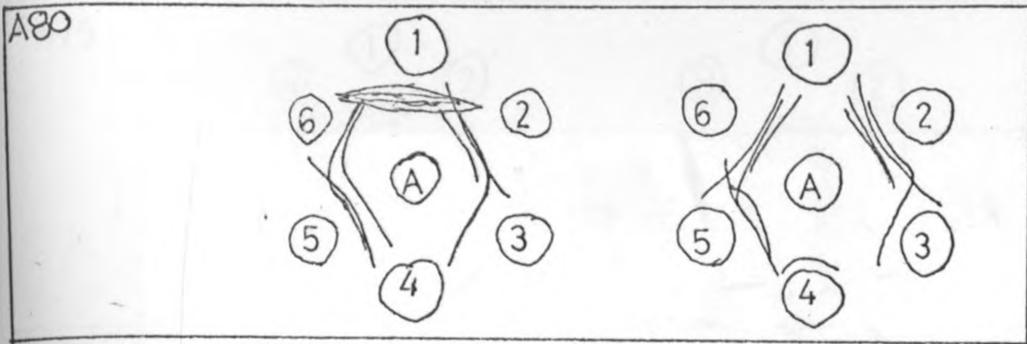
Fig. 3   Immunodiffusion test for cross reactivity
          between HCF and other parasites.

KEY:

Left pattern   Right pattern

Well   content   Well   content

A   Sheep anti-sheep HCF serum   A   Sheep anti-sheep
                                                HCF serum
1   Sheep HCF conc 10x   1   T. saginata

2   C. tenuicollis conc 20% w/v   2   C. tenuicollis

3   M. expansa   3   M. expansa

4   S. hepatica   4   T. saginata

5   C. tenuicollis conc 20% w/v   5   M. expansa

6   M. expansa   6   C. tenuicollis

RESULTS

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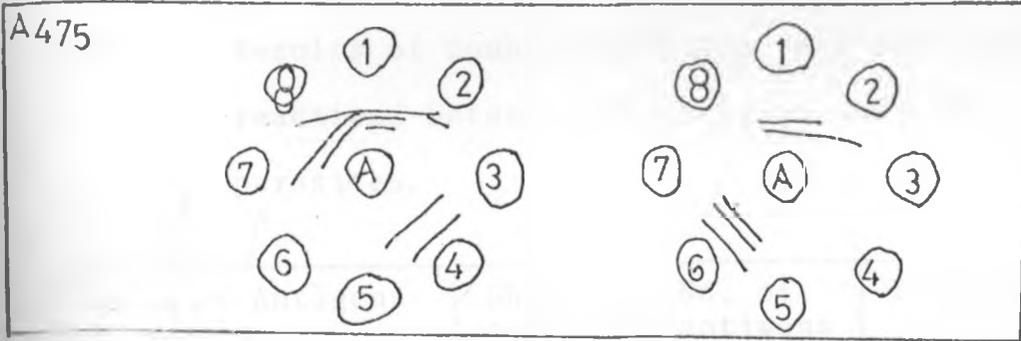
Fig. 4 Immunodiffusion test for cross reactivity
between HCF and other parasites.

KEY:

Left pattern		Right pattern	
Well	content	Well	content
A	Sheep anti-sheep HCF serum	A	Sheep anti-sheep HCF serum
1	Pool of HCF	1	C. tenuicollis
2	S. hepatica	2	B. phlebotomum
3	A. galli	3	P. microbothrium
4	A. centripunctata	4	F. gigantica
5	O. ostertagi	5	H. contortus
6	T. vulpis	6	C. bovis
7	A. suum	7	O. radiatum
8	T. saginata	8	S. lupi

RESULTS:

S. hepatica, A. centripunctata, T. saginata, C. tenui-
collis and C. bovis show cross-reactivity.

Table 6.   Results of Double Diffusion test for cross
           reactions between HCF antigens and other
           parasites.

Source of Antigens	Sheep anti HCF serum	No. of antigens in common	Ref. Figures
C. tenuicollis fluid	+	4	Fig. 2, Fig. 3
C. bovis bladder	+	3	Fig. 4
M. expansa	+	2	Fig. 2
T. saginata	+	2	Fig. 2
A. centripunctata	+	2	Fig. 3
S. hepatica	+	1	Fig. 3

4.8   <u>RESULTS OBTAINED AFTER ABSORPTION OF ANTI-
HCF SERUM WITH CRUDE PARASITE HOMOGENATES
USING DOUBLE DIFFUSION TEST</u>

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

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

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

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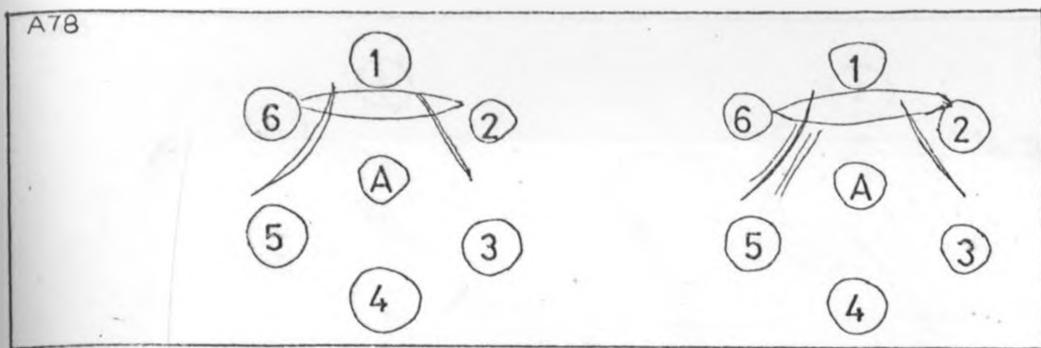
Fig. 5   Immunodiffusion test for the effect of absorbing
         sheep anti-SHCF serum with T. saginata and
         S. hepatica homogenates.

KEY:

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

RESULTS:

Absorption with T. saginata and S. hepatica was complete.
An antigen of HCF was not absorbed out in both cases.
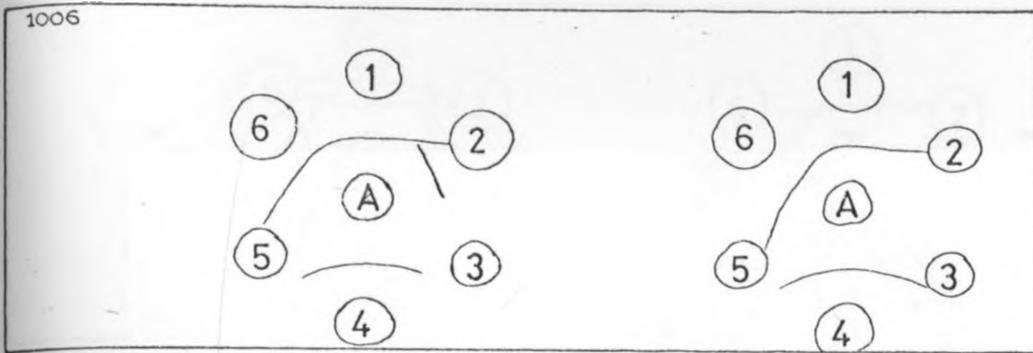
Fig. 6   Immunodiffusion test for the effect of absorb-

ing sheep anti-SHCF serum with T. saginata

and S. hepatica homogenate

KEY:

Left pattern	Right pattern

Well content	Well content
A Sheep anti-SHCFS absorbed with T. saginata	A Sheep anti-SHCFS absorbed with S. hepatica
1. SHCF conc 10x	1 SHCF conc 10x
2 M. expansa	2 M. expansa
3 P. microbothrium	3 P. microbothrium
4 C. tenuicollis 20% w/v	4 C. tenuicollis fluid
5 S. hepatica	5 T. saginata
6 Phosphotungstic acid/ magnesium chloride precipitate from HCF (880)	6 Phosphotungstic acid/ magnesium chloride precipitate from HCF (880)

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:

Left pattern		Right pattern	
Well	content	Well	content
A	Sheep anti-SHCFS absorbed with C. tenuicollis	A	Sheep anti-SHCFS absorbed with M. expansa
1	Sheep HCF conc 10x	1	Sheep HCF conc 10x
2	M. expansa	2	M. expansa
3	P. microbothrium	3	P. microbothrium
4	T. saginata	4	T. saginata
5	S. hepatica	5	S. hepatica
6	C. tenuicollis	6	C. tenuicollis

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.
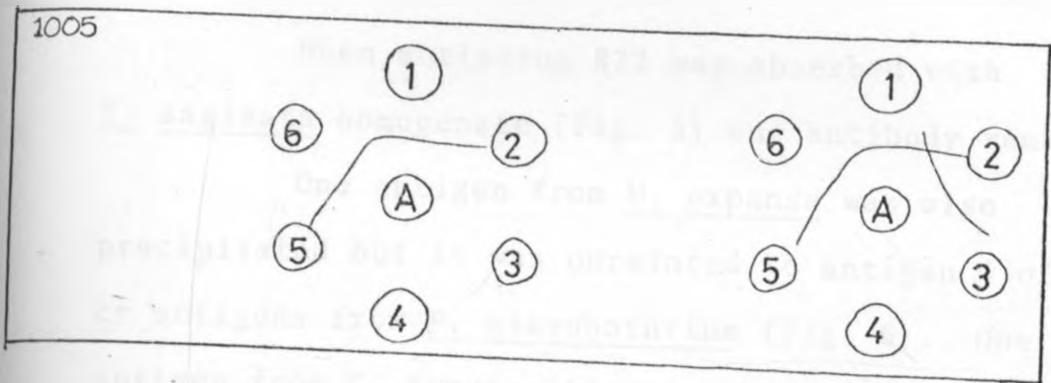
Fig. 8   Immunodiffusion test for the effect of
         absorbing sheep anti-SHCFS with C. tenuicollis
         or M. expansa

KEY:

Left pattern

Well   content

A   Sheep anti-SHCFS absorbed
     with C. tenuicollis

1   Sheep HCF conc 10x

2   M. expansa

3   P. microbothrium

4   T. saginata

5   S. hepatica

6   "880"

Right pattern

Well content

A   Sheep anti-SHCFS
     absorbed with M. expansa

1   Sheep HCF conc 10x

2   C. tenuicollis

3   P. microbothrium

4   T. saginata

5   S. hepatica

6   "880"

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.

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

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. hepatica.

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.

Fig. 10   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:

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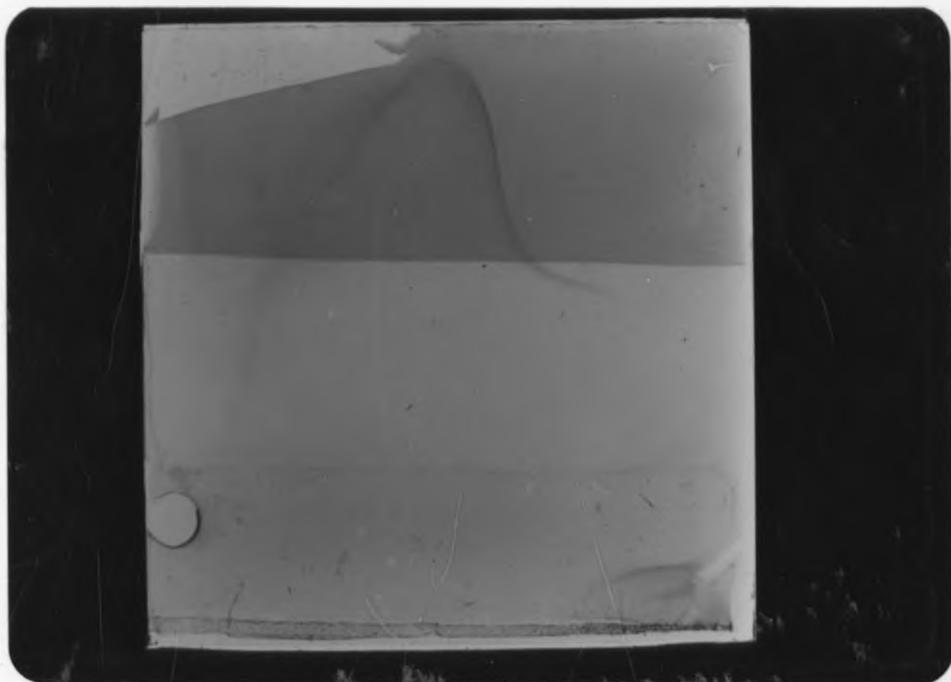
Fig. 11   The precipitation pattern obtained when
          HCF pool antigens were run against
          SA-HCFS which had been absorbed with
          five parasites.
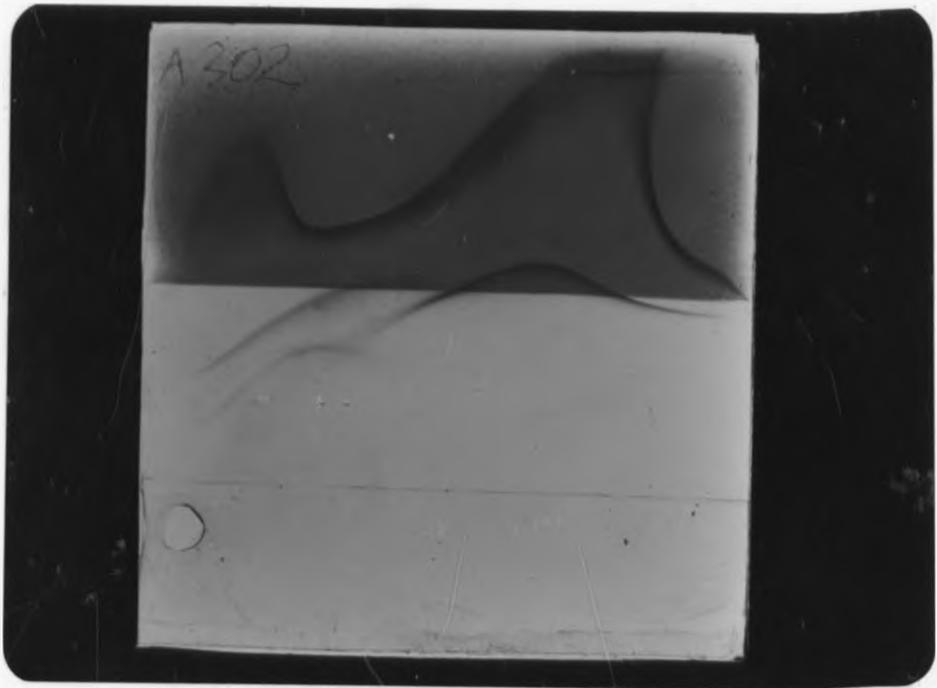
Fig. 12   The precipitation pattern obtained when
           the HCF pool antigens were run against
           concentrated SA-SHCFS which had been
           absorbed with five parasites in a series.
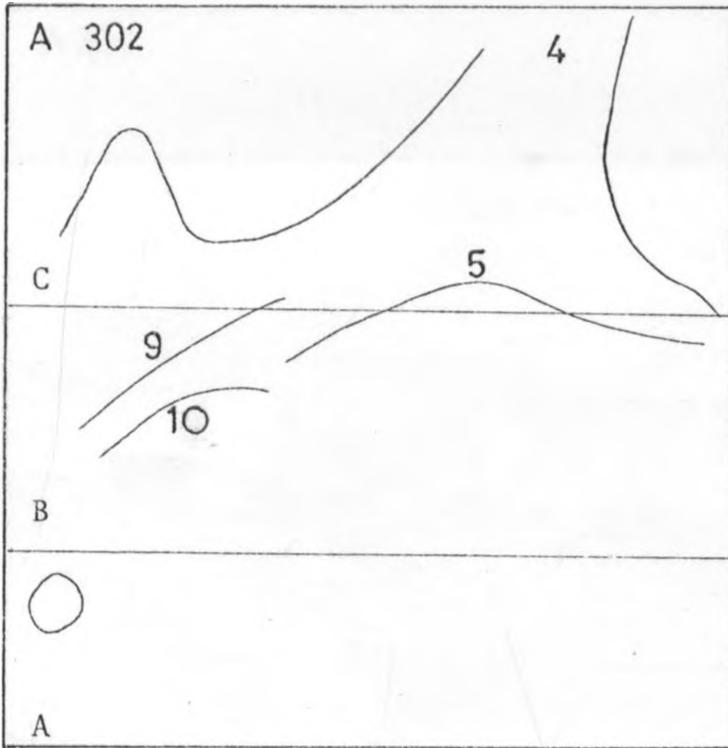
Fig. 12   The precipitation pattern obtained when the
           HCF pool antigens were run against concentr-
           ated SA-SHCFS which had been absorbed with
           five parasites in a series.

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.
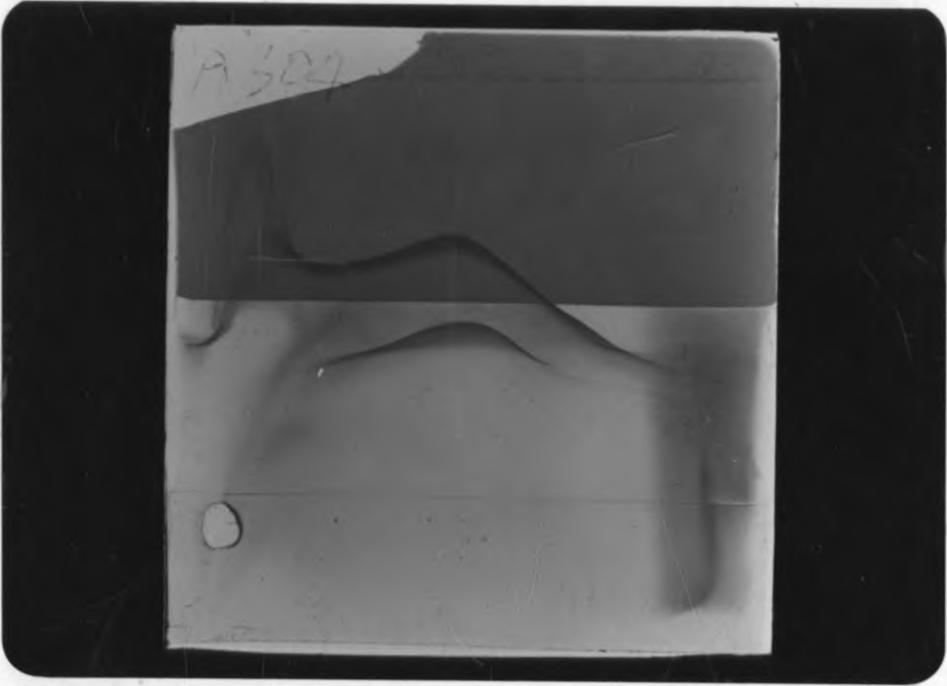
Fig. 13   The precipitation pattern obtained when
         HCF pool antigens were run against
         SA-SHCFS which had been absorbed with
         a pooled mixture of five parasites.
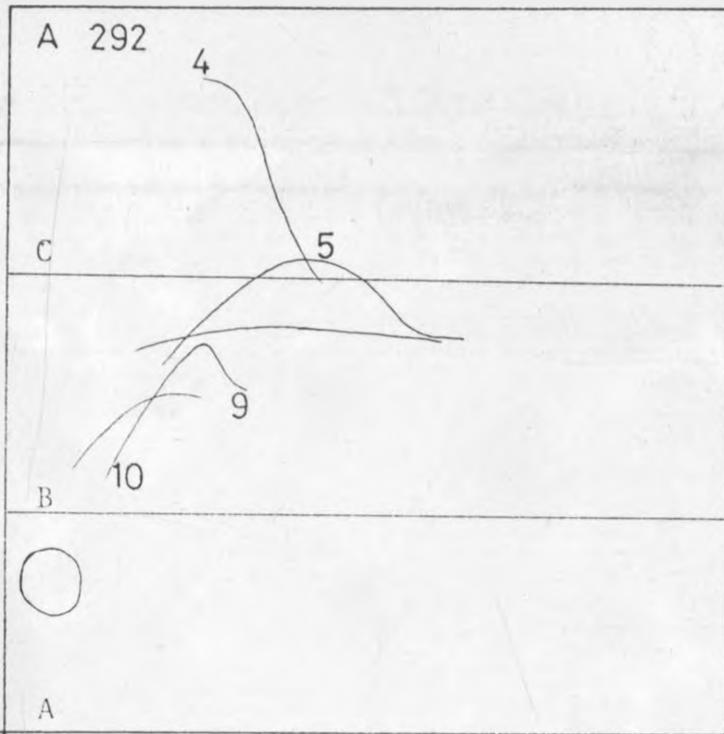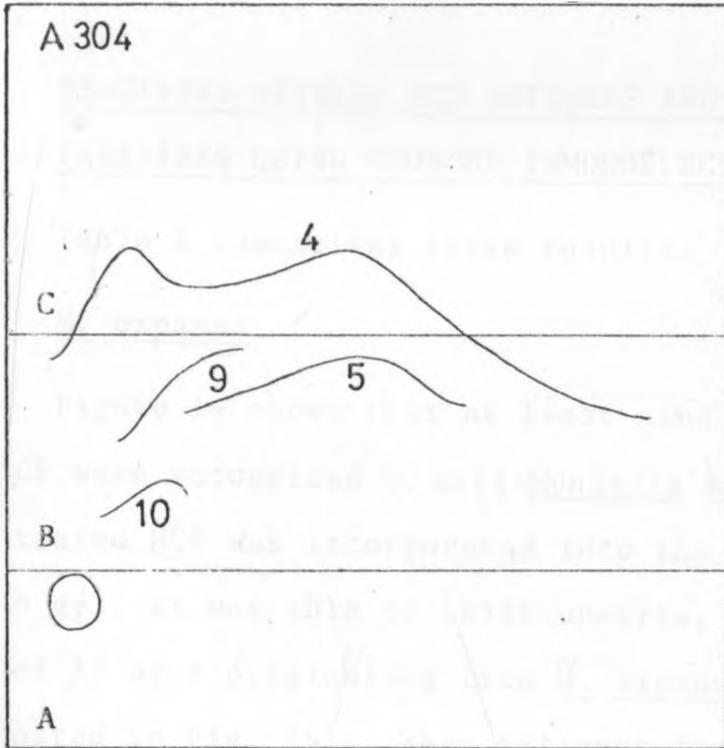
Fig. 13 The precipitation pattern obtained when HCF
pool antigens were run against SA-SHCFS which
had been absorbed with a pooled mixture of
five parasites.

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.
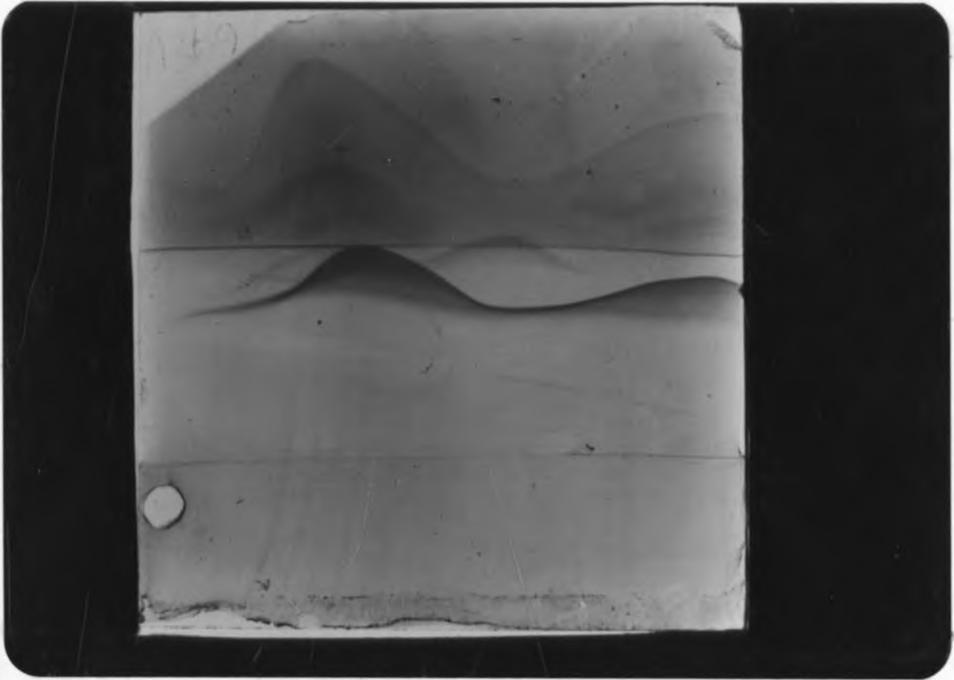
Table 8:   The distribution of HCF antigens in other common
parasites.

	HCF Antigen Numbers												
	1	2	3	4	5	6	7	8	9	10	11	12	13
M. expansa	-	+	-	+	+	-	+	+	+	+	+	-	-
C. tenuicollis	+	+	+	+	+	+	+	+	+	+	-	-	+
S. hepatica	+	+	+	+	-	+	+	+	+	-	+	+	+
T. saginata	+	+	+	+	+	+	+	+	+	+	+	+	-
C. bovis	+	+	+	+	+	-	+	+	+	+	-	-	+
H. contortus	-	-	-	+	-	-	-	-	-	-	-	-	-
F. gigantica	-	-	-	-	-	-	-	-	-	-	-	-	-
O. radiatum	-	-	-	+	-	+	-	-	-	-	-	-	-
T. vulpis	+	-	-	+	-	-	-	-	+	-	-	-	-
A. suum	-	-	-	-	-	-	-	-	-	--	-	-	-
P. microbothrium	-	-	-	-	-	-	-	-	-	-	-	-	-
A. centripunctata	-	+	-	+	-	-	+	-	-	-	-	-	-
A. galli	-	-	-	-	-	-	-	-	-	-	-	-	-
B. phlebotomum	-	-	-	-	-	-	-	-	-	-	-	-	-
S. lupi				+	+								

Fig. 14 The precipitation pattern obtained when
HCF antigens were run against rabbit
anti-M. expansa serum.

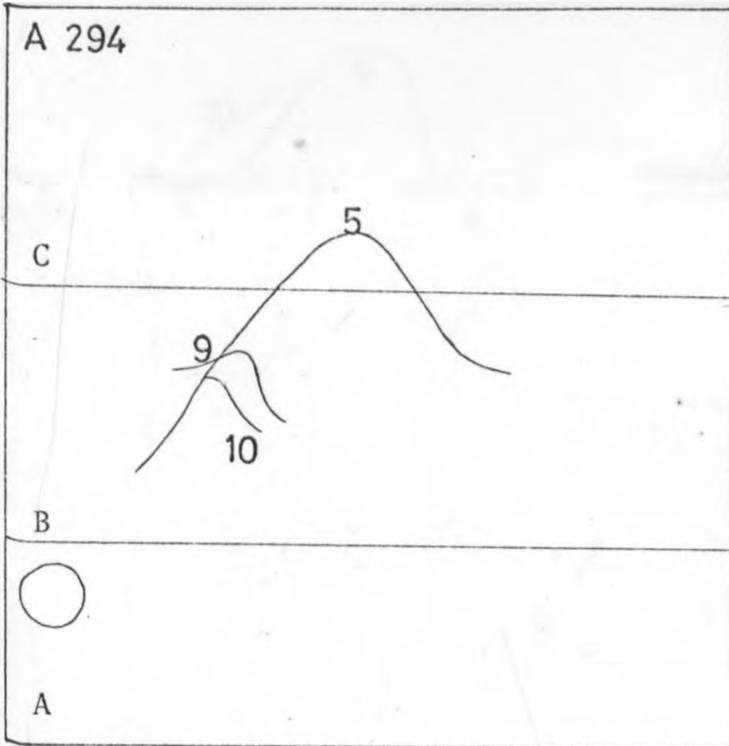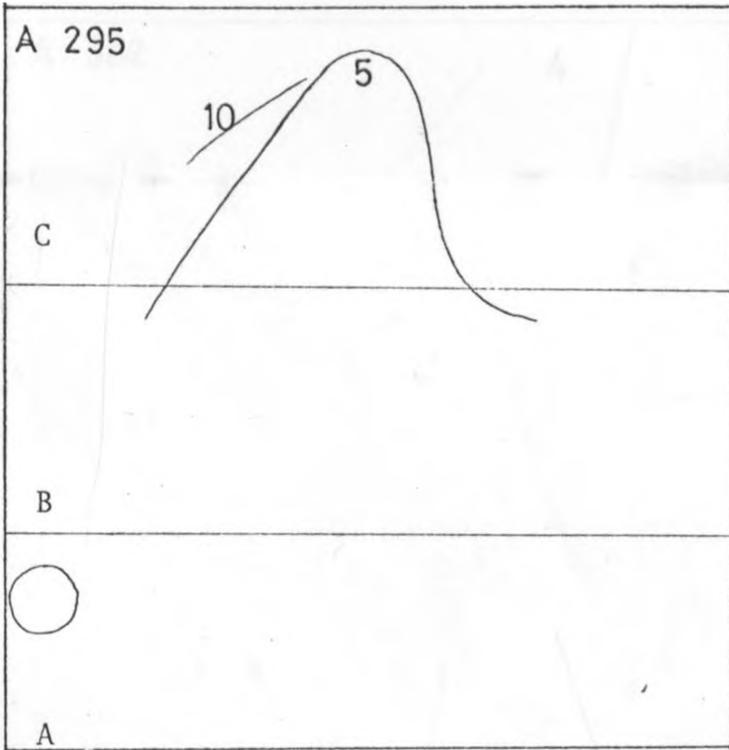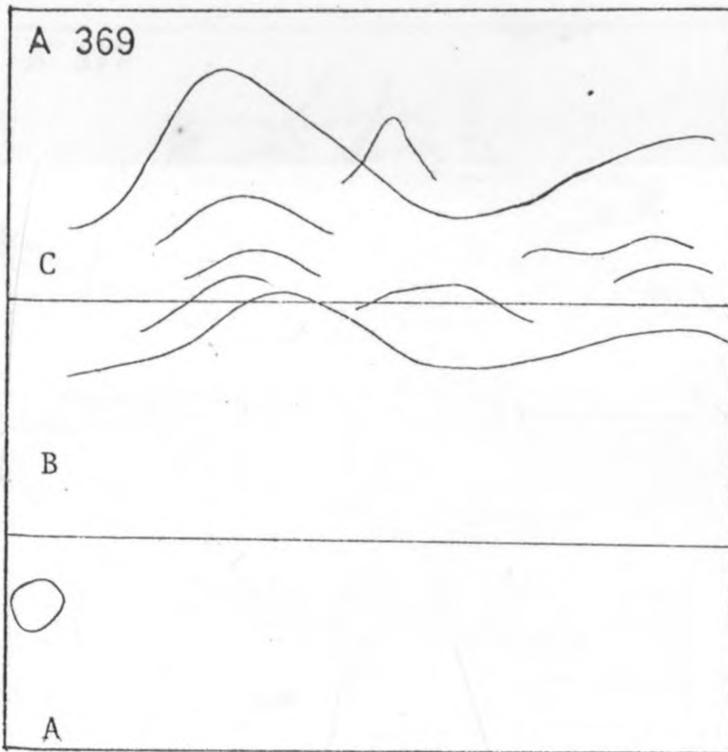Fig. 14   The precipitation pattern obtained when HCF
           antigens were run against rabbit anti-M. expansa
           serum.

           KEY:

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

           B - Blank agarose.

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

           RESULTS:

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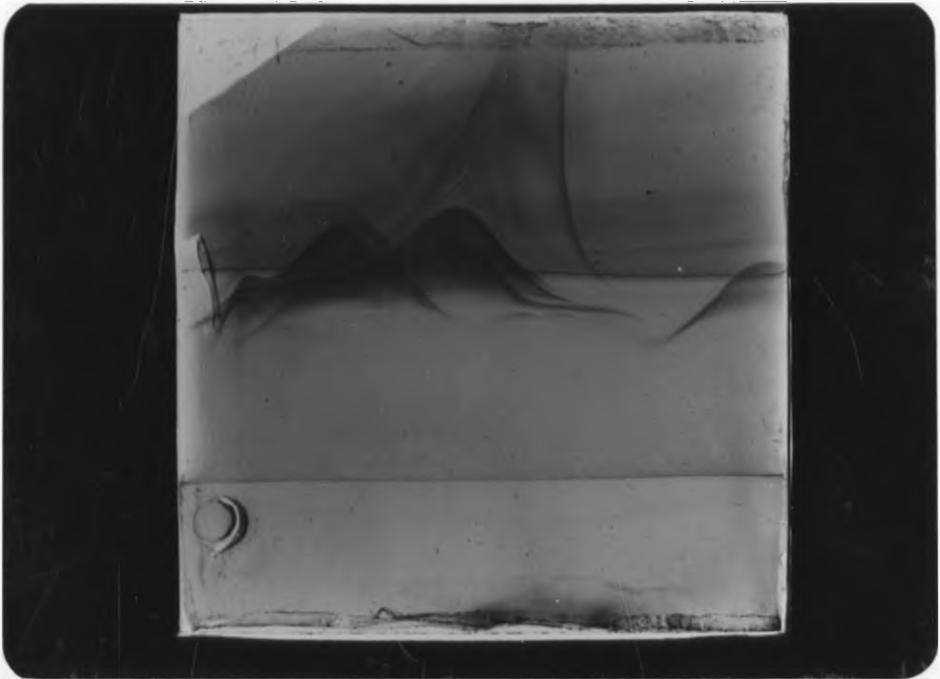
Fig. 15 The precipitation pattern obtained when
concentrated HCF pool antigens were
added to the intermediate gel.

Fig. 15 The precipitation pattern obtained when
concentrated HCF pool antigens were added
to the intermediate gel.

KEY:
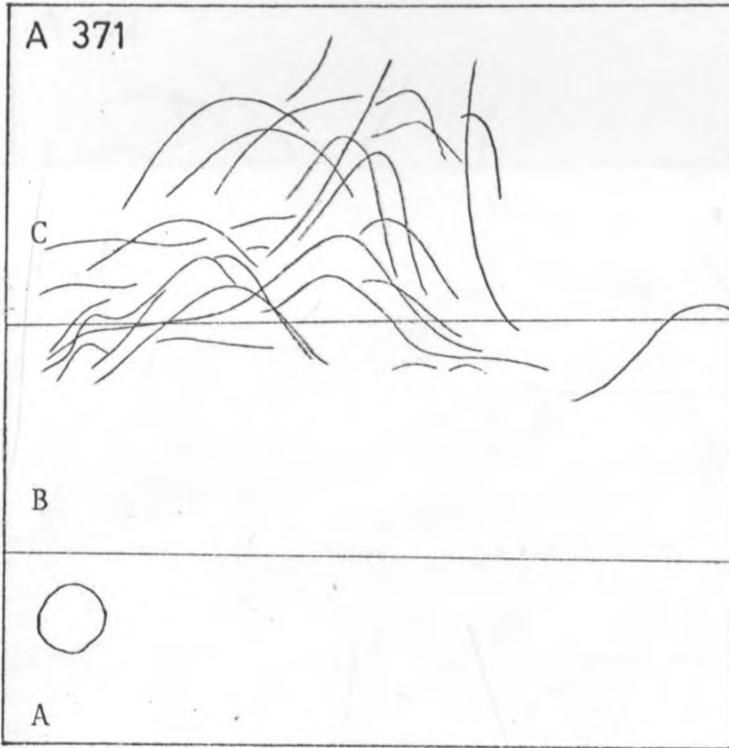
A - M. expansa saline extract was electro-
      phoresed in the first dimension.

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. 1   the precipitating pattern obtained when
                                           n against
                                         t HCF
                                         vel.

nsa serum.

levated.
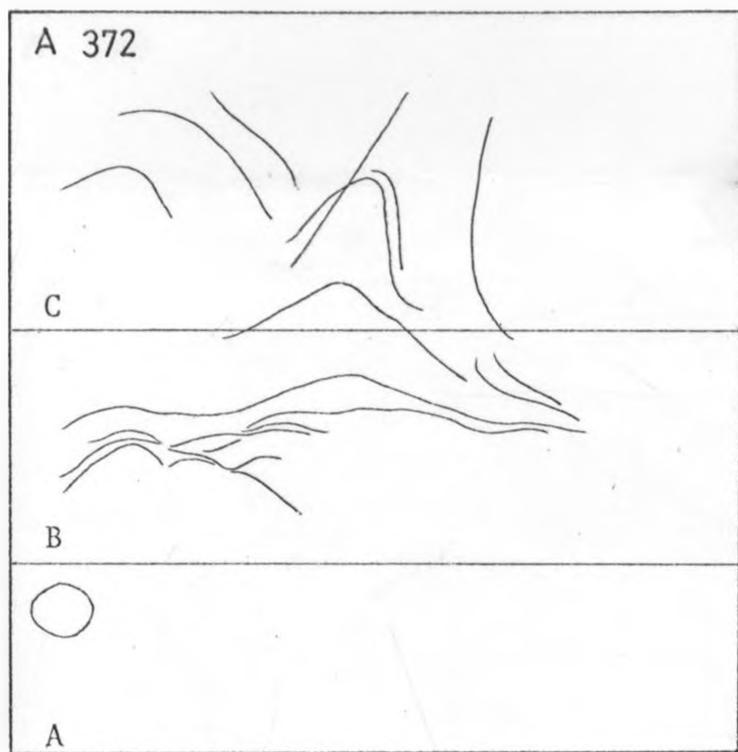
Fig. 16 The precipitation pattern obtained when
M. expansa antigens were run against anti
M. expansa serum without HCF antigens in the
intermediate gel.

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.
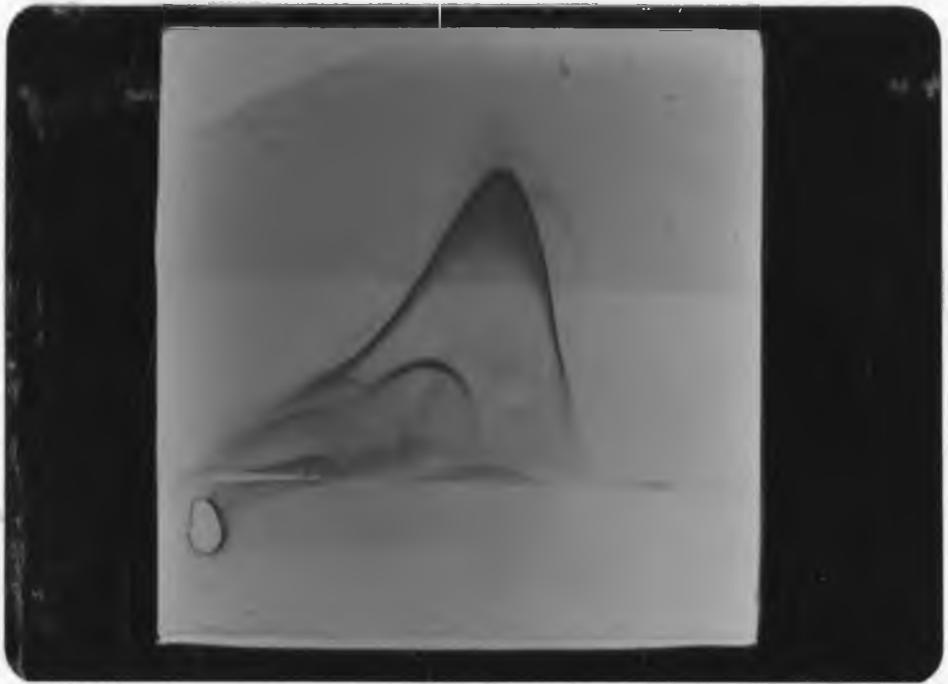
Fig. 17   The precipitation pattern obtained when
anti-M.expansa serum was added to the
intermediate gel between HCF antigens
and the homologous antiserum.
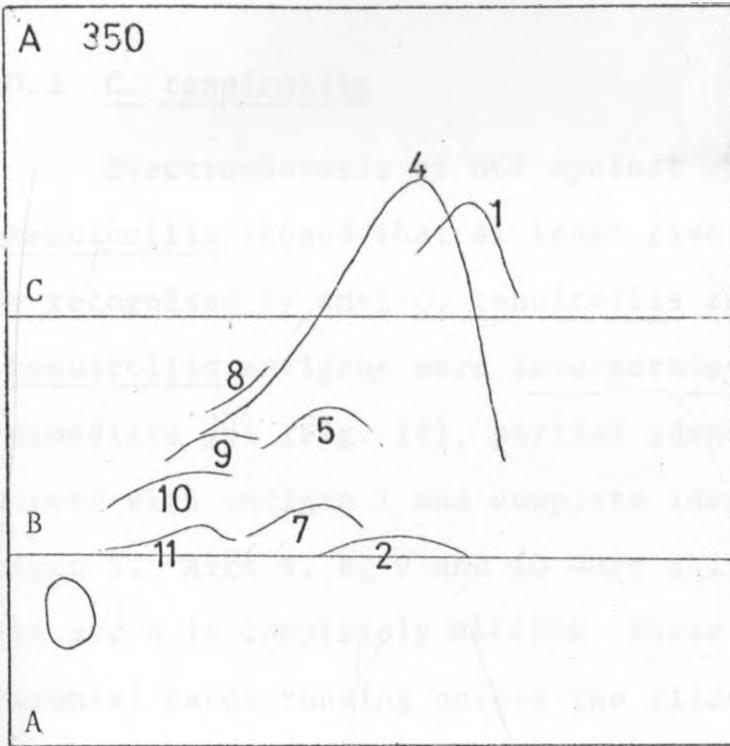
Fig. 17 The precipitation pattern obtained when anti-
M. expansa serum was added to the interme-
diate gel between HCF antigens and the homo-
logous antiserum.

KEY:

A - HCF pool electrophoresed in the first
dimension.

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

C - SA-SHCFS 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
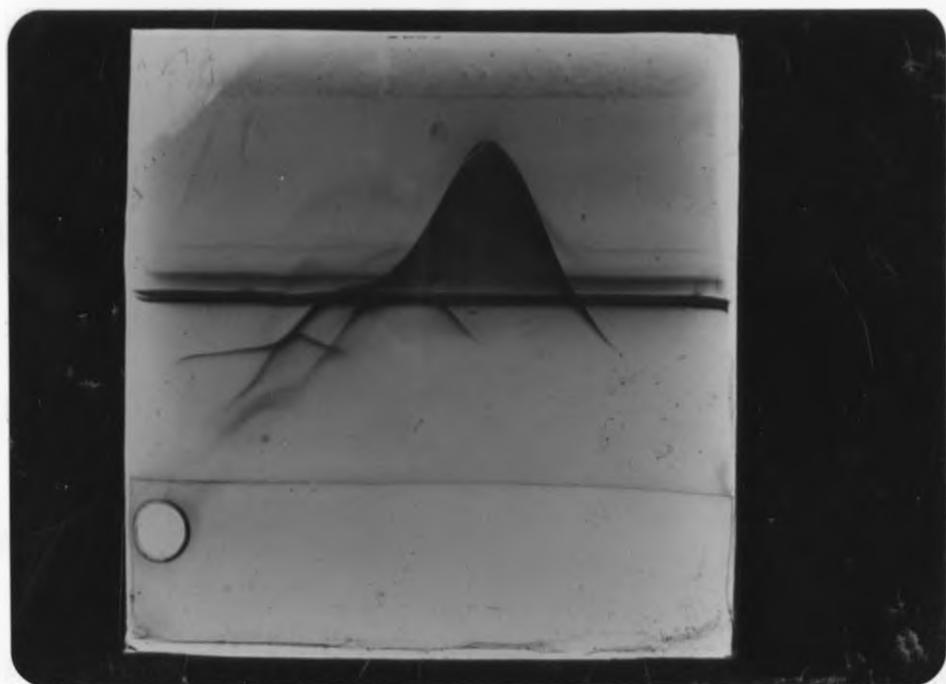
Fig. 18   The precipitation pattern obtained when
20% w/v C. tenuicollis was added to the
intermediate gel between HCF and
SA-SHCFS.

Fig. 18 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-C.tenuicollis was added to the
          intermediate gel between HCF and
          SA-SHCFS.

Fig. 19   The precipitation pattern obtained when goat
           anti-C. tenuicollis was added to the inter-
           mediate gel between HCF and SA-SHCFS.

           KEY:

           A - HCF pool electrophoresed in the first
               dimension run.

           B - Agarose containing anti-C. tenuicollis.

           c - Agarose containing anti serum to SHCF.

           RESULTS:

           Arcs 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-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 S. hepatica saline extract had at least five antigens which could be recognised by the reference 822 serum.

4.10.4 T. saginata

On incorporation of a T. saginata homogenate into the intermediate gel (Fig. 23) arcs 2, 3, 7, 11, 12 and 13 disapeared. An arc suspected to be arc 6 showed cross reaction and the precipitin line was prominently stained. On using hyperimmune anti-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 T. saginata was run against anti-HCF shows that the serum

Fig. 20   The precipitation pattern obtained when
          a S. hepatica saline extract was added
          to the intermediate gel between HCF
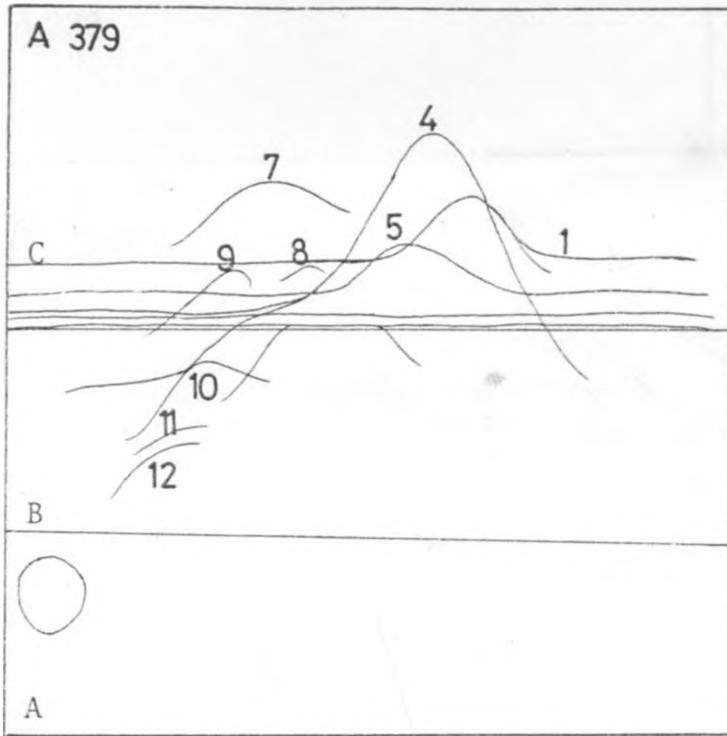   and SA-SHCFS.

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.

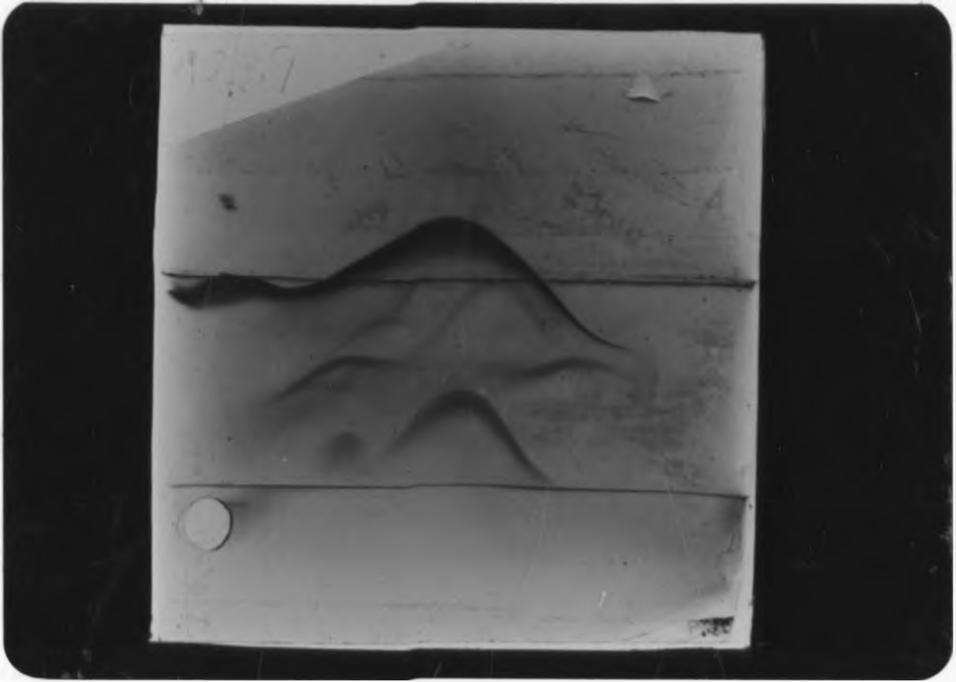RESULTS:

Arcs 4, 5 and 10 remained.

Fig. 21   The precipitation pattern obtained when
          anti-S. hepatica serum was added to the
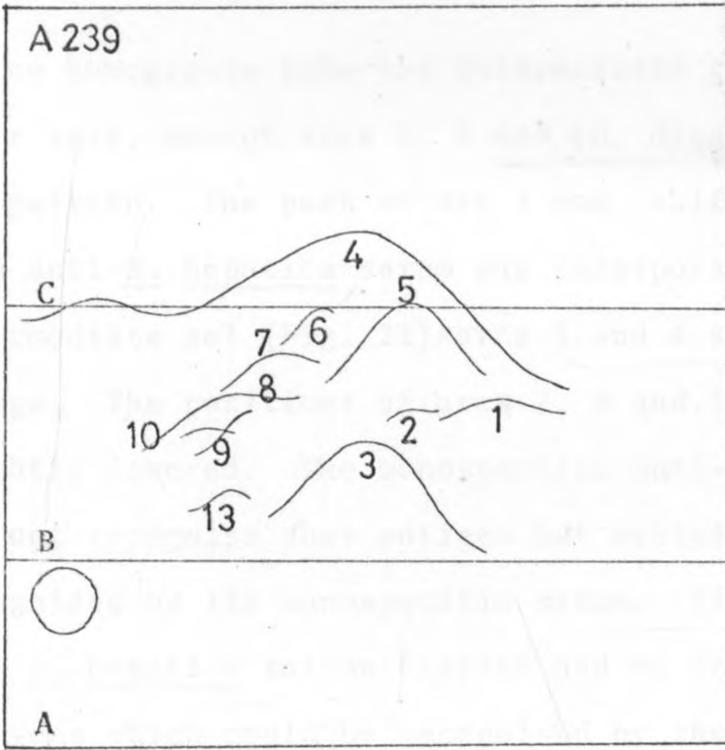   intermediate gel between HCF and SA-SHCFS.

Fig. 21   The precipitation pattern obtained when anti-
S. hepatica serum was added to the interme-
diate gel between HCF and SA-SHCFS.

KEY:

A - HCF pool electrophoresed in the first
    dimension run.

B - Agarose mixed with anti-S. hepatica serum.

C - Agarose mixed with SA-SHCFS.

RESULTS:

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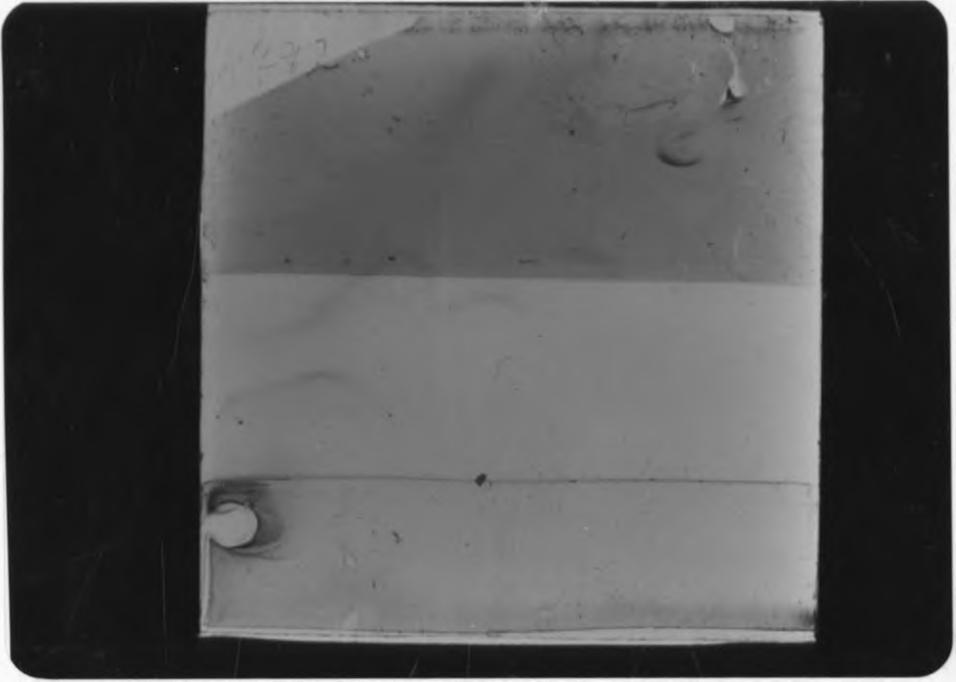
Fig. 22   The precipitation pattern obtained when
a S. hepatica saline extract was
electrophoresed against SA-SHCFS with
a blank intermediate gel.
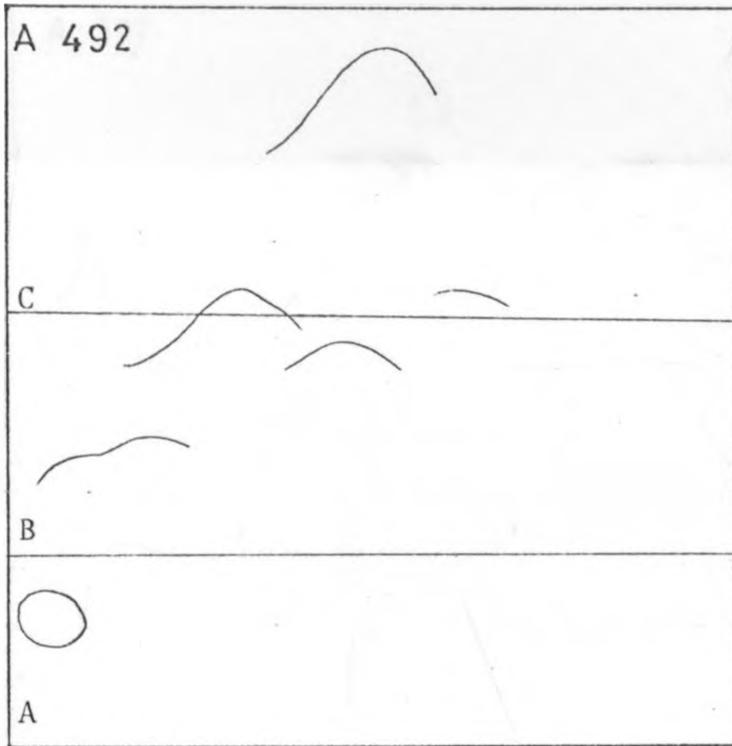
Fig. 22 The precipitation pattern obtained when a
S. hepatica saline extract was electrophoresed
against SA-SHCFS with a blank intermediate gel.

KEY:

A - S. hepatica saline extract was electro-
phoresed in the first direction.

B - Blank agarose.

C - Agarose mixed with SA-SHCFS.

RESULTS:

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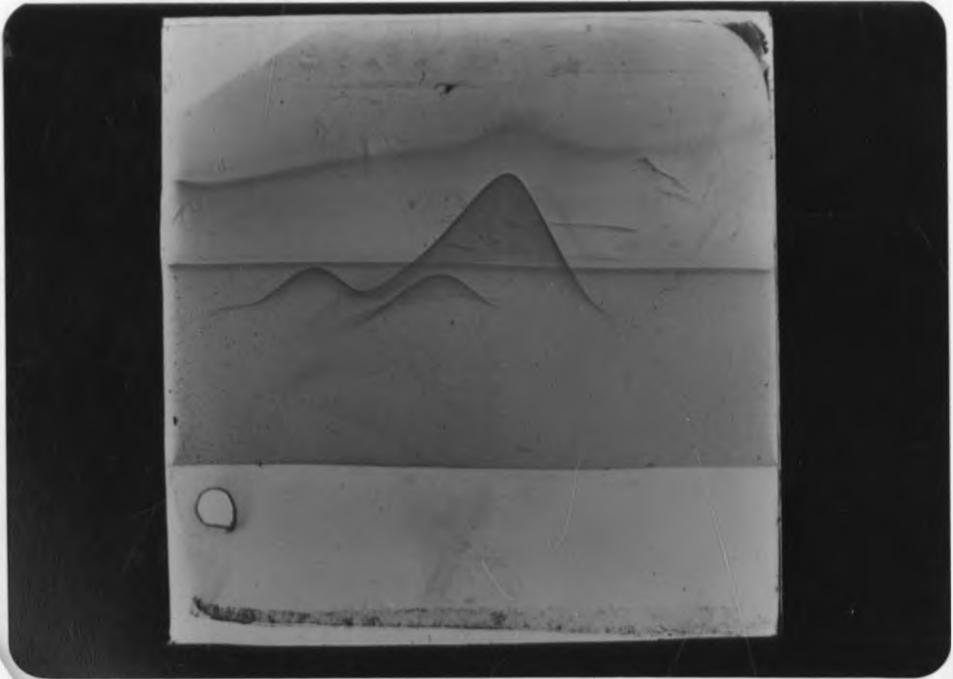
Fig. 23   The precipitation pattern obtained when
a T. saginata saline extract was added
to the intermediate gel between HCF
and SA-SHCFS.

Fig. 23 The precipitation pattern obtained when a
         T. saginata saline extract was added to the
         intermediate gel between HCF and SA-SHCFS.

KEY:
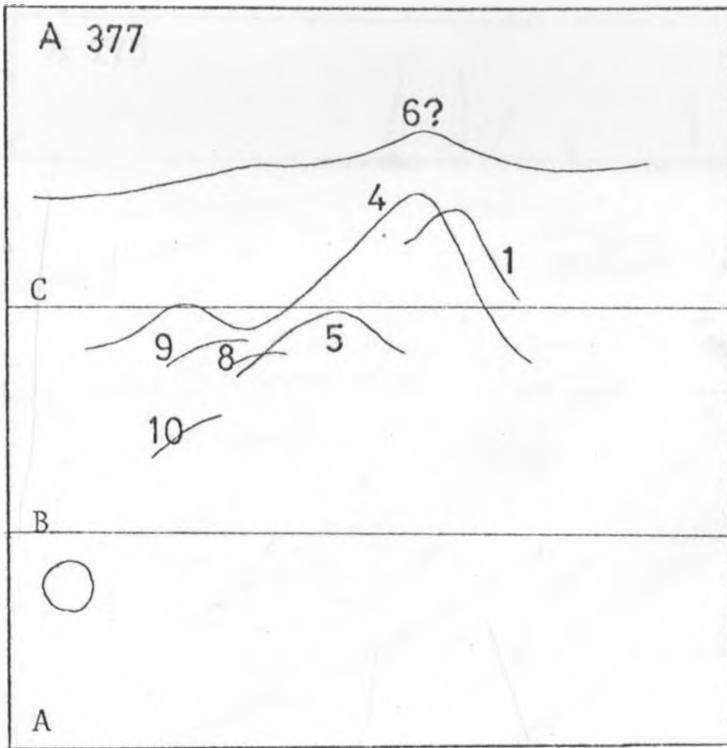
A - HCF pool electrophoresed in the first
    direction run.

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 IICF 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 incorpo-
ration of a whole extract of C. bovis into the inter-
mediate 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 inter-
mediate gel, the position of arc 4 was shifted upwards
(Fig. 28). When anti-H. contortus serum was added to
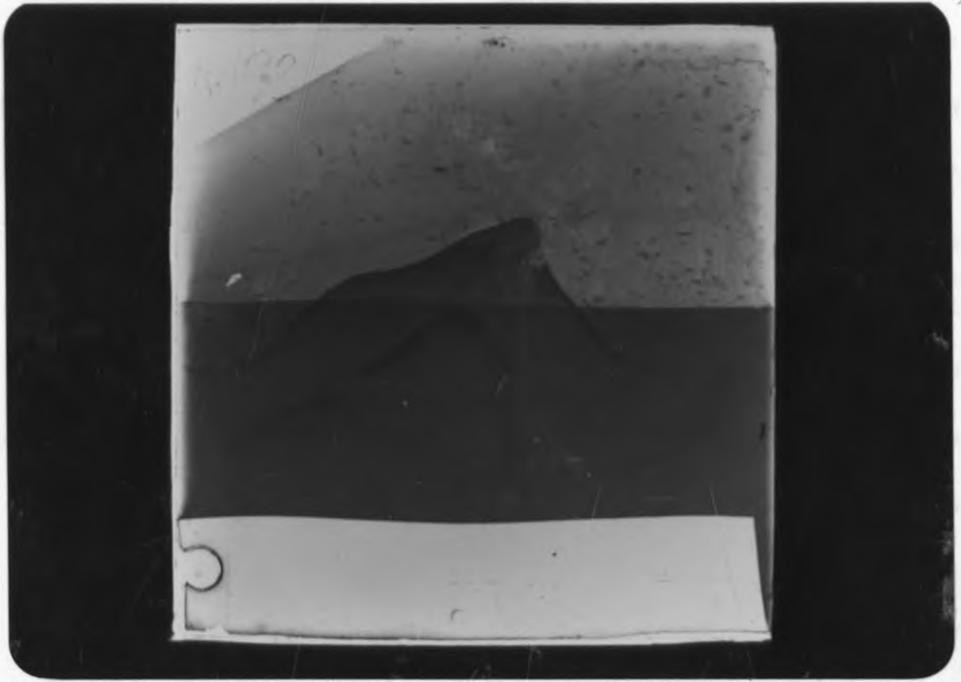the intermediate gel, arc 4 was retained. Monospecific
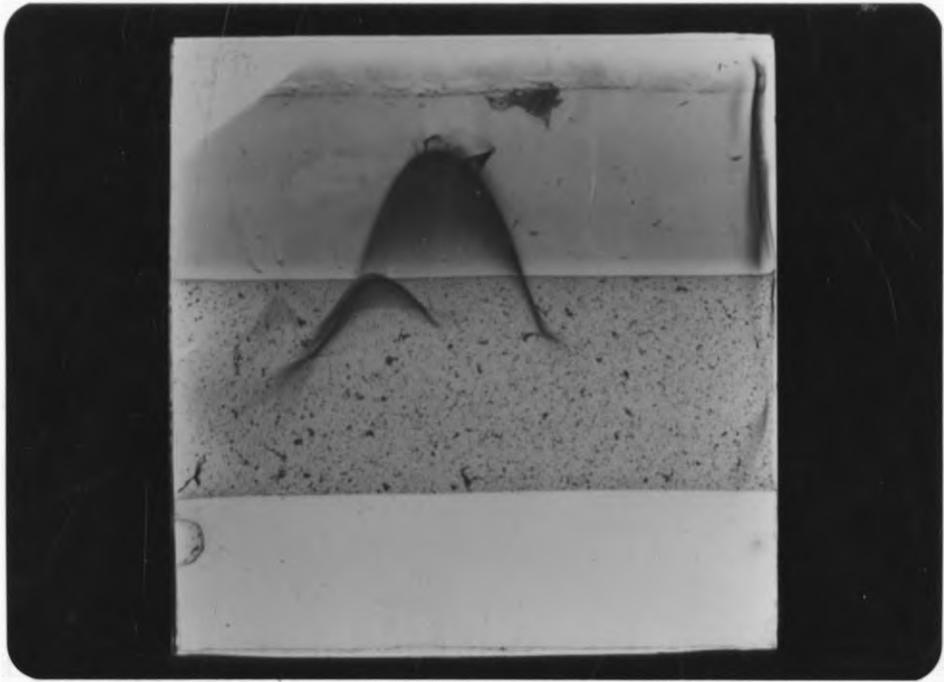
Fig. 25 The precipitation pattern obtained when
a C. bovis saline extract was added to
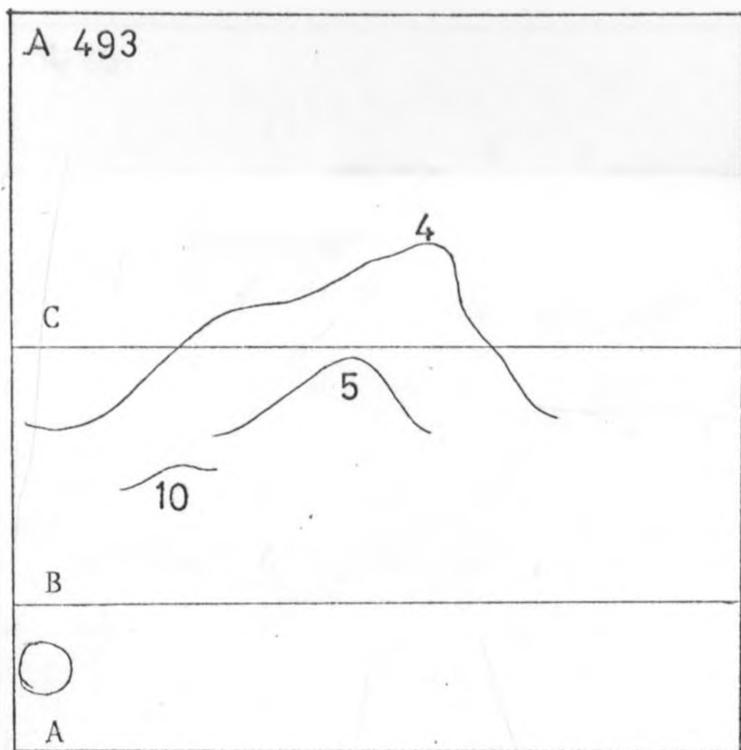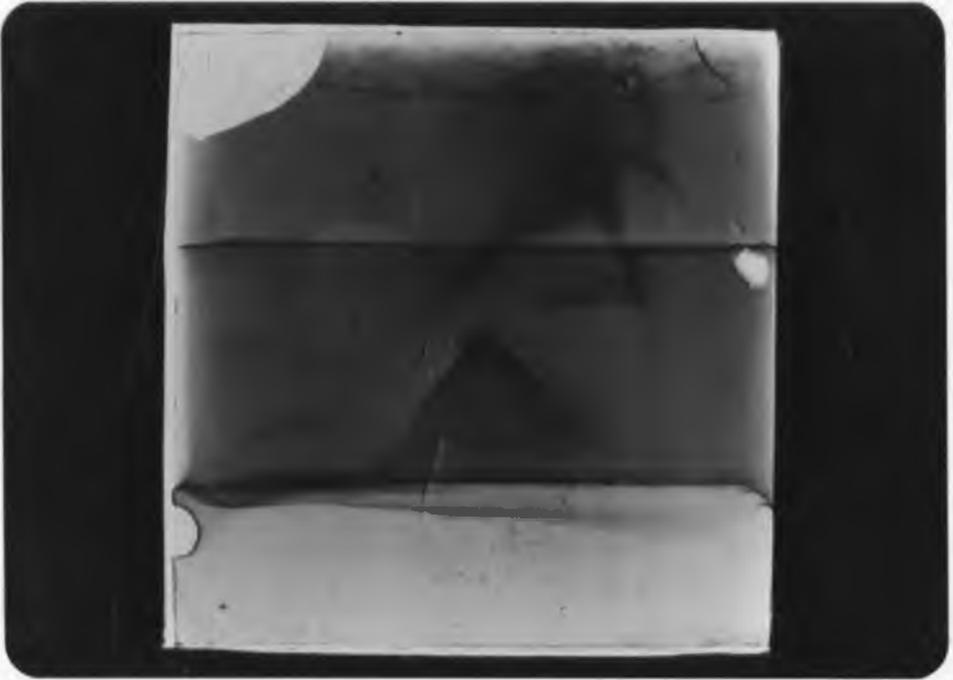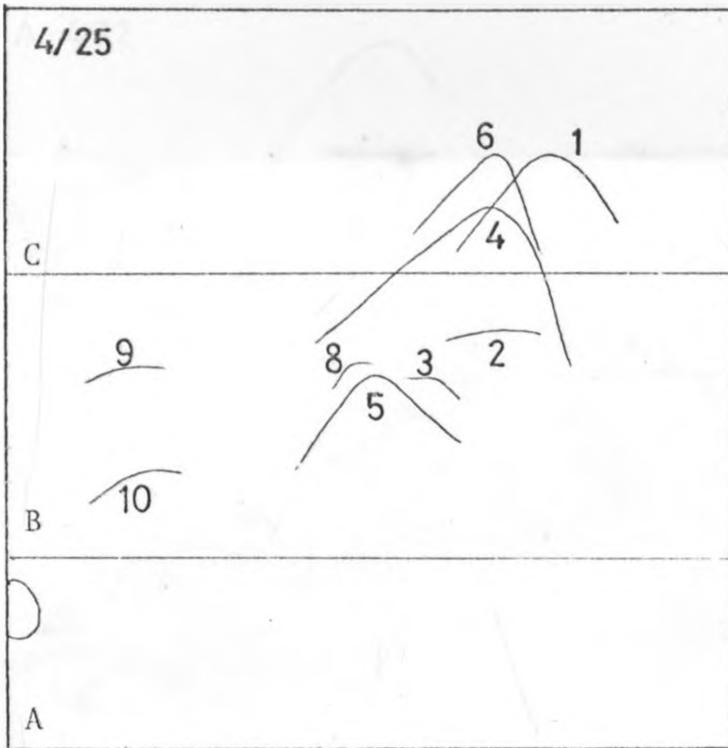the intermediate gel between HCF and
SA-SHCFS.
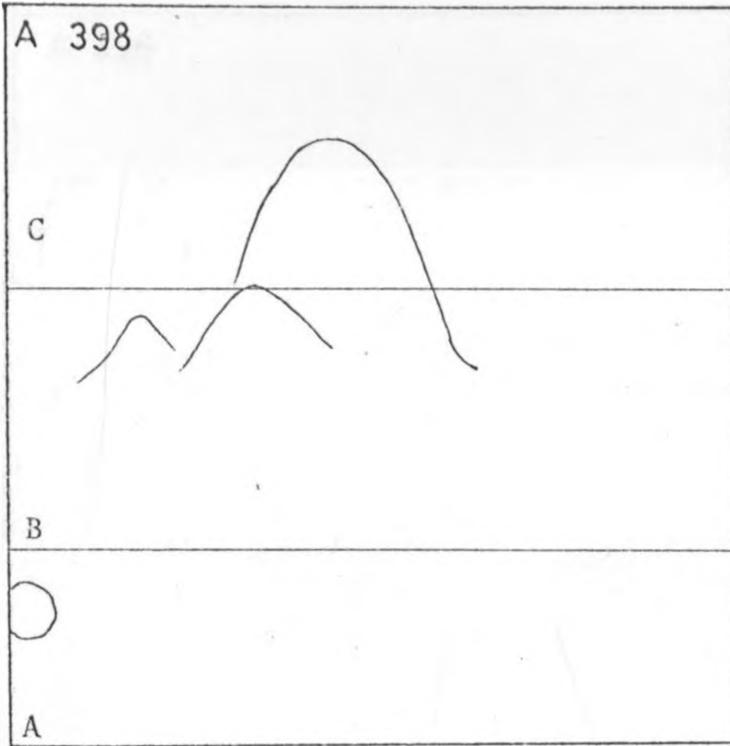
Fig. 25 The precipitation pattern obtained when a
C. bovis saline extract was added to the inter-
mediate gel between HCF and SA-SHCFS.

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.
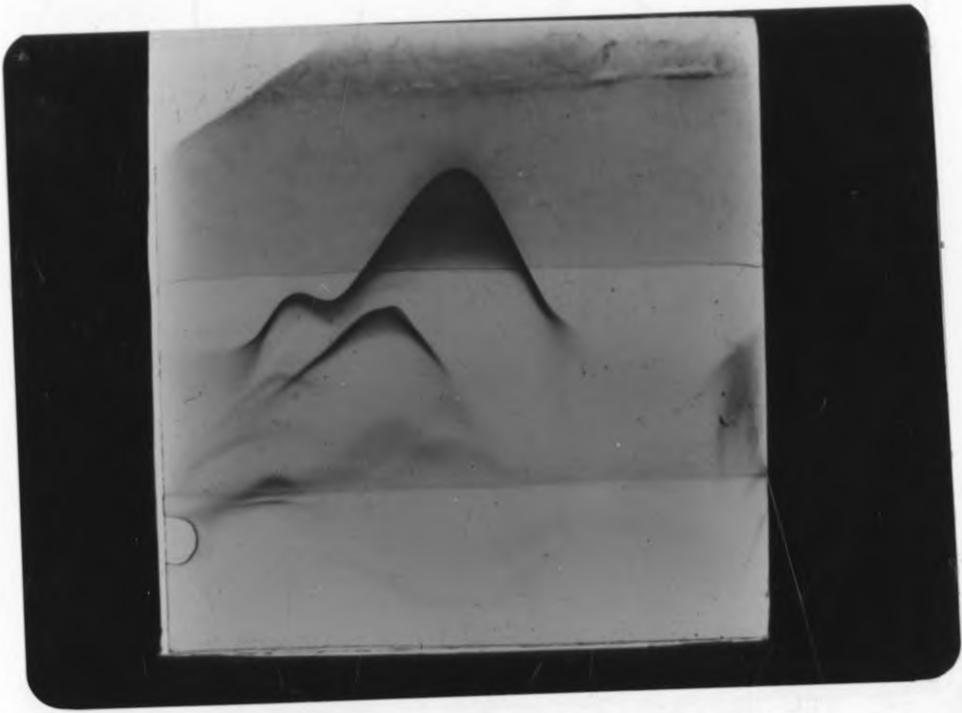
Fig. 26   The precipitation pattern obtained when anti-C. bovis was added to the inter-mediate gel between HCF pool and SA-SHCFS.

- 108 -
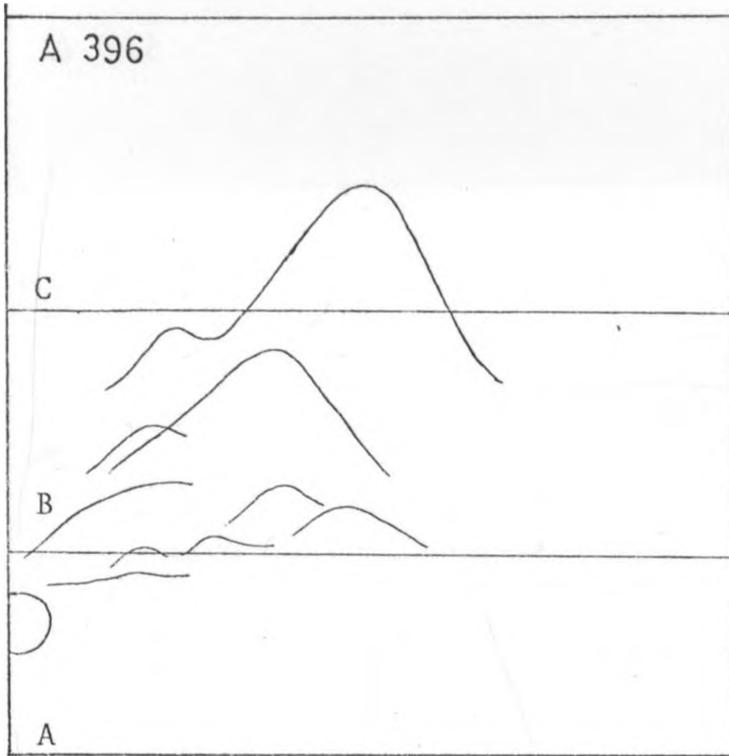
Fig. 26   The precipitation pattern obtained when anti-
C. bovis was added to the intermediate gel
between HCF pool and SA-SHCFS.

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.
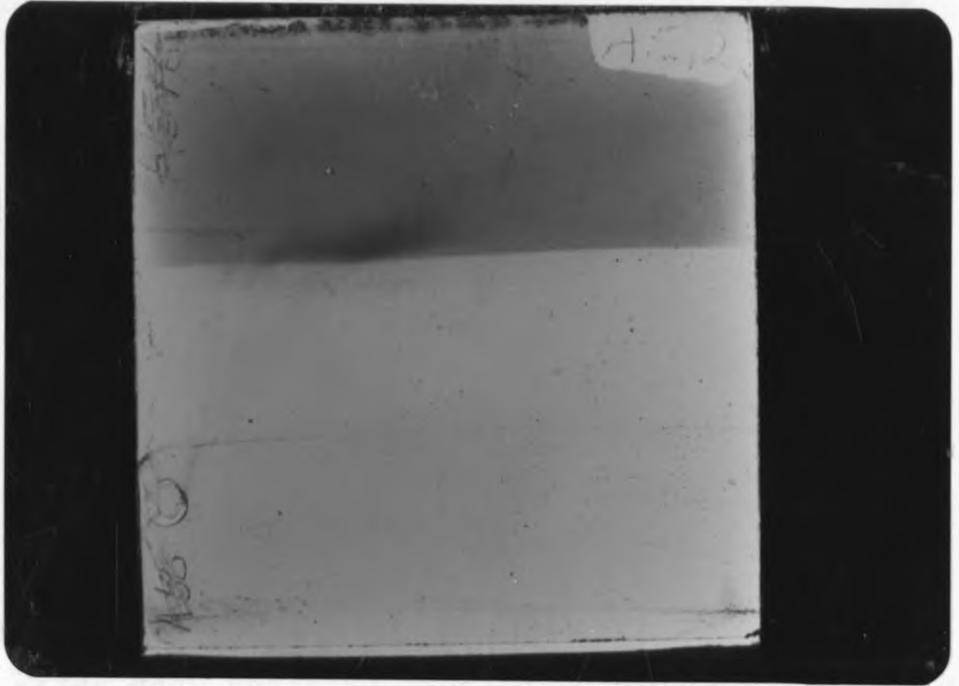
Fig. 27   The precipitation band obtained when
          a H. contortus saline extract was run
          against SA-SHCFS and a blank inter-
          mediate gel.
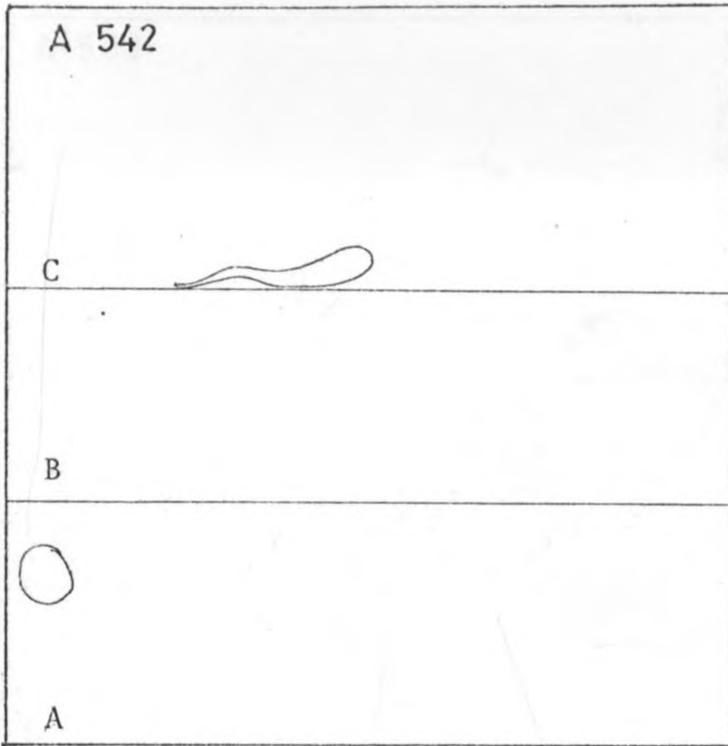
Fig. 27 The precipitation band obtained when a
H. contortus saline extract was run against
SA-SHCFS and a blank intermediate gel.

KEY:

A - H. contortus saline extract was electro-
      phoresed 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-SHCFS.

KEY:

A - HCF pool was electrophoresed in the first
direction.

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

C - SA-SHCFS 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-F. <u>gigantica</u> serum was added to
          the intermediate gel between HCF pool
          antigens and SA-SHCFS.

Fig. 29   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.

The photograph is the negative and appears when

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tly elevated.
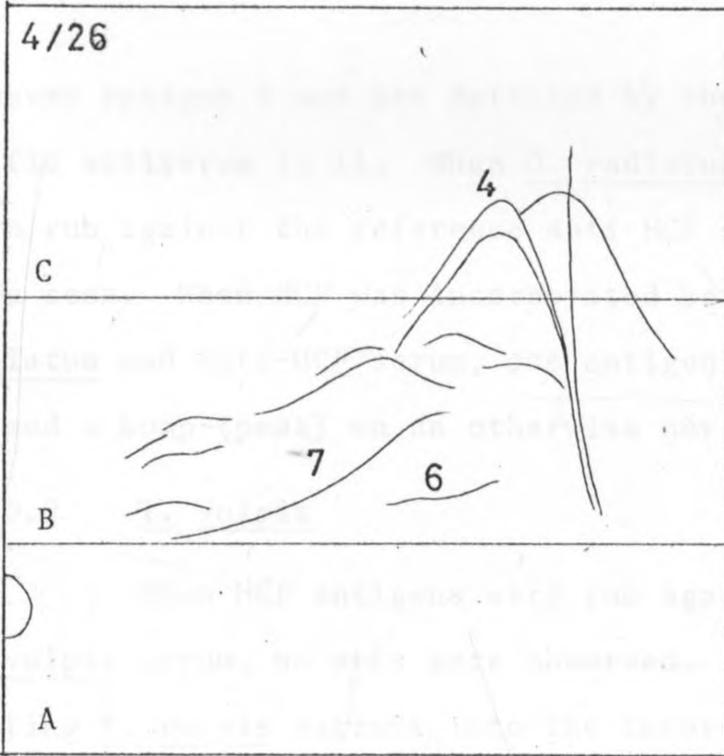
Fig. 31 The precipitation pattern obtained when
rabbit anti-O. radiatum was added to the

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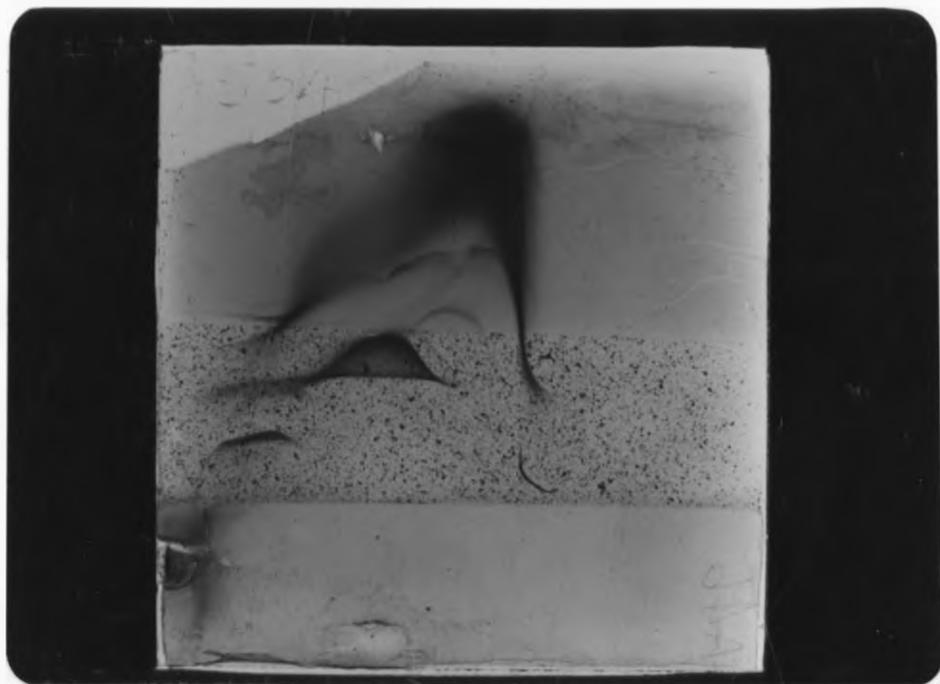
Fig. 32   The precipitation pattern obtained when
           a saline extract of T. vulpis was added
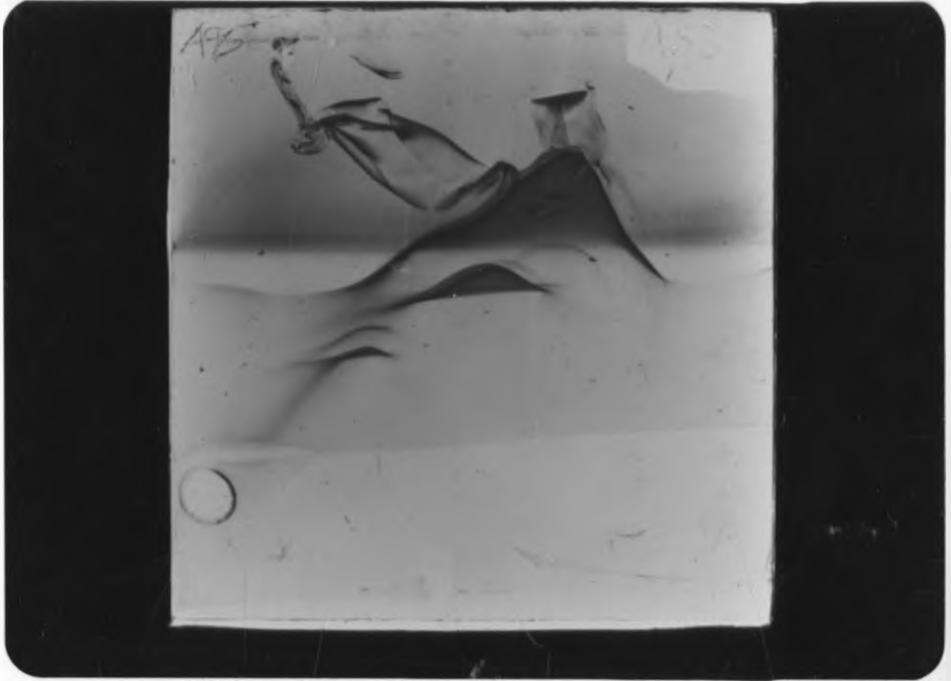   to the intermediate gel between HCF
           pool antigens and SA-SHCFS.
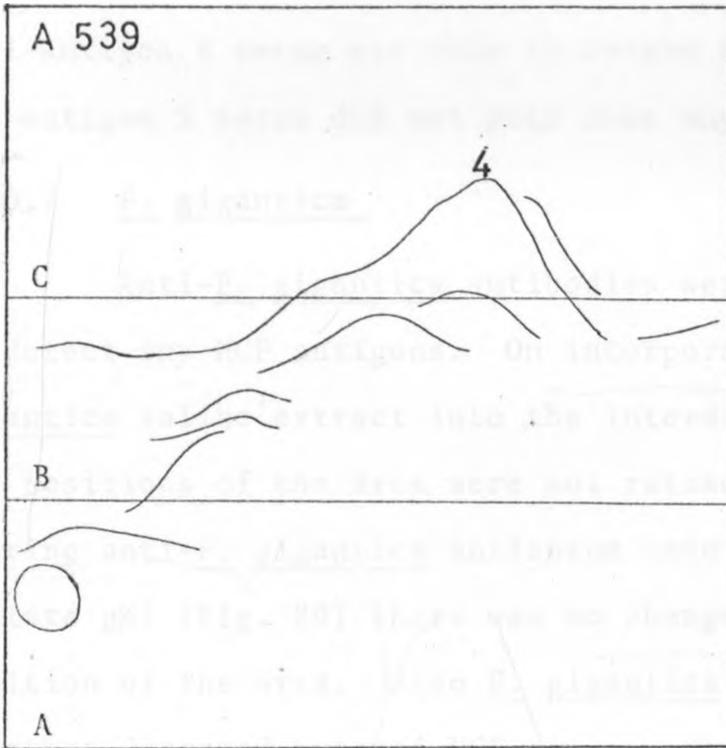
Fig. 32 The precipitation pattern obtained when a
saline extract of T. vulpis was added to the
intermediate gel between HCF pool antigens
and SA-SHCFS.

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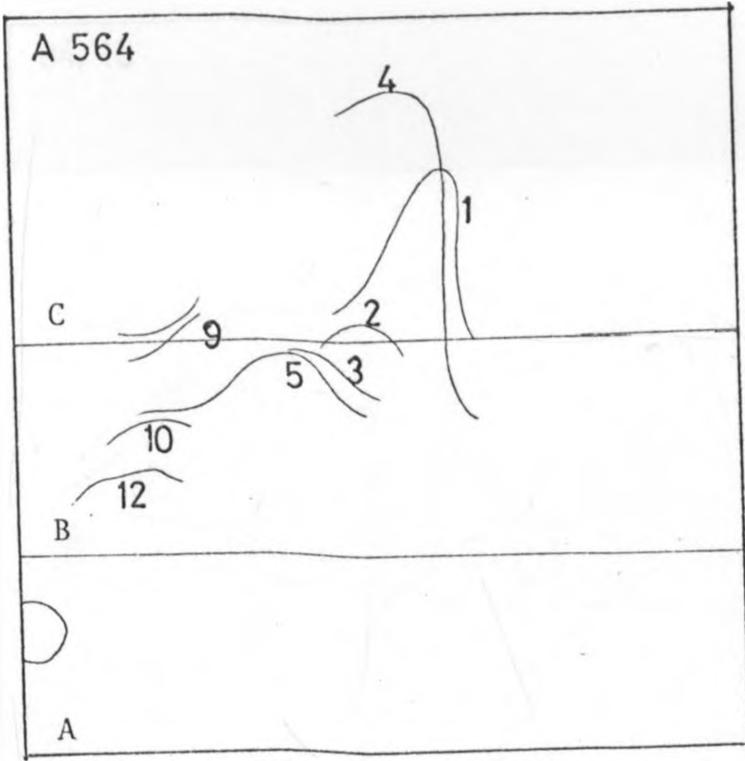
A - HCF pool antigens were electrophoresed in
the first direction run.

B - Agarose mixed with T. vulpis saline extract.

C - SA-SHCFS mixed with agarose.

RESULTS:

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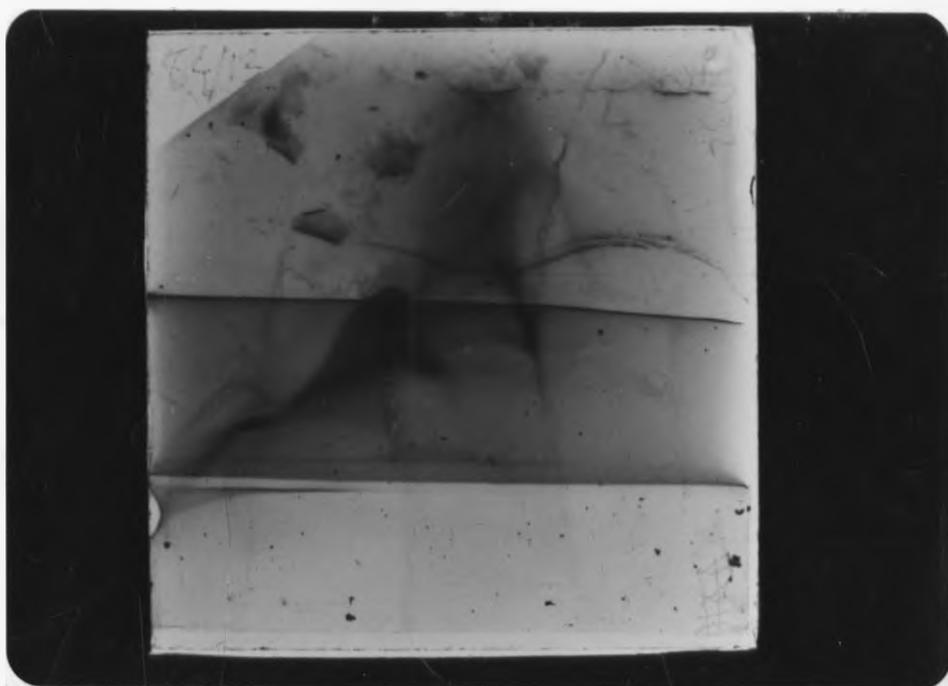
Fig. 33   The precipitation pattern obtained when
          rabbit anti-T-vulpis serum was added
          to the intermediate gel between HCF
          pool antigens and SA-SHCFS.

Fig. 33 The precipitation pattern obtained when
rabbit anti-T. vulpis serum was added to the
intermediate gel between HCF pool antigens
and SA-SHCFS.

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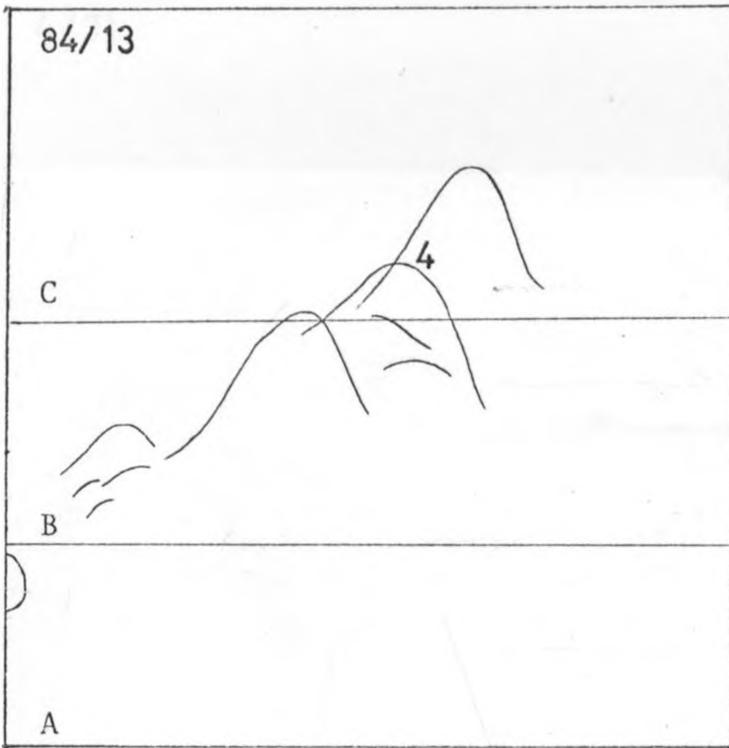
A - HCF pool antigens were electrophoresed in
the first direction run.

B - Rabbit anti-T. vulpis serum was added to
the intermediate gel.

C - SA-SHCFS mixed with agarose.

RESULTS:

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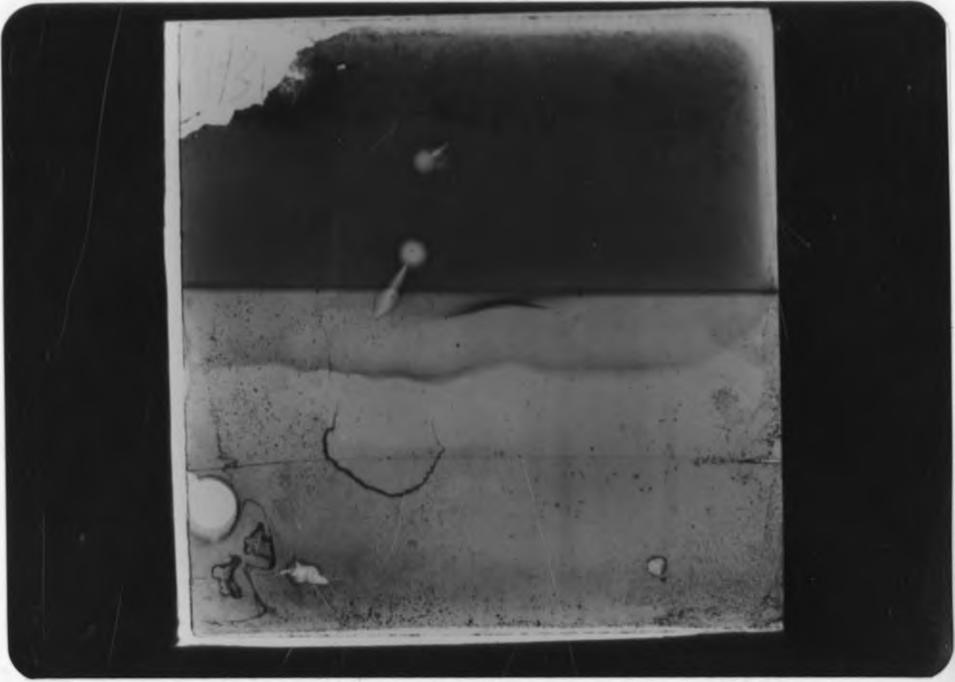
Fig. 34   The precipitation pattern obtained when
          HCF pool antigens were run against rabbit
          anti-A. suum.
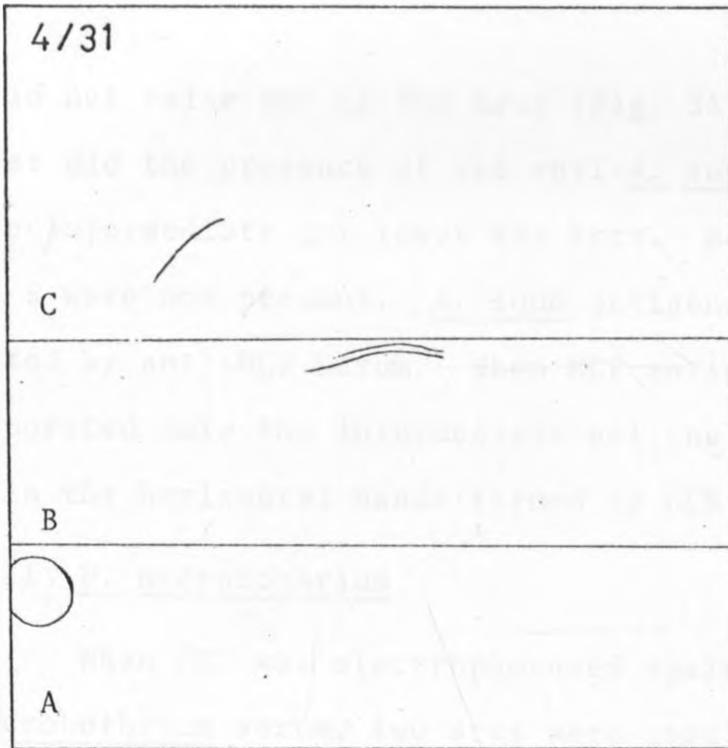
Fig. 34 The precipitation pattern obtained when HCF
pool antigens were run against rabbit anti-
A. suum.

KEY:

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

B - Blank agarose.

C - Rabbit anti-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-
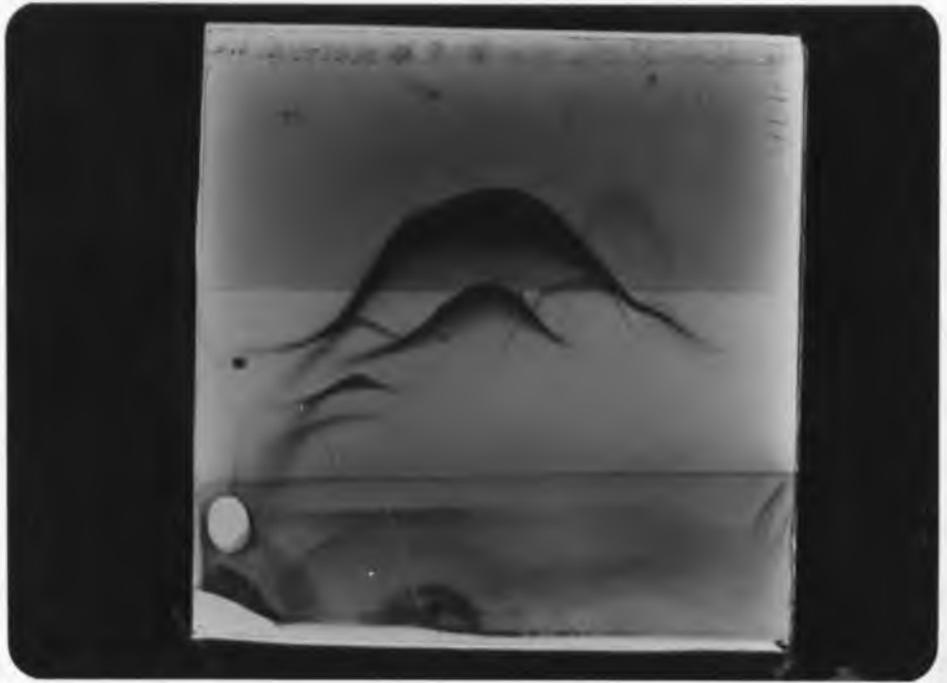antigen 5 serum did form an arc with A. centripunctata
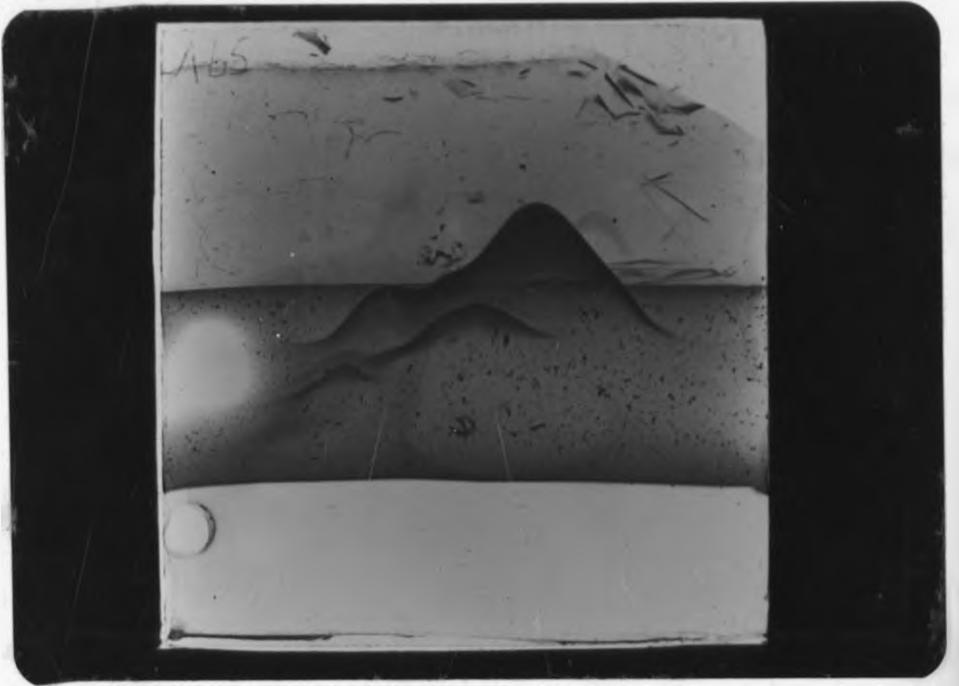
Fig. 35   The precipitation pattern obtained when
a saline extract of A. suum was added
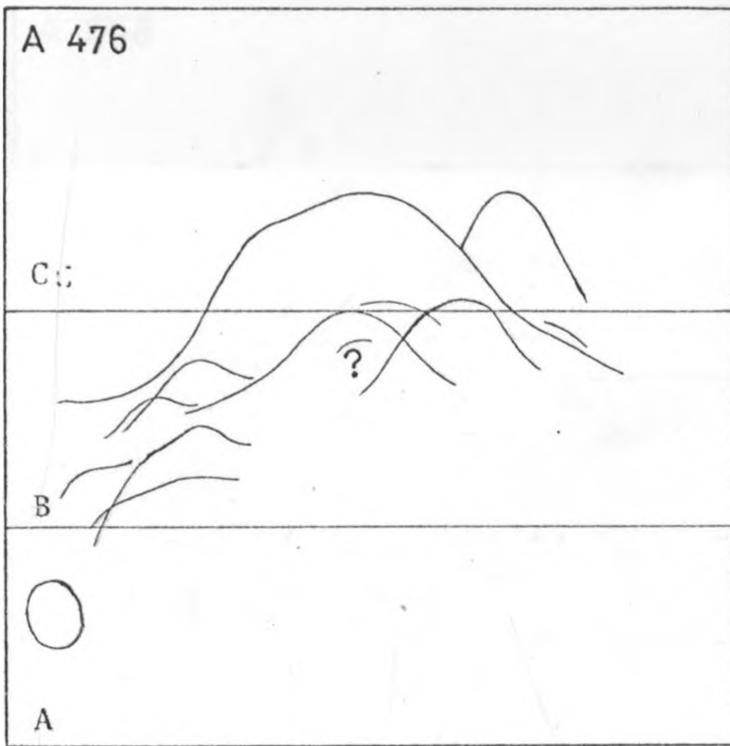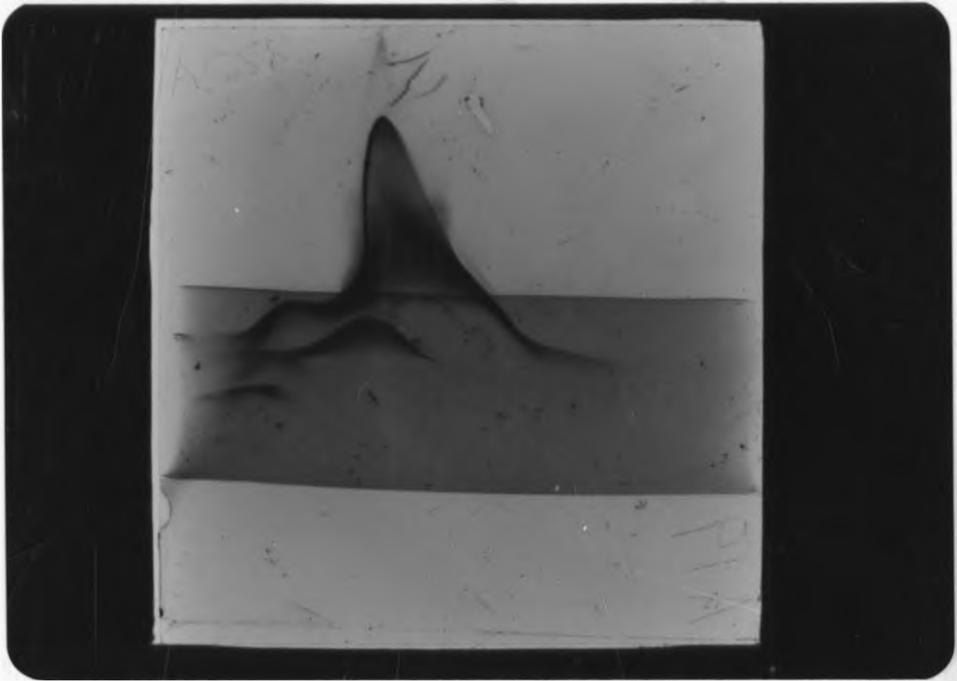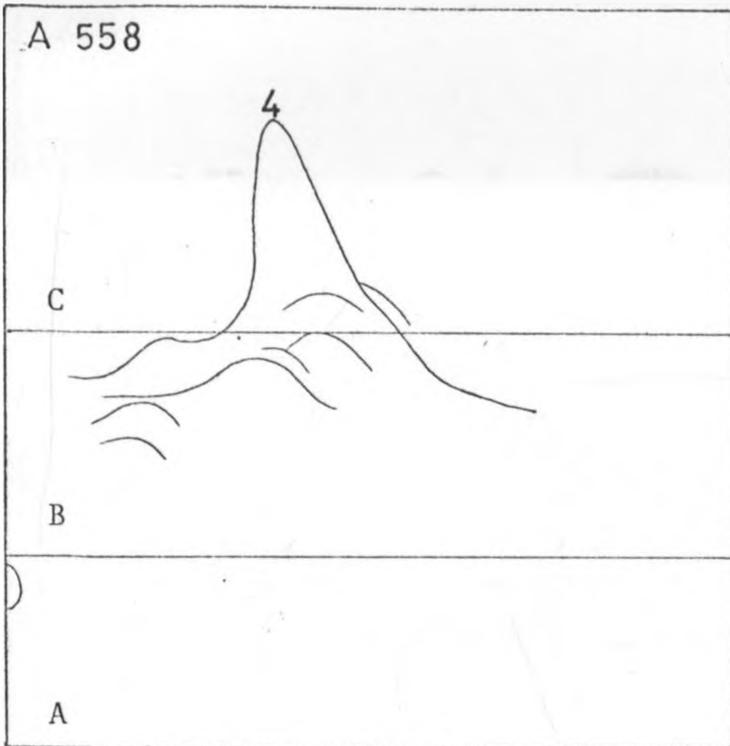to the intermediate gel between HCF pool
antigens and SA-SHCFS.

Fig. 35 The precipititation pattern obtained when
a saline extract of A. suum was added to the
intermediate gel between HCF pool antigens
and SA-SHCFS.

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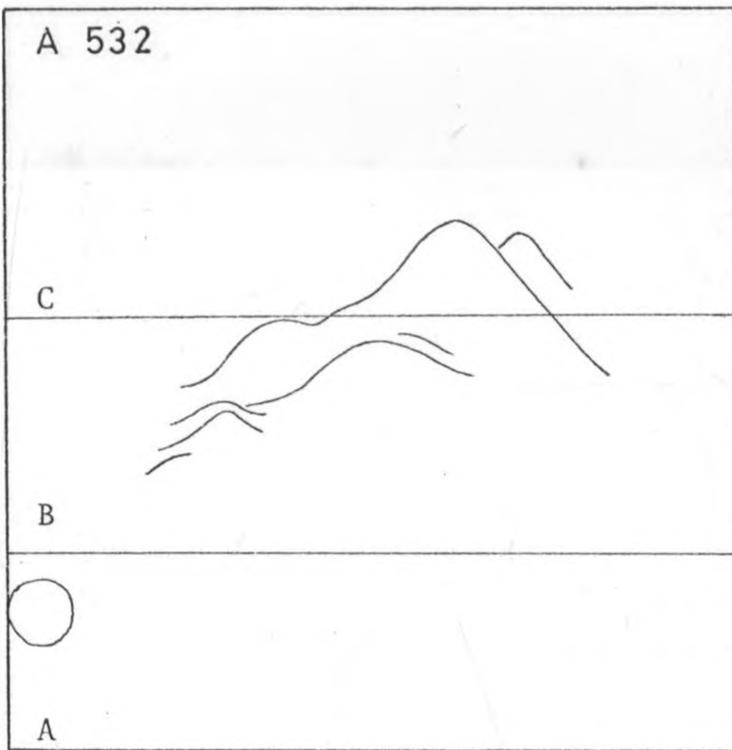
A - HCF pool antigens were electrophoresed
    in the first dimension run.

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.
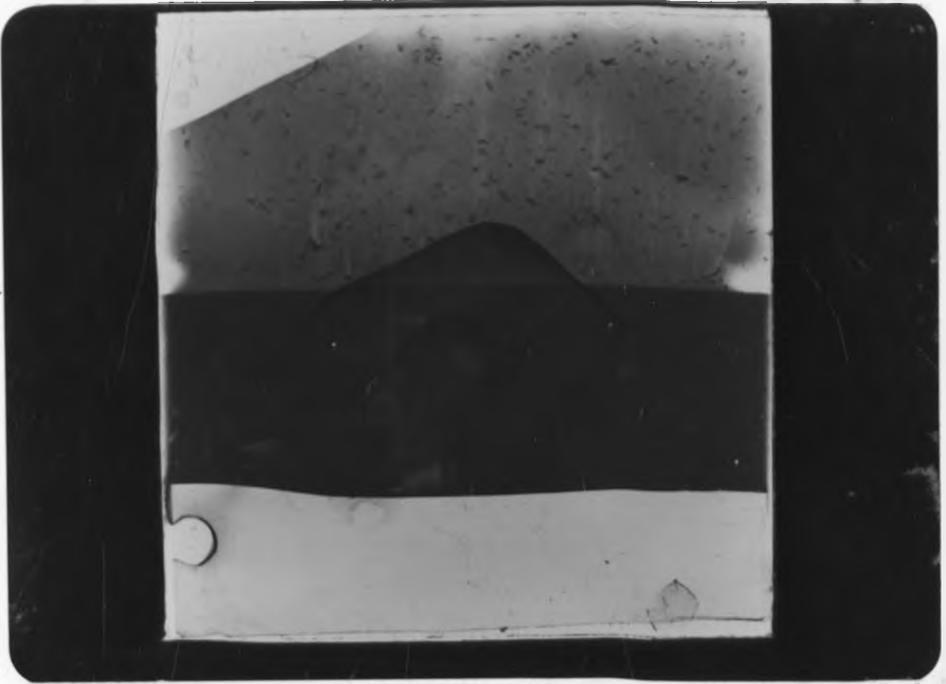
Fig. 36 The precipitation pattern obtained when
a saline extract of P. microbothrium was
added to the intermediate gel between
HCF pool antigens and SA-SHCFS.

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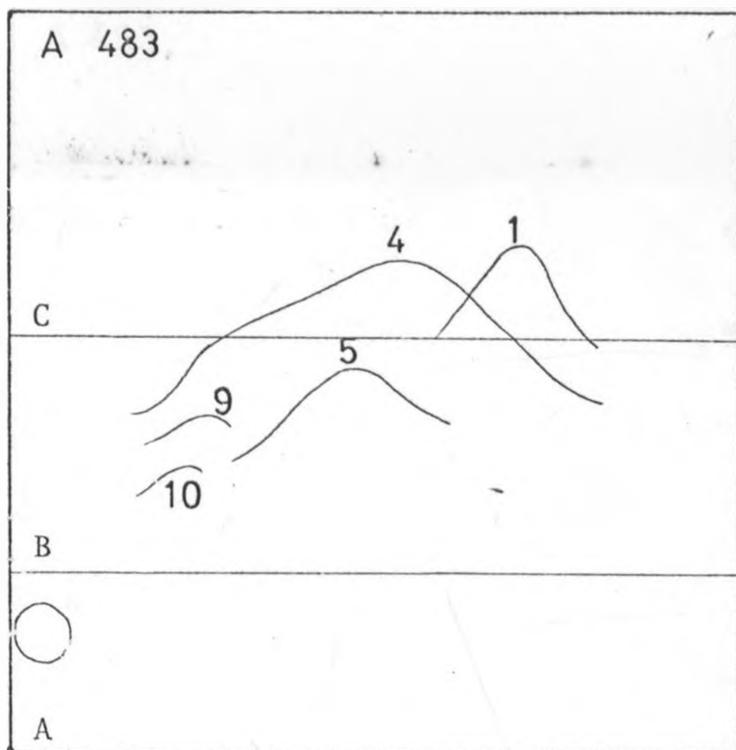
A - HCF pool antigens were electrophoresed in
the first dimension run.

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.
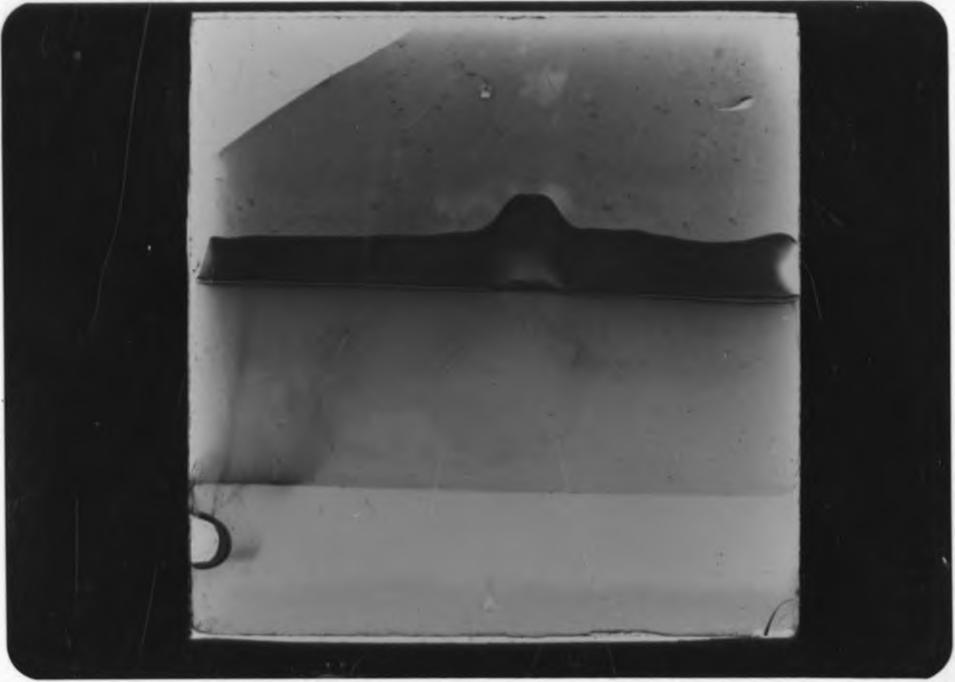
Fig. 37 The precipitation pattern obtained when
        P. microbothrium antigens were run
        against SA-SHCFS with HCF pool antigens
        in the intermediate gel.
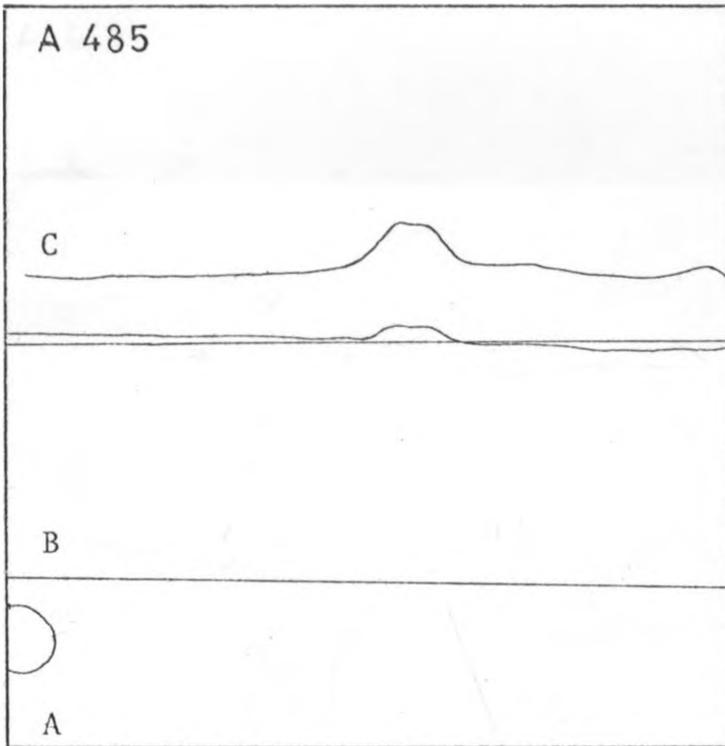
Fig. 37 The precipitation pattern obtained when
P. microbothrium antigens were run against
SA-SHCFS with HCF pool antigens in the inter-
mediate gel.

KEY:

A - A saline extract of P. microbothrium
was electrophoresed in the first dimen-
sion 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.
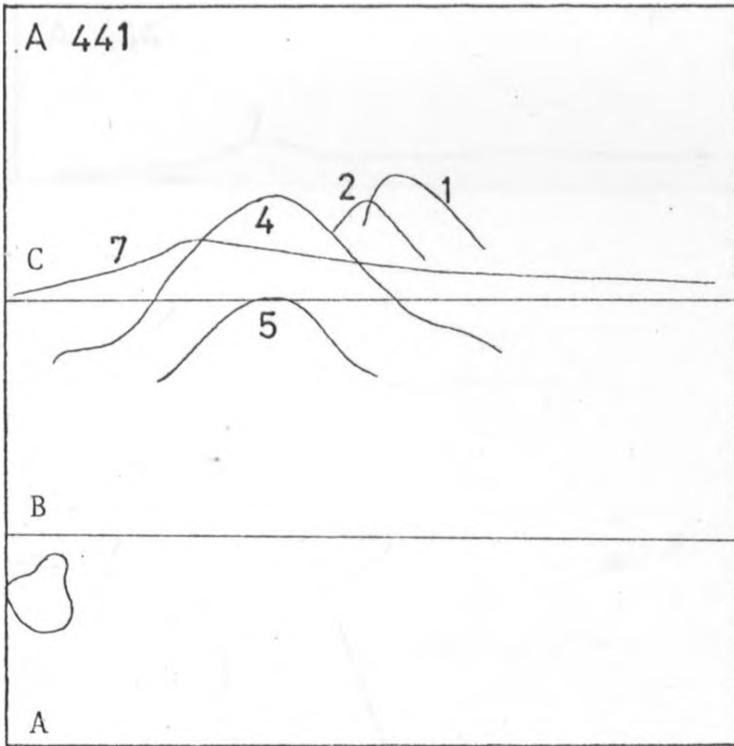
Fig. 38   The precipitation pattern obtained when HCF
          pool antigens were electrophoresed against
          SA-SHCFS.

          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.

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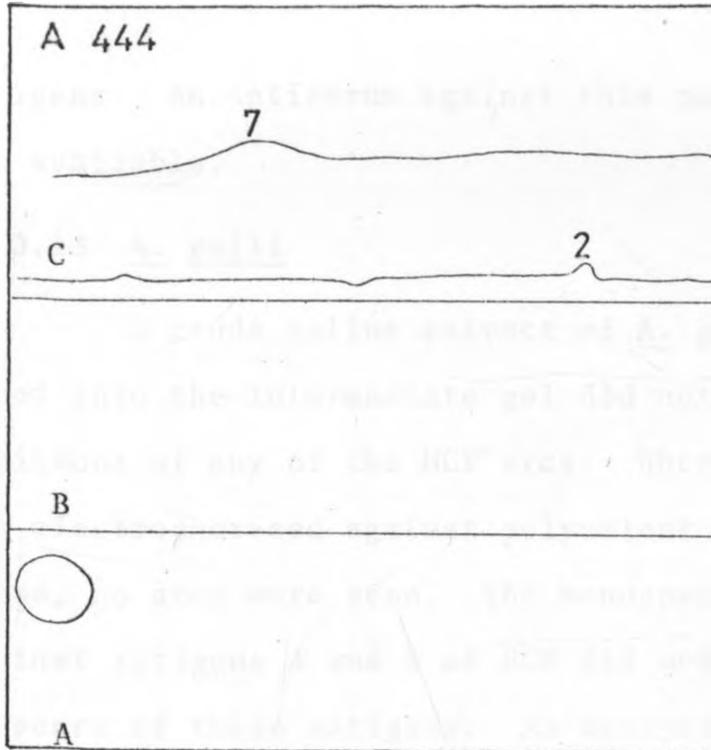
ediate

Fig. 39 The precipitation pattern obtai[...]
A. centripunctata antigens were [...]
SA-SHCFS while the intermediate [...]
HCF pool antigens.

KEY:

A - A saline extract of A. cent[...]
was electrophoresed in the [...]

B - HCF pool antigens were adde[...]
intermediate gel agarose.

C - SA-SHCFS mixed with agarose[...]

RESULTS:

Arcs 2 and 7 showed peaks on h[...]
Another horizontal line did no[...]

antigens. An antiserum against this parasite was
not available.

4.10.13 A. galli

A crude saline extract of A. galli incorpo-
rated 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
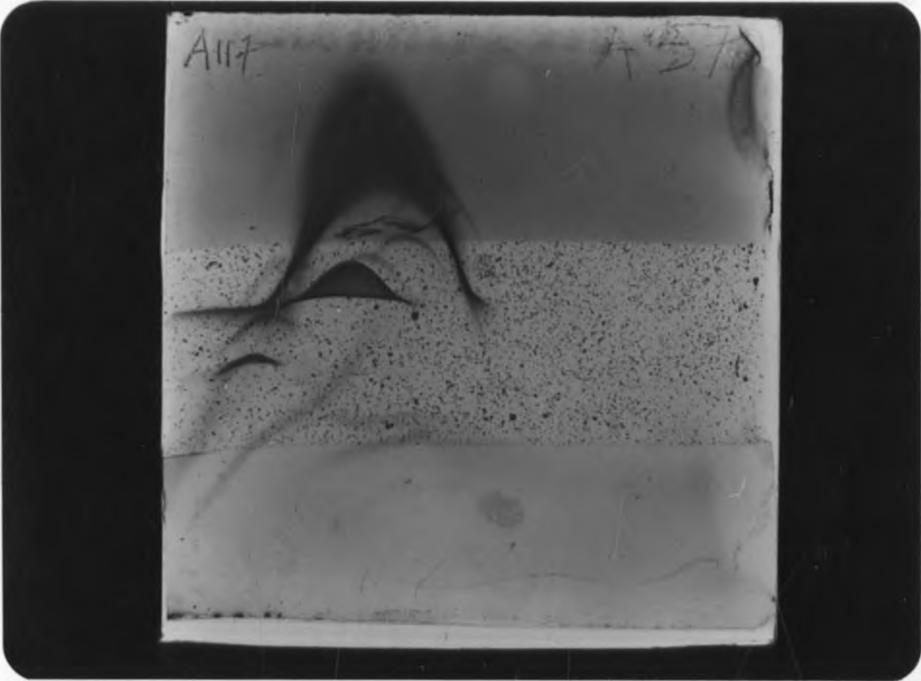
Fig. 40   The precipitation pattern obtained when
          HCF pool antigens were run against
          SA-SHCFS while the intermediate gel
          contained a saline extract of S. lupi.
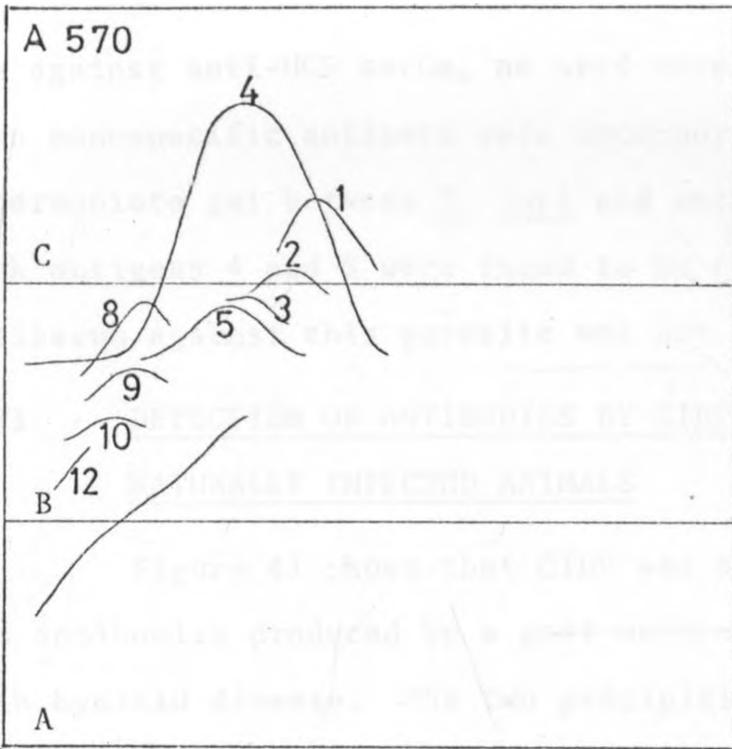
Fig. 40 The precipitation pattern obtained when HCF
pool antigens were run against SA-SHCFS while
the intermediate gel contained a saline
extract of S. lupi.

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.
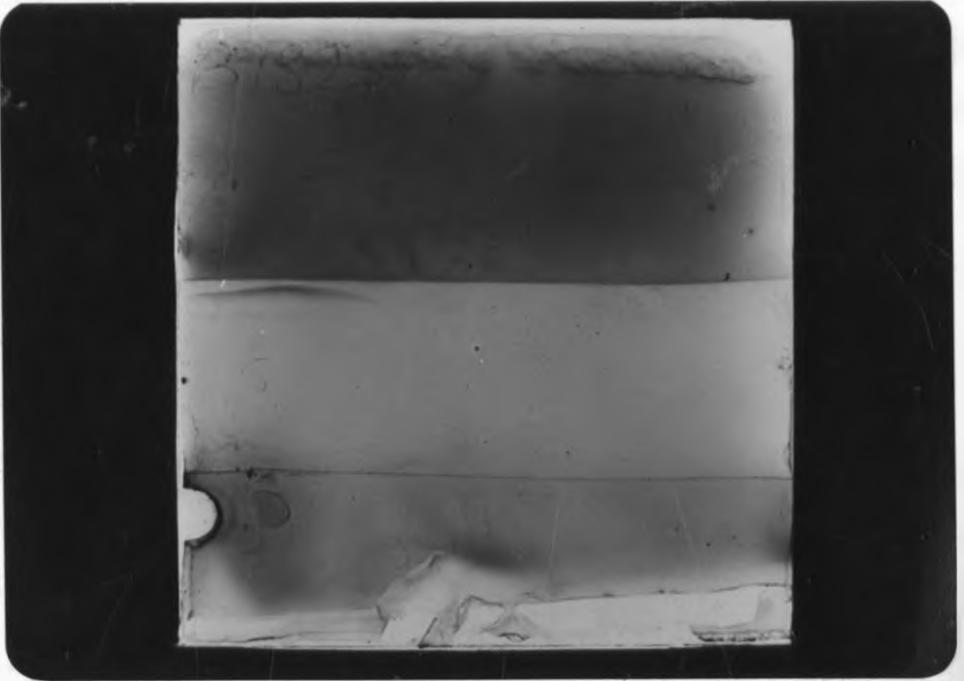
Fig. 41   The precipitation pattern obtained when
          HCF pool antigens were run against
          serum from a goat naturally infected
          with hydatidosis.
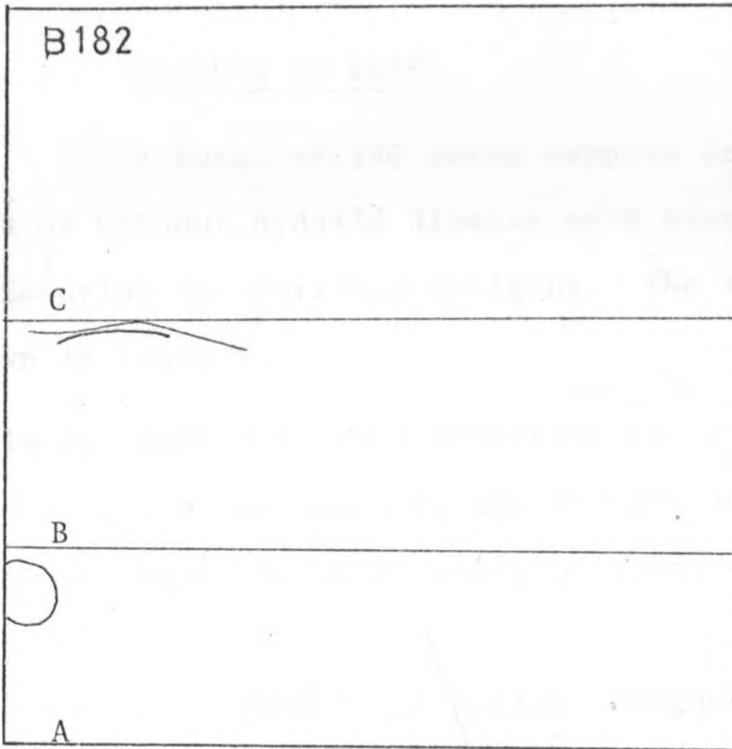
Fig. 41   The precipitation pattern obtained when HCF
          pool antigens were run against serum from a
          goat naturally infected with hydatidosis.

          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.

True (post mortem) Diagnosis

		+	−	Total
	+	127	15	142
ELISA	−	3	35	38
		130	50	180

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

Specificity = $\frac{35}{50}$ x 100 = 70%

Predictive value = $\frac{127}{142}$ x 100 = 89.4%

Using both antigens 4 and 5, the sensiti-
vity 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%.

5.   DISCUSSION

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 hydati-
dosis in livestock too can be detected by immunodiagn-
ostic technics. A major drawback to immunodiagnostic
tests in hydatidosis of livestock is the presence of

5. DISCUSSION

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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

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 immuno-diagnosis of hydatid disease. Farag et al. (1975) utilised ELISA, a fairly sensitive test, in the diag-nosis 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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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

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. lepraemurium 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 demon-
strated 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 sophist-
icated equipments which may not be within the reach of
particularly rural hospitals or veterinary investi-
gation laboratories where hydatidosis is endemic.
Another obstacle in this test is the need to concen-
trate 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 concent-
rated 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 intra-muscularly 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 character-
istic 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 25 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 migrat-
ing 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 gener-
ally 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 structrual groupings within their molecule.
Thus cross reactivities pose a problem in immuno-
diagnosis.

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 amd 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 activited
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. multi-
locularis (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 inter-
mediate gel in CIEP method.

Fig. 42 Reproduced from Closs et al. (1975)

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 $a_1 - a_n$. $AbA \rightarrow a_1 + a_2 \ldots + a_{n-m}$ – the reference antibody is directed against the determinants $a_1 - a_{n-m}$.

I $AgA = a_1 + a_2 + a_3 \quad + a_n$.
$AbA \rightarrow a_1 + a_2 + a_3 \quad + a_{n-m}$

II $AgA = a_1 + a_2 \quad + a_n$. $AbA \rightarrow a_1 + a_2$
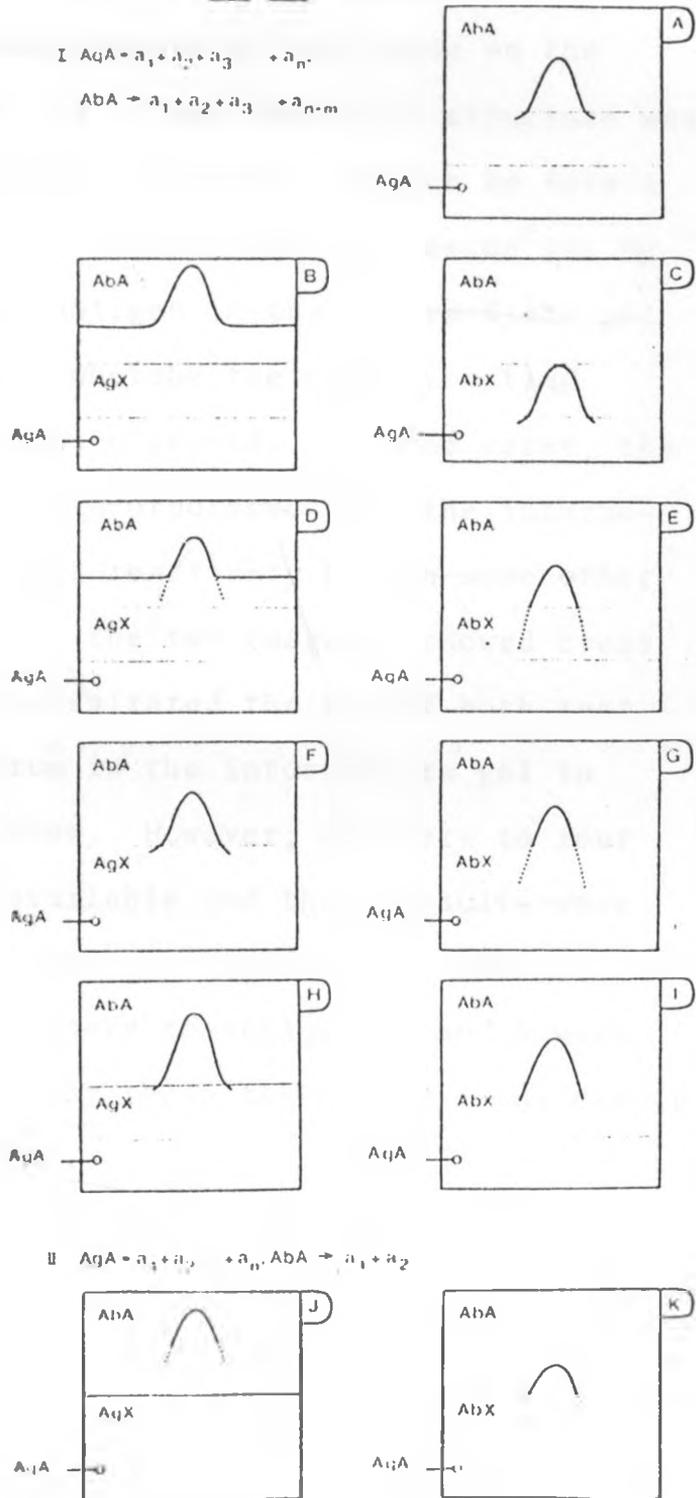
With this method it was possible to study
the effect of a test antigen or antiserum on the
reference pattern. If a test antigen's structure was
not close enough to the reference antigen to form a
precipitate, its cross reactivity was tested for by
the use of the test antigen in the intermediate gel
(Closs et al., 1975) whereby the cross reacting
antigen's arc would be elevated. In some cases, the
antisera and antigen incorporated into the interme-
diate 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 conclu-
sive 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 lipo-
protein 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 β-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 instestinal worms are only weakly immunogenic
(Weinmann, 1966), their antibody titres are bound to
be low and not be a serious obstacle for the immuno-
diagnosis 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 immunodia-
gnosis 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.

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

7. 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. giganti

8. 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.

9. 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 seroligcal tests.

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APPENDIX 1.

Buffers and solutions used in the preparation
of insoluble immunosorbents.

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

> where R = required concentration
>
> V = total volume needed
>
> O = original concentration

R x V ÷ O = amount of O to be mixed with distilled
water to make V.

1.2 0.1M sodium bicarbonate (NaHCO₃) 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 stir-
ring. 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.

APPENDIX 2.

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 dissoved 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 immuno-
electrophoresis)
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
disstilled water and the pH adjusted to 8.4 to
8.6.

2.2.1   <u>For electrophoresis chambers.</u>
        The above buffer was used.

2.2.2   <u>For preparation of agarose.</u>
        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 immuno-
electrophoresis.

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

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 (NaN$_3$) Preservative	0.2 g

APPENDIX 4.

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

APPENDIX 5.

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 NaHPO$_4$: 21.3g dissolved in
1000mls of distilled water.

Solution B - 0.15 M NaH$_2$PO$_4$. 2H$_2$O: 23.4g dissolved
in 1000 mls 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 NaH$_2$PO$_4$.2H$_2$O: 78.0g dissolved in
in 1000 mls of distilled water.

Solution B - 0.5 M Na2HPO$_4$: 70.98 g dissolved in
1000 mls 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 mls

The pH was adjusted to 5.0 using concentrated
ammonia. Distilled water was added to make
1000 mls.

5.4 <u>Diluent for the HCF antigen used to coat the</u>
   <u>ELISA plates</u>.

   0.015M 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 <u>Diluent for the test serum</u>.

   0.05M 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 <u>Diluent for HRPO conjugate</u>

   0.05M 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
O-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 mls
Tween 80	5 mls
Distilled water	10,000 mls
Sodium azide	2 g