"PARTIAL CHARACTERIZATION OF THE ANTIGENS OF CYSTICERCUS BOVIS"

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DEDICATED TO MY PARENTS AND ALL MY FRIENDS

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S U M M A R Y

SUMMARY

Although the modes of transmission of Cysticercus bovis to cattle and Taenia saginata to man have been known for a number of years, bovine cysticercosis and human taeniasis still exist as a world-wide problem.

The magnitude of the economical and nutritional losses caused by bovine cysticercosis is unknown but can be estimated to be considerable, especially in highly endemic areas.

Many factors may account for the lack of effective control measures, one of them being the lack of a reliable antemortem diagnostic procedure in cattle.

Several attempts have been made to establish a specific and sensitive immunodiagnostic procedure which will serve as an adjunct to the present meat inspection procedures. Several achievements have been recorded in this regard, despite the disappointments encountered.

Work described in this thesis attempts to define the antigens of C. bovis and T. saginata and their relationship to some common parasites. Some of the antigens were shown to possess a high degree of specificity which is deemed necessary for immunodiagnostic procedures.

This work can be divided into four parts. The first part describes the immunological characterization of the antigens of C. bovis and T. saginata. The antigens were compared by the double immunodiffusion method in gel,

using specific antisera produced in goats, rabbits and calves. Three antigenic components were found to be common to C. bovis and T. saginata but only one of these was unique to these parasites. The other two components showed cross reactivity with other parasites. As the unique antigen was detected at the end of this study, time did not allow further work on this promising finding.

The antigens of C. bovis origin were further examined by crossed immunoelectrophoresis. A reference pattern was established using pooled rabbit anti-C. bovis serum and a homogenate of "Inner membrane-scolex" of C. bovis (bladder worm from which the fluid had been removed) as the reference antigen. The precipitin lines were arbitrarily numbered starting with the most anodic component. Fifteen antigen-antibody precipitating systems were defined by this method.

Antigen preparations derived from C. bovis and C.tenuicollis were also examined for the presence of host components, since it has been established that many cystic stages of parasites contain such components which may be responsible for non-specific reactions in several serodiagnostic tests for parasitic infections. The major contaminating host components in C. bovis and C. tenuicollis were shown to be IgG and albumin. All the antisera prepared against C. bovis and C. tenuicollis antigens were, therefore, absorbed with insoluble whole serum immunoadsorbents to remove reactivity with host components. Since other workers have described the presence of blood group antigens, such as P_1-active substances and ABO(H)-like substances, it was

considered necessary to absorb the antisera with red cells of appropriate groups also.

In the second part of this study, the cross-reactions between C. bovis, T. saginata and more than 16 other parasites were examined by immunodiffusion in agar gel. A wide range of cross-reactivity was found, but some antigens of C. bovis gave cross-reactions with only a few parasites, notably C. cellulosae, C. tenuicollis and T. hydatigena, using antisera to C. bovis and T. saginata. Similar reactions were observed when goat anti-C. tenuicollis serum was used. The precipitin line which was common to C. bovis, T. saginata, T. hydatigena, C. tenuicollis and C. cellulosae was cut out of agar gel, sonicated and inoculated into a goat to make a specific antiserum to this antigen which showed a limited cross-reactivity. Using the crossed immunoelectrophoresis reference pattern established for C. bovis antigens and the specific antiserum in the intermediate gel, the cross-reacting antigen was shown to be identical with Antigen 11 of the reference system.

The third part of this work involved the isolation and characterisation of Antigen 11. The antigen was eluted from an affinity column prepared by coupling the IgG fraction from the Antigen 11-specific antiserum. Antigen 11 was further characterised by isoelectric focusing and polyacrylamide gel electrophoresis into which sodium dodecyl sulphate was incorporated (SDS-PAGE). Antigen 11 was found to have a molecular weight between 63,000 and 78,000 daltons by SDS-PAGE analysis and was isoelectric at pH 4.47 to 5.50.

This antigen was used in the last part of this work.

In the fourth and last part of the study, an attempt was made to determine whether Antigen 11 could be of serodiagnostic value. Serum samples from known experimentally infected and non-infected cattle were tested for the presence of antibodies to Antigen 11 using the solid-phase radioimmunoassay. High levels of IgG and comparatively low levels of IgM antibodies were detected. The IgG antibody levels were detected 5 weeks post infection and they reached the peak at week 24. Thereafter, the antibody levels showed a gradual fall up to week 64 post infection when the last sample was tested. There was a clear-cut antibody level difference between the known infected and non-infected animals from non endemic areas, which shows that Antigen 11 could be of diagnostic value. The ability of Antigen 11 to detect naturally infected animals has not yet been investigated and more work is needed to define its reactions with sera from animals infected with other parasites.

The possible use of Antigen 11 of C. bovis which shows a narrow range of cross-reactivity may reduce substantially the occurrence of non-specific reactions in serodiagnostic tests. However, further studies are required on the other 14 antigens, especially the one which appears to be unique to C. bovis and T. saginata.

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INTRCDUCTION

1. Introduction

The problem of bovine cysticercosis caused by *Cysticercus bovis*, the larval stage of *Taenia saginata* is of world-wide importance, both as a zoonosis and for economic reasons. The economic importance of cysticercosis is particularly felt in developing countries where attempts are being made to establish a cattle rearing industry so as to export fresh meat and meat products.

A very conservative estimate gives the number of infected animals on the African continent alone to be not less than 20 million head (Mann, 1978). The following countries have a higher than 10% incidence of bovine cysticercosis:- The Congo Republic, Guinea, Uganda, Kenya, Libya, Sierra Leone, Somalia, Ivory Coast, Angola, Nigeria and Tanzania (Froyd, 1965; Dada and Belino, 1978).

The incidence of *C. bovis* is also increasing in several European countries (Pawloski, 1971). Table 1a shows the prevalence rates obtained in several European slaughterhouses and Table 1b shows the prevalence rates in several countries in the world. At present, more than 3% of cattle slaughtered in the German Democratic Republic harbour *C. bovis*, while more than 1% of the human population are infected with *T. saginata* (Hiepe and Buchwalder, 1978).

Studies carried out in the United States of America (U.S.A.) over a five year period (1963-67) by Schultz and his co-workers (1970) gave numbers of cases of *C. bovis* ranging between 12,000 and 16,000 per annum. The

Table 1a: Percentage of infected carcasses found at routine meat inspection in European slaughter-houses (Data from Pawloski, 1971).

Slaughter House	Year of Survey					
	1945	1951	1953	1955	1962	1964
Prague	0.34	NR	NR	1.6	NR	3.5
Genova	NR	3.4	8.0	NR	NR	NR
Pozman	NR	NR	NR	0.5	2.3	NR

NR = Not Recorded

Table 1b: Prevalence of C. bovis in cattle in various countries (Data from Pawloski and Schultz, 1972)

Country	% of cattle infected
Australia	0.21
Argentina	0.62
Bulgaria	0.62
Brazil	1.0
Cuba	0.16
Czechoslovakia	1.39
Equador	0.70
France	0.19
German Democratic Republic	2.63
Federal Republic of Germany	2.06
Guatemala	1.72
Holland	1
Hungary	0.224
Polland	0.58 ± 0.02
Sweden	1
India	1.4
Ireland	1.0

endemic areas of South Western U.S.A. have a constant level of infection of about 1%. One single sporadic epidemic in the feed lots caused a loss of about $250,000 (Schultz, 1974). While such epidemics had been thought to be a hazard only in the South Western U.S.A., a recent outbreak in Ontario (Canada) showed that similar incidents may occur in unsuspected locations (Shultz, 1974).

The renewed interest in bovine cysticercosis in the U.S.A. has led to federal legislation prohibiting the trimming of lightly infected carcasses, thus placing a greater economic burden on the beef producer. However, Schultz and his collegues (1970), indicated that there was no evidence of a decrease inspite of these new regulations.

The economic loss resulting from the cost of freezing and down-grading of lightly infected carcasses or total condemnation of heavily infected carcasses is unknown, but estimated at approximately US$ 10 million per year in East Africa alone, and this is increasing (Urquhart, 1958; Mango, 1971; Mann, 1974; Taylor, 1975; Gathuma, 1979; Wilwig et al., 1978).

With regard to the control of this disease, the following methods are available:

(a) Treatment of the human reservoir combined with education in proper sanitation.

(b) Improvement of hygienic standards.

(c) Centralized and properly supervised meat

inspection.

(d) Antemortem diagnosis

(e) Development of an efficient drug which will kill the larval stages in the muscle without lowering the quality of meat (Pawlowski et al.,1978).

As a zoonosis, however,bovine cysticercosis presents a challenging problem, since current methods of control are of limited efficacy, especially in poorly developed countries where the hygienic standards are low (Sewell and Gallie, 1974). There is, for instance, conflicting evidence to date on the efficacy of drugs which can kill the larval stage in the musculature without reducing the quality of the meat (Kondela and Schanzel, 1978; Graig and Ronald, 1978; Walther and Koske, 1979). Elimination of T. saginata from man has likewise proved to be deficient, and it has been estimated that more than 40 million people in the world are infected with this parasite (Stoll, 1947).

The method based on meat inspection is inadequate, since it depends only on the examination of a small portion of musculature. Moreover, there is a practical limit as to the number of incisions which are permissible to avoid gross mutilation of the carcass which would lower its marketability (Thornton, 1968a). Such a technique can therefore fail to detect the lightly infected but epidemiologically significant infective carcass (Mann and Mann, 1947).

Theoretically, the problem of taeniasis and cysticercosis can be solved by improvement of hygienic standards, most important of which is the proper disposal of human faeces, but this cannot be considered fully adequate since mature segments of T. saginata are known to be able to move actively out of the anus without the patient's notice. Therefore, contamination of water and pastures by such a patient need not necessarily occur by indiscriminate defaecation (Mönnig, 1941). Mass chemotherapy of Taenia carriers is applicable, but unless it is combined with efficient disposal of excreta after treatment such a method may increase the incidence since it is known that mature segments expelled after therapy are infective (Urquhart, 1958).

In Europe, sewage effluent which is an important source of irrigation of pasture, is also an important factor in the epidemiology of bovine cysticercosis. Various methods of sewage treatment have been found to be inefficient in killing mature eggs of T. saginata (Silverman and Griffiths, 1955; Arkipova, 1977, Hammerberg et al., 1978) and the ova can survive up to one year in damp vegetation (Duthyl and Van Someren, 1948).

One of the major drawbacks to the control of cysticercosis is the absence of a reliable technique for antemortem diagnosis. It was, therefore, the objective of the work described in this thesis to develop such a technique.

It seemed likely that a diagnostic technique based on immunological principles may offer the simplest and most reliable method for antemortem diagnosis of cysticercosis. The development of a reliable immunodiagnostic technique, however, would require a thorough understanding and elucidation of the immune response of the infected animals to various antigens of C. bovis or T. saginata. Because of the complex nature of the antigenic structure of the parasite, animals may exhibit a strong immunological response to one or more antigens, but these may be common to, or cross-react with antigens of other parasites or micro-organisms. Although many serological tests will give positive results with such antigens, the specificity must be expected to be poor. If, on the other hand one or more specific antigens could be isolated from C. bovis or T. saginata to which infected animals respond regularly, a reliable method could be worked out, provided the technique chosen possesses the required sensitivity.

This investigation was therefore designed:

(i) To characterise immunologically the antigens of C. bovis and T. saginata and to elucidate the antigenic relationship between various fractions of this parasite.

(ii) To select the antigens which are specific for C. bovis by examining the cross reactions with various common parasites.

(iii) To isolate and characterize the potentially specific antigen(s).

(iv) To examine if such antigen(s) elicit(s) an immune response in experimentally infected animals.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Immunity to cestode infections

It is generally observed that exposure of the host to different forms of Taenia antigens induces resistance to subsequent challenge (Flisser et al., 1979). Protection of cattle against C. bovis has been attempted in many ways using artificially hatched oncospheres (Wilkerhauser et al., 1971; Wilkerhauser, 1972), irradiated embryos (Wilkerhauser et al., 1974) viable or irradiated eggs (Lucker and Vegors, 1965; Gallie and Sewell, 1972; 1974), secretions and excretions of cysticerci in culture (Kosminkov, 1973a; Rickard and Adolph, 1976), or whole cysticerci, (Kosminkov, 1973b). In general an exposure to these various antigens reduced the number of cysticerci found after challenge.

In a study of the immune response in pigs to infection with eggs of T. solium, it was observed that animals which received a single dose of eggs had more cysticerci than those that received two doses, indicating that larger doses gave better immunity (Herbert and Oberg, 1974). Sheep were also partially protected against C. ovis and C. tenuicollis when immunized with either live, formalin-treated or frozen embryos of T. ovis and T. hydatigena, respectively (Gemmell, 1969; Rickard and Bell, 1971a, & b; Rickard and Coman, 1977). As regards T. taeniaformis, T. pisiformis and Hymenolepis nana experiments in laboratory animals have demonstrated that resistance could be induced artificially with crude homogenates of strobillate, larval tissue, live cysticerci

or embryos (Miller, 1931; Miller and Kerr, 1932; Hunninen, 1935; Campbell, 1936; Larsh, 1944; Nemeth, 1971; Gemmell,1973; Heath, 1973; Musoke and Williams, 1976).

Antibodies have been shown to be involved in protection against cestode infections as demonstrated by serum transfer studies (Miller, 1934, 1935; Miller and Gardiner, 1932, 1934; Campbell, 1938a, b,c; Hearin,1941; Larsh,1942,1951). In the study by Campbell (1938a), rats were protected following serum transfer from donors with up to 28 days old infection with C. fasciolaris while sheep acquired complete resistance to C. tenuicollis within 14 days of immunization as demonstrated by the markedly reduced larval cyst counts (Blundell et al., 1968; Gemmell et al., 1968, 1969). Rickard and Adolph (1976) then immunized calves with antigens collected from in vitro cultures of T. saginata and showed that they were highly resistant to homologous challenge infection. Furthermore, calves which received colostrum from cows similarly vaccinated were also shown to be highly resistant to infection (Rickard et al.,1977). With regard to H. nana, simultaneous administration of immune serum and implantation of activated embryos of this cestode into the tissues, inhibited the development of activated oncospheres. The active serum contained immunoglobulin-G (7S) and immunoglobulin-A (7S-17S) (Di Conza, 1970).

In subsequent studies on the role of antibodies in cestode infections, Leid and Williams (1974) showed that in rats infected with larvae of T. taeniformis, IgG_{2a} was involved during the first 4 weeks of infection. Reaginic

skin-sensitizing antibodies were also detected in the sera of rats, mice and rabbits infected with T. pisiformis (Leid and Williams, 1974; Musoke and Williams, 1975). Generally the reaginic antibodies in the rat and rabbit appeared in the circulation within the first 2 to 3 weeks, reached a peak in 4 to 6 weeks and then declined. It has been shown by the same workers, that there is a coincidental development of IgG_{2a} and IgE antibodies to T. taeniformis infection in rats. This led to the speculation that IgE and IgG_{2a} act synergistically (Musoke et al., 1978).

Cell mediated immune mechanisms may also be involved in cestode immunity. Transfer of spleen cells from immune donors induced early death of oncospheres of various species (Friedberg et al., 1969; Blundell, et al., 1969). Thymectomy at birth depressed immunity in some "strains" of mice, and this immunity could be restored by implantation of thymus tissue (Okamoto, 1968; 1970). Anti-mouse thymocyte antibodies completely abolished acquired immunity to H. nana (Okamoto and Koizumi, 1972), while whole body irradiation depressed it. Immunity in such animals was restored effectively with bone marrow cell graft from isogeneic immune donors (Friedberg et al., 1969).

Whether death of the challenging dose occurs during hatching in the intestine after the organism has penetrated into the mucosa or after the metacestode has been translocated to the site of predilection is unknown, but several investigators working with C. pisiformis and C. bovis

(Leonard and Leonard, 1941; Froyd and Round, 1960) believe that immunity to cestodes can be divided into two phases namely, an intestinal phase and a parenteral phase; and that both cell mediated immunity and humoral immunity are important. With regard to the intestinal phase, there is a rapid digestion of oncospheres in artificial intestinal fluid, which suggests that any event which may hinder the penetration may lead to the digestion of naked embryos before penetration can be accomplished (Gemmell, 1962; 1964;). This is supported by the following evidence.

Firstly, the oncospheres of H. nana and E. granulosus, will not readily penetrate the intestinal mucosa of immune mice (Bailey, 1951) or sheep (Heath, 1971). Secondly, immune serum has been observed to form a precipitate around the "penetration glands" of T. pisiformis, which suggests that the failure to penetrate intestinal mucosa may have an immunological basis (Silverman, 1955).

Evidence from experimental and natural infection with adult tapeworms has shown that a specific antibody response does occur and that immunologically mediated effector mechanisms probably contribute to the outcome of host-parasite interaction in the gut (Williams, 1979). Although contact between the intestinal mucosal surface and the attachment organs of adult cestodes may be very close (Smyth and Smyth, 1968), it is not necessary to propose that this is a prerequisite for the stimulation of antibodies. There is simple evidence of antibody formation against orally or intraintestinally administered non-invasive antigens

(Thomas and Parrot, 1974; Walker and Isselbacher, 1974; Warsaw et al., 1974), including those derived from cestodes (Ayuya and Williams, 1979). Circulating antibodies to H. diminuta have been detected in experimentally infected rats as demonstrated by complement fixation test, indirect fluorescent antibody test and passive cutaneous anaphylaxis (Coleman et al., 1968; Harris and Turton, 1973). Skin sensitizing antibodies have also been described in dogs naturally infected with E. granulosus (Williams and Perez-Esandi, 1971), while hemagglutinating antibodies were present in sera examined by Machnika (1974) from human patients infected with T. saginata.

The role of circulating antibodies in the regulation of the development of tapeworms or their elimination is not clear because of insufficient evidence of passive transfer protection by serum from immune donors. Whether or not local antibody production is an important determinant remains to be proved. Some support for this possibility comes from the evidence of the binding of immunoglobulins and the third component of complement (C3) to the tergument of H. diminuta in mice (Befus, 1974) and the demonstration of membrane lesions that may be the result of antibody mediated damage (Befus and Threadgold, 1975). The significance of these observations is still difficult to assess because immunoglobulin and C3 are also demonstrated on the surface of H. diminuta in rats, in which the worms survive for prolonged periods in the lumen of the intestine

(Befus, 1974). Furthermore, immunoglobulin is bound to the surface of H. microstoma in mice (Befus, 1974), even though the worms persist up to 2 years in the bile duct lumen. Hopkins et al., (1977) however, have suggested that an immunological effector mechanism manifested by detrobilation and rejection of adult worms may account for their observations on H. diminuta in mice. In their study, tapeworms were rejected from 9 to 14 days after primary exposure and in secondary infection fewer worms were recovered while those that developed were markedly stunted.

2:2. Some considerations of antigens used in the immunodiagnosis of parasitic infections

In the immunodiagnosis of parasitic diseases it is important to consider the characteristics of the antigen employed, since these determined the specificity, sensitivity and the feasibility of the test. One of the major problems involved in the quality of some parasite antigens is the presence of host components in the antigens. This complicates the analysis, the standardization and the interpretation of the immunodiagnostic tests (Chordi and Kagan, 1965; Oriol et al., 1971).

Mordern physico-chemical fractionation techniques, however, have improved the quality of many antigens used in the serodiagnostic tests. Various investigators have used different procedures for isolation and purification of antigens. These have included the following methods: Chloroform gel fractionation (Fife and Kent, 1960), isoelectric precipitation (Melcher, 1943; Kagan and Bargai,

1956; Reiber et al., 1961; Tarrant et al., 1965), ethanol fractionation under controlled pH and ionic strength (Sleeman, 1960, 1961), alcohol extraction (Fairly and Williams, 1923; Minning and McFadzen, 1956), ion exchange, gel filtration chromatography (Kent, 1963; D'Antonio et al., 1966; Ohira, 1967; Morris et al., 1968; Khan and Meerovitch 1970; Gross-Klaus and Walther, 1970; Gathuma and Waiyaki, 1980), pyridine fractionation (Labsoffsky et al., 1959) and ammonium sulphate precipitition (Urquhart et al., 1954).

Another consideration which is important is the developmental stage of the parasite which is used as the antigen source for immunodiagnosis. It is both tempting and practical to use the stages which are easily obtainable in large quantities. These stages, however, may not provide the best antigen preparation since the antigenic composition of one parasite stage may differ significantly from that of another stage (Williams and Soulsby, 1970; Fife, 1971). Murkin (1969), for instance, observed that there were antigenic differences between various parts of the same strobila of T. saginata at different stages of development, in that only the distal mature segments contained similar antigens. Ballad (1971) observed that different, purified antigen fractions from both adult and larval stages of T. saginata were more suited for detecting antibodies produced against different stages of C.bovis. This led Enyenihi (1974) to suggest that purified antigens for detecting both human and animal cysticercosis should contain a mixture of these stage-specific antigens.

2.3. Immunodiagnostic procedures which have been applied to cestode infections

Many methods have been applied to establish a sensitive and specific immunodiagnostic method for cestode infections. The following are the methods which have been applied:- Immnodiffusion (Trawiniski, 1936, 1937; Rydezewski et al., 1975), complement fixation test (Frick and Susse, 1970a; Lamina and Hein,1970), bentonite flocculation test (Norman et al., 1959), Latex agglutination test (Norman et al., 1959; Leikina et al., 1966; Frick and Susse, 1970b), indirect haemagglutination test (Alferova, 1969; Walther and Grossklaus, 1972; Gallie and Sewell, 1974), intradermal test (Bugyaki, 1961; Froyd, 1963; Machnicka-Roguska, 1965), indirect fluorescent antibody test (Daoc, et al., 1972; Machnicka, 1973; 1974; Rydzewiski et al., 1975; Gathuma et al., 1978), immunoelectrophoresis and counter immunoelectrophoresis (Betran and Gomez-Priego, 1973; Soulsby and Sanquini, 1974; Greet et al., 1979), and the enzyme linked immunosorbent assay (Arambulo et al., 1978; Van Knapen et al., 1979a,b). In the sections that follow the merits and demerits of these methods will be reviewed.

2.3.1. Immunodiffusion: Gel diffusion is one of the fastest and easiest method to perform, but it is very unreliable (Trawinski, 1936, 1937; Biagi and Tay, 1958; Dewhirst et al., 1960; Maddison et al., 1961). The

technique can detect only heavily infested animals (Proctor et al., 1966; Kagan, 1974). Enyenihi (1970) failed to detect precipitating antibodies in the sera of mice infected with C. longicollis until 30 days post infection, while Nemeth (1971) recorded positive results in rabbits infected with C. pisiformis 10 days after infection. This inability to detect early antibody response to infection is an important weakness of this technique. According to Enyenihi (1974), this may be due to a low concentration of precipitating antibodies at this early stage of infection. Morris et al. (1968), however, stated that the lack of sensitivity of immunodiffusion tests in porcine cysticercosis was caused by the complexity of the crude antigen preparation employed in the past. When specific fractions were used, positive reactions were recorded in every proven case of cysticercosis. These findings indicate that the characterization of various types of antigens in C. bovis and T. saginata is essential.

2.3.2. The complement fixation test (C.F.T.):

The complement fixation test has been shown to be more sensitive than indirect haemagglutination test, but less specific (Frick and Susse, 1970a). The greatest shortcoming of this test is that it can only detect antibodies to bovine cysticercosis within a particular period of infection in calves; false negative results are recorded in animals older than six months. Thus, this test is unreliable for the diagnosis of bovine cysticercosis

(Lamina and Hein, 1970). Enyenihi (1974), suggests that this may be due to change of type of immunoglobulin produced by the host at different stages of infection. He further suggests that work similar to that of Nemeth (1971, 1972, 1973) on rabbits infected with C. pisiformis is necessary to elucidate the dynamics of the production of complement-fixing antibodies in the bovine host.

2.3.3. The bentonite floculation test (B.F.T.):

Norman et al.(1959) used B.F.T. in their studies of human hydatidosis. B.F.T. was shown to be more sensitive than C.F.T. but less sensitive than I.H.A. in the diagnnosis of human disease (Kagan, 1968, 1978). Gonzalez-Castro and Chordi (1960), using a modified B.F.T. where the sensitized particles were emulsified in absolute alcohol, found that B.F.T. was more sensitive than C.F.T. However, the specificity of the test was poor.

Antienza-Fernandes et al., (1969) recorded a higher sensitivity in B.F.T. with cysticerci extract antigen than with cyst fluid antigen when used in the diagnosis of cysticercosis in sheep and goats. They too, however, recorded a high degree of false positive reactions. These limits thus undermine the usefulness of B.F.T. as a practical immunodiagnostic test.

2.3.4. The latex agglutination test(L.A.T.):

Fischman (1960) reported good sensitivity using L.A.T. in the diagnosis of hydatidosis. But Svividenko-Stepankovskaya (1973)found that L.A.T. had a lower degree of sensitivity

and specificity when compared with the indirect haemagglutination test.

L.A.T. is a popular immunodiagnostic test for cysticercosis due to its speed, sensitivity and specificity, but there are still several things which have to be standardized before results obtained in different laboratories can be effectively compared (Frick and Susse 1970b). Filippov (1971),for instance, showed that the use of purified scolex antigen eliminated cross reaction with fasciolaris, while Alferova et al., (1972) recorded that the size of the latex particles seemed to affect the results in the case of diagnosis in cattle. Castagnari and Pozzuoli (1969) also showed that the size of latex particles affected the specificity of the test in the diagnosis of human hydatidosis. Another shortcoming of this test in diagnosis in humans is that it gives false positive results in case of liver cirrhosis and non-parasitic cysts (Enyenihi,1974).

2.3.5. The indirect haemagglutination test (I.H.A.):

The Indirect Haemagglutination Test is one of the most common methods used in the immunodiagnosis of parasitic diseases. Kagan (1968), reviewing the serological tests used for the diagnosis of hydatidosis, indicated that IHA was mong the three best tests which utilize inert particles. Since then microhaemagglutination has been employed extensively in the diagnosis of taeniasis and animal cysticercosis.

Schultz and Ismagilova (1969) found the method to be the most sensitive of seven immunological tests employed in the diagnosis of cysticercosis in sheep. Other workers (Proctor and Elsdon-Dew, 1966; Gomez-Garcia et al., 1969; Frick and Susse 1970b; Grossklaus and Walther 1972; Nemeth, 1972, 1973; and Gathuma and Waiyaki, 1980), also obtained excellent results using IHA for diagnosis of cysticercosis in pigs, rabbits and cattle. Gathuma and Waiyaki, (1980) detected antibodies from the second week up to four months post infection. Their findings were similar to those reported by Alferova (1969), Enyenihi (1970), and Grossklaus and Walther (1972). Gathuma and Waiyaki, (1980) used T. saginata antigens separated by sephadex G200 gel filtration. They showed that fraction F1 had a higher degree of sensitivity and specificity than the crude antigen. This partial purification also reduced cross reactivity with sera from animals infected with hydatid cysts. These findings are similar to those of Morris et al., (1968) who showed a high degree of sensitivity when they used partially purified antigen in the diagnosis of porcine cysticercosis.

2.3.6. The intradermal test (I.D.):

The intradermal test was introduced by Casoni (1911) for the diagnosis of hydatidosis in man. Since then, this test has been widely used for the diagnosis of a variety of parasitic diseases (Fife, 1971). It is among the most sensitive immunodiagnostic procedures and the rapidity with which results are obtained is an additional advantage.

The ID test for diagnosis of cysticercosis measures the immediate-type hypersensitivity reaction, and the results are therefore, obtained within 1-2 hours after the injection of the antigen (Dewhirst et al., 1967).

Despite its wide usage, however, the intradermal test has limited specificity, especially in cases of concomitant helminthic infection (Dewhirst et al., 1967; Kagan, 1968; Fife, 1971). The intradermal tests for cysticercosis performed in the caudal fold of cattle by Bugyaki (1961) thus gave a high rate of positive reactions in infected animals but false positive reactions were frequent. Froyd (1963) used 57 different crude antigens, prepared from C. bovis, T. saginata, F. hepatica, E. granulosus and C. fasciolaris in the intradermal tests for diagnosis of C. bovis infection. None of the antigens used proved to be specific. Schoop and Lamina (1970) recorded positive intradermal reactions 21 days post infection in 10 calves experimentally infected with eggs of T. saginata, but the reactions were not specific. The animals continued to be positive 94 days after infection and were slightly reactive 11 months post infection although lung cysticerci were still present. Herbert and Oberg (1974) used the intradermal test for diagnosis of cysticercosis in pigs, employing crude antigens and they too encountered both false positive and false negative results.

When T. saginata antigens fraction $C_{"alk"}$, isolated

from whole worm (T. saginata)was used for the diagnosis of human infection,however, a high rate of specific reactions was recorded in 36 persons with T. saginata infection. Only 4 out of 34 patients cured of taeniasis gave positive reactions (Machnicka-Roguska and Zwierz, 1970). Zapart et al., (1974) then tried the ID test with an acid soluble protein fraction of T. saginata, E. granulosus, and C.cellulosae in the diagnosis of cerebral cysticercosis in man. They found that intradermal reactions were more marked with homologous than with heterologous antigens in proven cases of cerebral cysticercosis. The intradermal test thus proved to be a valuable tool in the diagnosis of cerebral cysticercosis, but it should be noted that the antigen used was a complex mixture of polysaccharides and protein, each of which could show different antigenic reactivity (Kent, 1963). The use of purified antigens which are free of host components and blood group-like substances in the diagnosis of cysticercosis in man and animals may therefore, prove to be very specific and sensitive in ID tests (Enyenihi,1974).

2.3.7. The indirect fluorescent antibody test (I.F.A.):

The indirect fluorescent antibody test has been used with some success in the immunodiagnosis of a variety of parasitic diseases, for example, Chaga's disease (Sadun et al., 1963), amoebiasis (Boonpucknavig and Nairn, 1967), trichinosis (Sadun et al., 1962) and malaria (Wilson et al., 1970).

The IFA is a sensitive serodiagnostic procedure. It can be performed using serum or whole blood (Anderson et al., 1961). The use of finger prick blood samples dried on filter paper facilitates collection of specimens in the field and greatly enhances the suitability of the IFA procedure for large scale epidemiological studies.

The IFA procedure has received considerable attention and application in the serodiagnosis of parasitic diseases. However, its most useful application appears to be in the serodiagnosis of malaria, American trypanosomiasis, leishmaniasis and toxoplasmosis (Fife, 1971).

There are few reports on the use of IFA for the diagnosis of cysticercosis. Grossklaus and Walther (1971) observed non specific reactions in their immunofluorescence studies and found it difficult to distinguish between reactions of known positive and negative sera. Soulsby and Sanquini (1974) observed similar reactions. In a study of human patients, Daoc et al. (1972), employing frozen sections of T. solium as antigens, reported a good sensitivity but poor specificity of the IFA test. Patients with cysticercosis reacted with hydatid antigens and the diagnosis had to be made on clinical grounds. In the case of cattle, a differential diagnosis between C.bovis and hydatid cysts on clinical ground would be impossible because cattle do not show clinical signs in either of these parasitic infections.

Calamel and Soule (1972) employed 17 antigens prepared from different stages of C. bovis, T. saginata C. pisiformis and C. tenuicollis. Antigens prepared from a concentrated solution of T. saginata embryos provided a sensitive test which gave the most consistent results. However, cross-reactions occurred between different preparations of T. saginata antigens and those of C. pisiformis and C. tenuicollis. Cross reactions also

occurred with the scolices of E. granulosus and it was impossible in these experiments to differentiate between bovine cysticercosis and hydatidosis.

Rydzewski et al. (1975) compared IFA, IHA and agar gel diffusion using 28 sera from persons with proven or suspected cysticercosis, 7 from persons with hydatid disease, and 8 from healthy individuals.
The results showed that IFA test using paraffin sections of T. solium was as sensitive as IHA in detection of human cysticercosis, while double diffusion in agar gel lacked sensitivity.

2.3.8. The indirect fluorescent antibody test using activated oncospheres of T. saginata as antigens

Machnicka (1973 and 1974) employing activated oncospheres as antigens in the diagnosis of cysticercosis of calves and man, observed specific immunofluoresence of cells of the hexacanth embryos of T. saginata. She considered that the fluorescence shown by the membrane of oncospheres was not specific. Gathuma et al., (1978), using artificially hatched and activated oncospheres fixed in gelatin as antigen, obtained a high sensitivity and specificity in C. bovis infected animals, but there was cross-reactivity with serum from animals with hydatid cysts.

2.3.9. The immunoelectrophoresis (IEP) and Counter-immunoelectrophoresis (C.I.E.):

Immunoelectrophoresis as a test for human hydatid disease was developed by Capron et al., (1967). They based their criterion for positivity on the detection of a precipitin line called "arc 5" after immunoelectrophoresis of concentrated sheep hydatid cyst fluid as antigen and the patient's serum sample in the trough.

There are a few reports on the use of IEP in the diagnosis of cysticercosis. Morris et al., (1968) compared IEP and double diffusion test in agar gel with IHA for the diagnosis of cysticercosis in animals and reported poor specificity in IEP test. More recently, Greet et al. (1979) analysed the antigenic components of T. saginata proglottides in IEP. They defined 22 antigenic components, but most of these were of host origin and were not specific for the cestodes. The same workers also observed that calves experimentally infected with T. saginata eggs showed 3 precipitin lines, depending on the evolutionary stage of the infection. The precipitating antibodies could be detected 3 weeks post infection, but the major drawback of IEP, according to these authors, was that of false negative reactions in animals with less than 50 cysts.

Betran and Gomez-Priego (1973) reported the use of counter immunoelectrophoresis (C.IE.) in both human and experimental cysticercosis employing cyst fluid, secretion and excretion antigens, complete and incomplete somatic

antigens. They reported a good correlation between the clinical condition of the cyst in the host and the number of precipitating bands observed.

CIE for the serodiagnosis of cysticercosis in cattle has been evaluated by Soulsby and Sanquini (1974). They used fresh and stored sera from experimentally and naturally infected animals and the antigens from the adult and the larval stages of T. saginata and larval stages of T. crassiceps prepared by the method described by Kent (1963). Encouraging results were obtained with the CIE technique using sera obtained from naturally infected cattle showing light(1-4 cysts) moderate (5-20 cysts) heavy (over 20 cysts) infections as assessed by carcass inspection. The CIE test permitted a classification of the degree of infection based on the number and intensity of precipitin lines observed. However, false positive reactions were observed with sera from adult normal cattle. These false positive reactions posed problems similar to those encountered with other serodiagnostic tests.

2.4.0. The enzyme linked immunosorbent assay (ELISA):

Arambulo et al., (1978) described a miro-Elisa technique for the serodiagnosis of human cysticercosis. They obtained results which correlated remarkably well with IHA results. The ELISA results were easily reproducible, but the major drawback was the false positive results due to hydatidosis.

Van-Knapen et al., (1979a, b) used ELISA to demon-

strate antibodies and circulating antigens in bovine cysticercosis. The method was shown to be rather insensitive during early and late infection in both naturally and experimentally infected animals but the sensitivity of ELISA was comparable to that of IHA. The major disadvantage of ELISA was that positive reactions were found in animals infected with Fasciola hepatica This, however, could be due to the crude nature of the T. saginata antigen used (Craig and Rickard, 1980).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

A. ANTIGENS

A.1. Taenia saginata

A.1.1. Collection of T. saginata

Children in various Maasai "Manyatta" in Kajiado District were treated with Yomesan (2,5-dichloro-4-nitrosalicylanilide) followed by magnesium sulphate as cathartic. Whole tapeworms and segments were recovered from the stools of these children.

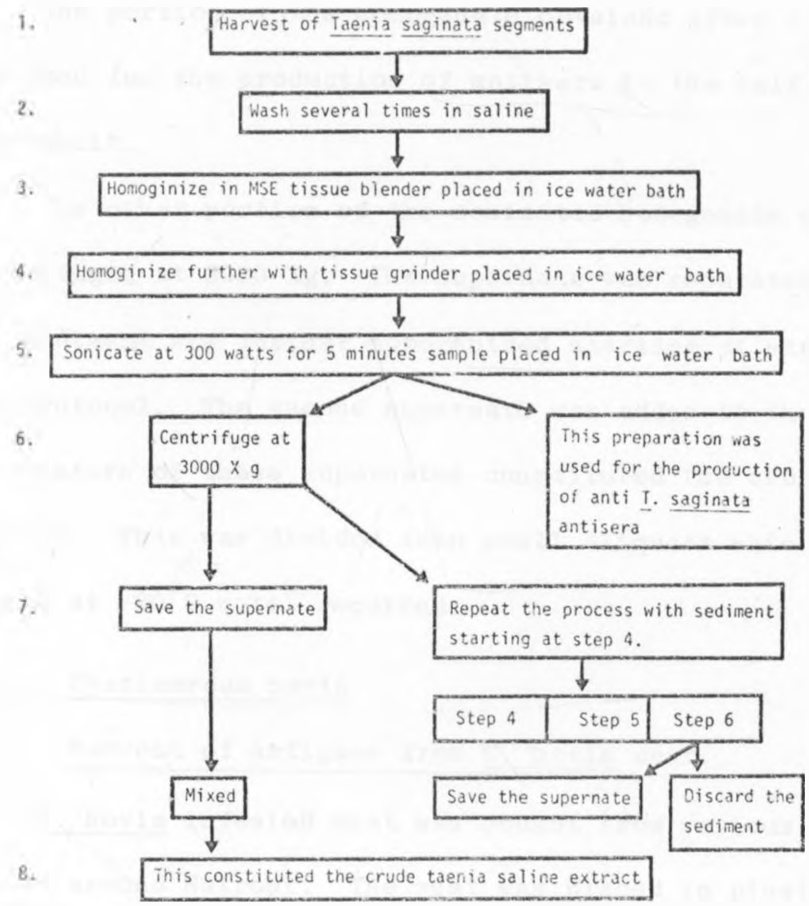
The tapeworm segments were washed clean of intestinal contents using clean water; the proglottides were placed in 2 litre plastic containers containing normal saline in which 0.1% sodium azide (BDH-Chemical Poole, England) was added. They were then stored in an ice-box until delivery to the laboratory.

The other source of tapeworm segments was Kariobangi sewage treatment plant in Nairobi. Tapeworms were collected just before they entered the treatment plant, and they were treated as described above.

A.1.2. Preparation of crude Taenia antigens

The tapeworm segments were rinsed several times in normal saline upon arrival in the laboratory. Preparation of crude Taenia saline extract was performed following the protocol shown in Fig.1. Briefly, the following procedure was followed: Clean tapeworm segments were homogenized with a MSE (Measuring and Scientific Equipments, London) tissue blender placed in an ice water bath at $0^{o}C$. The homogenate

Figure 1. Preparation of Crude Taenia Extract



was further homogenized with Potter-Elvehjem tissue grinder (Kontes Glass Company, New Jersey, U.S.A.) placed in an ice-water bath. The homogenate obtained was sonicated at 300 watts for 5 minutes in an ice-water bath using Brausonic 1510 sonicator (Braun Messungen AG, Germany).

The portion of the homogenate obtained after sonication was used for the production of antisera in the calf, goat and rabbit.

The other portion of the sonicated homogenate was centrifuged at 3000 Xg. The supernate was separated, and the sediment was further homogenized starting at step 4 of the protocol. The second supernate was added to the first. The mixture of these supernates constituted the crude saline extract. This was divided into small aliquots which were stored at -20^{o}C until required.

A.2. Cysticercus bovis

A.2.1 Harvest of antigens from C. bovis cyst

C. bovis infested meat was bought from various slaughter houses around Nairobi. The meat was placed in plastic bags placed in an ice-box, and delivered to the laboratory where the cysts were carefully removed from the meat on the same day.

A.2.2. The "Outer membrane" and the "Outer fluid" of C. bovis

Figure 2 shows a drawing of C. bovis cyst and the location of various antigens harvested for use in this study.

The cysts were placed in petridishes. The outer connective tissue capsule, which is derived from host components (Thornton, 1968b), was carefully dissected from the inner bladder and the scolex. The fluid between these two membranes was harvested and was termed the Outer fluid (F1); this was placed in universal bottles and stored at -20°C. The outer connective tissue was termed the Outer membrane (O.M). This was washed extensively with normal saline, dried with blotting paper and placed in universal bottles.

A.2.3. The "Inner Membrane-Scolex" and the"Inner fluid" of C. bovis

The intact bladder and the scolex was blotted dry and placed in the petridish. The bladder was then punctured using dissecting needles, the fluid obtained from the bladder was termed the Inner fluid of the cyst (F2). This was placed in vials and stored at -20°C.

The bladder and the scolex were washed several times in saline and dried on filter paper. The bladder and scolex were termed "the Inner Membrane-Scolex".

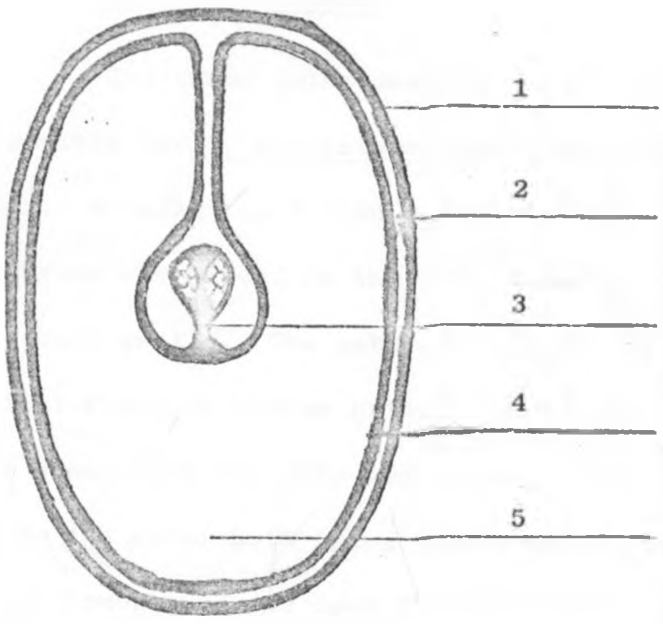


Figure 2: Drawing of Cross section of C. bovis

Key 1: Outer membrane

2: Outer fluid (F_1)

3: C. bovis scolex

4: Inner membrane

5: Inner fluid (F_2)

Note: - (3) and (4) constitutes "the Inner membrane-scolex"

- (3), (4) and (5) constitutes "The bladder worm"

A.2.4. Preparation of the crude saline extracts of Outer membrane, and the crude saline extract of Inner membrane - scolex

The crude Outer-membrane extract was prepared as described for T. saginata extract but with slight modification. Briefly the method was as follows: The frozen outer membrane was placed in the frozen mortar and ground using a frozen pestle. The material was further homogenized using Potter-Elvehjem tissue grinder placed in an ice water bath. The homogenate was then sonicated at 300 watts for 5 minutes in an ice water bath using the Brausonic 1510 sonicator. This sonicated homogenate was used for the production of an antiserum in a calf. The homogenate was then centrifuged at 3000Xg, the supernate divided in aliquots and stored at -20°C.

The crude Inner membrane-scolex extract was prepared as described for the crude Outer membrane extract.

A.3. Other parasites

A.3.1. The parasites collected:

The following parasites were harvested from different slaughter houses around Nairobi and in Kajiado District.

i) C. tenuicollis from goats and sheep.

ii) Fasciola gigantica from goats, sheep and cattle.

iii) Moniezia species from goats, sheep and cattle.

iv) Stilesia hepatica from goats and sheep.

(v) Paramphistomum species from goats, sheep and cattle.

vi) Hydatid cyst from sheep, goats and cattle

vii) Trichuris species from cattle

viii) Oesophagostomum species from cattle

ix) Haemonchus contortus from cattle

x) C. fasciolaris from mice

xi) Ascaris suum from pigs

The parasites were washed several times in water, then placed into bottles containing normal saline and 0.2% sodium azide. They were delivered to the laboratory in an ice-box, where extraction of different parasite antigens was performed on the same day.

A.3.2. Parasite/antigens obtained from other laboratories.

T. hydatigena from dogs and Coenurus cerebralis from goats were obtained from the Turkana District, through the courtesy of Dr. A. Wilson of the Department of Veterinary Pathology and Microbiology, University of Nairobi.

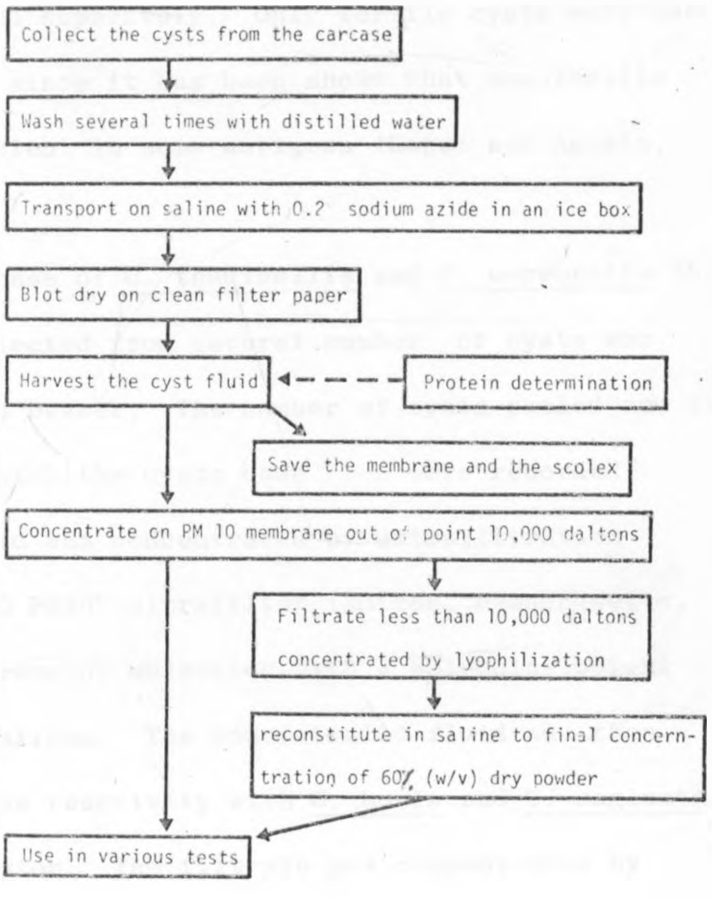
Trypanosoma brucei - 221 and Schistosoma mansoni purified antigens were obtained through the courtesy of Dr. V.M. Nantulya of ILRAD and Dr. Z. Ahmed of Department of Human Pathology, University of Nairobi, respectively.

C. cellulosae antigen was obtained from Mexico through the courtesy of Dr. A. Flisser of University of Mexico.

A.3:3: Harvest of parasite cyst fluid antigens

The following cyst fluids were collected: C. tenuicollis, C. cerebralis and hydatid cyst fluid from goats, sheep and cattle. The cyst fluid antigens were prepared following the protocol shown in Fig.3.

Figure 3: Preparation of cyst fluid antigens



Briefly, the cysts were blotted dry on clean filter paper and the bladder was then punctured. The fluid was harvested into beakers. In the case of hydatid cysts, the individual cysts were harvested into separate containers, the fluid was then centrifuged at 3000Xg for 15 minutes and examined for hydatid sand. Fertile and unfertile cysts were then pooled separately. Only fertile cysts were used in this study, since it has been shown that non-fertile cysts are deficient in some antigens (Kagan and Agosin, 1968).

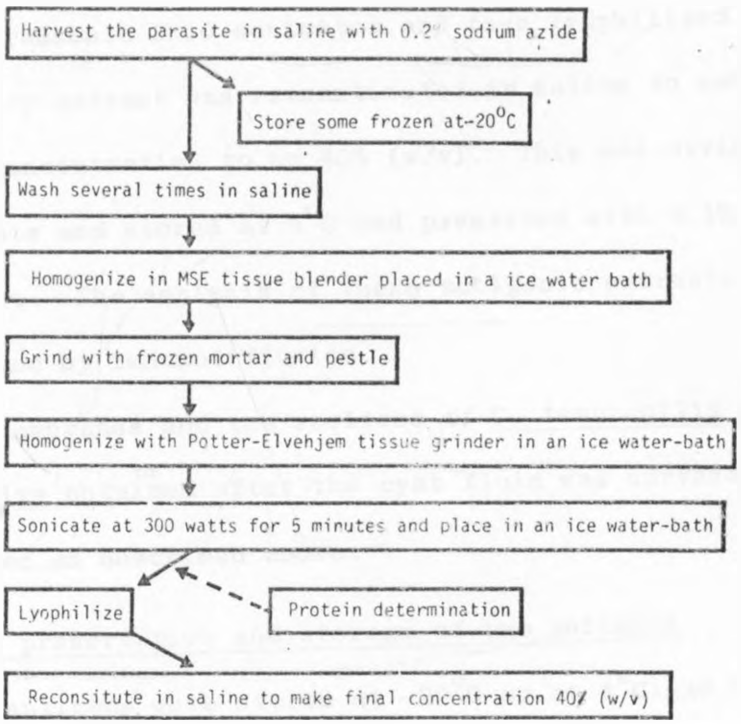
In the case of C. tenuicollis and C. cerebralis the cyst fluid collected from several number of cysts was pooled into one beaker. The number of cysts pooled and the species from which the cysts came were recorded.

The fluid was concentrated by ultrafiltration through "DIAFLO PM10" ultrafilter (Amicon, Masschusetts, U.S.A.) which retains molecules with a molecular weight above 10,000 daltons. The concentrated fluid was then tested for cross reactivity with C. bovis and T. saginata by immunodiffusion. The filtrate was concentrated by lyophilization and re-constituted in saline. This was also used for testing the cross reactions.

A.3:4: Preparation of crude saline extracts from other parasites

The extraction was performed following the protocol shown in Fig.4. Briefly, the following procedure was

Figure 4 Preparation of crude parasite saline extracts



followed: After washing several times in saline, the parasites were homogenized in an ice water bath, using the MSE tissue blender. The homogenate was further homogenized using frozen mortar and pestle, and finally with the Potter-Elvehjen tissue grinder placed in an ice water bath. The homogenate obtained was sonicated at 300 watts for 5 minutes using the Brausonic 1510 sonicator and then lyophilized.

The dry extract was reconstituted in saline to make the final concentration to be 40% (w/v). This was divided into aliquots and stored at 4°C and preserved with 0.1% sodium azide. The analysis of these antigenic extracts was performed by immunodiffusion.

The membranes and the scolices of C. tenuicollis and C. cerebralis obtained after the cyst fluid was harvested, were treated as described above.

A.4: The preservation and storage of the antigens

All antigens were stored at -20°C or at 4°C, and 0.1% sodium azide was used as a preservative in all antigens used for purposes other than immunization of animals. Antigens once thawed were not frozen again but stored at 4°C.

A.5 Protein determination of antigenic extracts

Protein determination was performed on all antigens extracted. The protein was estimated by the Folin-Ciocalteau reaction (William and Chase, 1968), a modification of the method described by Lowry et al., (1951).

B ANTISERA

B:1 Animals used

The antisera used in this study were prepared in calves, rabbits and goats.

B.1.1. Calves

Five Friesian bull calves, ranging in age between six to seven months old were bought from the Faculty of Veterinary Medicine Farm, Kanyariri.

The calves were ear-tagged for the purpose of identification. They were then dewormed at two weeks intervals using thiolbenzole (Merck Sharp Dohme, Germany) and sprayed with Bacdip (Bayer, Germany). After the first deworming the calves were bled. This constituted the preimmunization bleeding.

The calves were housed in isolation and fed on hay and concentrates (early weaner calf pellets, calf meal and calf pencils, Unga Ltd., Nairobi). They were also provided with salt lick (Maclick; Welcome Laboratories, Nairobi) and water.

B:1:2 Goats

Three maasai goats were purchased from Ongata-Rongai in Kajiado District. They were dewormed, sprayed and bled as described for the calves.

The goats were housed in isolation and fed on hay and concentrates (Ewe and Lamb nuts; Unga Ltd., Nairobi). They were provided with water and salt lick (Macklick-

Wellcome Laboratories, Nairobi) ad-libitum.

B:1.3 Rabbits

Four Newzealand Large White rabbits were bought from Veterinary Research Laboratories, Kabete. The rabbits were treated for coccidiossis using Amprol (Soulsby Co., U.S.A.). They were put in isolation and fed on concentrates (Rabbit pellets, Unga Ltd., Nairobi). They were also provided with water ad-libitum. They were bled before the start of the immunization schedule.

B:2 Antisera prepared

The list of the antisera prepared are shown in Table 2.

Table 2. The Antisera Prepared

Identification (Animal species and No.)	Preparation used for Immunization	Antisera Prepared
Calf No.10	Homogenate of outer membrane of C.bovis	Anti outer membrane C.bovis
Calf No.118	Outer fluid of C. bovis	Anti outer fluid C. bovis
Calf No.835	Inner fluid of C. bovis	Anti inner fluid C. bovis
Calf No. 846	Homogenate of inner membrane-scolex of C. bovis	Anti inner membrane scolex C. bovis
Calf No.900	Homogenate of T. saginata	Anti T. saginata
Goat No.824	Homogenate of T. saginata	Anti T. saginata
Goat No.888	Homogenate of C. tenuicollis membrane scolex and fluid (goat)	Anti C. tenuicollis
Goat No. 879	Sonicate of precipitin line of C.bovis	Anti "precipitin Line"
Rabbit No.140	Homogenate of inner membrane scolex of C. bovis	Anti inner membrane scolex C. bovis
Rabbit No.154	Homogenate of T. saginata	Anti T. saginata
Rabbit No.158	Homogenate of inner membrane scolex C. bovis	Anti inner membrane scolex C. bovis
*Rabbit No. 79	Homogenate of C. bovis scolices	Anti C. bovis scolices.

Table 2 (Cont'd) The Antisera Prepared

Identification (Animal species and No.)	Preparation used for Immunization	Antisera Prepared
Rabbit No. 146	Inner fluid of C. bovis	Anti inner fluid
[1] Rabbit No.87	Whole bovine serum	Rabbit anti bovine serum
[2] Rabbit No.39	Whole goat serum	Rabbit anti goat serum
[3] Goat No.878	Bovine IgG_1 and IgG_2	Anti bovine IgG

* Rabbit anti C. bovis scolices was obtained through the courtesy of Prof. K. Lindqvist.

1,2 and3. These antisera were prepared with the help of Mr. H.F. Kaburia and Mr. J.B. Ndunda (The technicians in the Department of Public Health, Pharmacology and Toxicology).

In the text all the antisera are referred to by the identification number given to the animal in which the antisera were prepared. e.g. antiserum number 10 refers to calf anti-outer membrane of C. bovis.

B:3 Production of antisera

Pre-immunization bleeding was performed on all animals. The priming dose of each antigen was emulsified in Freund's complete adjuvant (DIFCO Laboratories Detroit, U.S.A.) mixed in the ratio of 3:1.

The antigens were injected into the lymphnodes and into the muscles according to the method described by Newbould (1965). The subsequent booster doses were emulsified in Freund's incomplete adjuvant (DIFCO Laboratories, Detroit, U.S.A.), and were injected intramuscularly.

The immunization and test bleeding was done at two weeks intervals until the antisera gave distinct precipitin lines when tested with homologous antigens in double immunodiffusion in the gel. (The total immunization and bleeding was for the period of 18 months). The preparation of monospecific antiserum to a component of C. bovis inner membrane scolex is described in Section B:4.

The pools of different antisera were prepared by pooling together several serial bleedings from each immunized animal. In the case of anti-C. bovis antiserum used in immunodiffusion, however,pools of Antisera Nos. 140 and 158 were combined. Similarly the antiserum used for crossed immunoelectrophoresis consisted of pools of Antisera Nos.79, 140 and 158.

B.4. Preparation of monospecific antiserum to a component of C. bovis inner membrane scolex

Immunization of experimental animals with immunoprecipitates obtained either by immunoelectrophoresis or crossed immunoelectrophoresis in order to produce a monospecific antiserum has been performed by several workers (Goudie et al., 1966; Harboe et al., 1976). This method has the advantage of production of monospecific antibody to the antigen required without preliminary chemical or physical purifications of the

antigen from a complex antigenic mixture such as parasite antigens (Goudie et al., 1966).

In this study, a monospecific goat antiserum against a component of C. bovis (Antigen 11) was prepared by immunizing a goat with a single precipitin line obtained by immunodiffusion between goat anti T. saginata (antiserum No.824) and, inner membrane-scolex of C. bovis (See B:4:1). This line also showed complete identity with other lines obtained when inner fluid and outer fluid of C.bovis and T. saginata were used as antigens in immunodiffusion using goat anti-T. saginata as the antiserum. T. saginata antigens were not used in the production of antiserum to Antigen 11 because the identical line which was produced in the immunodiffusion using the homologous antisera was weak.

B:4:1. Preparation of precipitin line for immunization of goat No.879

Twenty five microscope slides were prepared for immunodiffusion (described in Section C:1). The diffusion was allowed to proceed for 18 hours, after which a single precipitin line was clearly visible. The slides were then pressed and washed as described in C:5, but they were not stained. The precipitin line was cut out of the gel using a clean razor blade as close to the line as possible. The agar strips containing this line were then washed at $4^{o}C$ with a large volume (200 times volume excess) phosphate buffered saline (P.B.S.), pH 7.4, with several changes of buffer for five days.

For each immunization about a 100 strips were used. The PBS was sucked off and strips sonicated in a cooling bath with ice by Brausonic 1510 (Braun Messungen AG, Germany) with an effect of 100W in 1 sec. pulses until the agar had visibly been dispersed. Freund's complete adjuvant (DIFCO Laboratories, Detroit, U.S.A.) was then added in ratio 1:3 (sonicated agar strips: adjuvant). The mixture was sonicated as before until the material became a cream. This was used for immunization. The subsequent booster injections were prepared as described except that Freund's incomplete adjuvant (DIFCO Laboratories, Detroit, U.S.A.) was used.

B:5 Absorption of antisera with the insoluble immuno-adsorbents

Insoluble immunoadsorbents were used in this study to remove the anti-host components which might have been produced in the immunized animals. There were two types of insoluble immunoadsorbents used in this study:

(i) Those prepared by coupling a specific protein to cyanogen bromide activated sepharose 4B (CNBr-sepharose);

(ii) The glutaraldehyde preserved erythrocytes.

B:5:1 Preparation of CNBr-sepharose immunoadsorbents

CNBr-sepharose was obtained from Pharmacia Fine Chemical Co. (Uppsala, Sweden). The immunoadsorbent was prepared according to the manufacturer's recommendations. Briefly,

the method was as follows: A required amount of CNBr-sepharose was weighed out. The sepharose was then washed with 1mM HCl in the ratio of 200 mls per gram of dry sepharose. This was followed by rinsing with excess distilled water. The protein to be coupled was dissolved in coupling buffer (0.1M sodium bicarbonate buffer pH 8.3, containing 0.5 M sodium chloride; BDH, Poole, England). The protein solution was mixed with the suspension of the gel in end over end mixer (Voss of Maldon, Essex, England) for 2 hours at room temperature (27^{o}C) and then for 18 hours at 4^{o}C. The uncoupled protein was then washed off with the coupling buffer, and the remaining active groups were blocked by 1M ethanolamine at pH 9 for 2 hours at room temperature. The blocking reagent was washed off using coupling buffer followed by 0.1M acetate buffer pH 4 containing 0.5M sodium chloride and finally rinsed with coupling buffer and saline.

The uncoupled protein was determined following the method of Lowry et al. (1951) and the percentage of coupled protein was then determined. The protein sepharose conjugate was stored at 4^{o}C as a 50% suspension in saline to which 0.1% sodium azide was added.

B:5:2 Immunoadsorbents

The immunoadsorbents used in this study are shown in Appendix 1, which also shows the percentage of protein coupled to the sepharose.

B:5.3 Preparation of red blood cells used in the absorption of antisera

The blood was collected from animals slaughtered in various slaughterhouses around Nairobi. The blood was pooled together according to the species, viz. cattle, goats and sheep. The red cells were then treated with glutaraldehyde by the method briefly described below:

B:5:4 Preservation of erythrocytes using glutaraldehyde

Sixty millilitres of packed red cells which had been washed five times in physiological saline were mixed with 1.2 litres of phosphate buffer 0.15 m, pH 7.2 in a 2 litre conical flask. 120 mls of 2.5% solution of commercial glutaraldehyde (25% glutaraldehyde in water - Koch Light Laboratories Ltd., Colnbrook Buckshire, England) were added slowly under agitation using a clinical rotator (Arthur H. Thomas, Philadelphia, Pa, U.S.A.), for two hours at room temperature (25^{o}C). Then 2 times molar excess solid glycine (Koch Light Laboratories, Colnbrook, Buckshire, England) was added directly into the flask and the agitation continued overnight. After overnight agitation, the red cells were allowed to settle at room temperature. The supernatant was sucked off, and the red cells washed five times with saline.

The preserved cells were then resuspended in saline and sodium azide added to give a final concentration of 1.0%. The cells were stored at 4^{o}C.

B:5:4 Procedure for immunoabsorption of antisera using insoluble immunoadsorbents

Appendix 2 shows the schematic absorption of the antisera. Briefly, the absorption was done as follows: The antiserum was mixed with the immunoadsorbent and the mixture was mixed in an end over end rotator at room temperature ($25^{o}C$) for 2 hours, then in cold ($4^{o}C$) for 18 hours.

The immunoadsorbent was then separated from the antiserum by passing the serum through sintered glass (G3-Pyrex, England). The immunoadsorbent was regenerated by washing with 0.2M glycine/HCl buffer, pH 2.8, followed by the coupling buffer and saline. The immunoadsorbents were re-used several times.

B:6 The harvesting and the storage of the antisera

The preparation of the antisera was done as described by Campbell et al.,(1970). Briefly, the method involved the following procedure:-

The freshly drawn blood was allowed to stand for 3 hours in the incubator at $37^{o}C$. The clot was then carefully removed from the wall of the siliconized tubes using an applicator stick and placed in the refrigerator at $4^{o}C$ for 18 hours to permit the clot to contract.

The serum was decanted into clean test tubes and centrifuged at 3000 Xg for twenty minutes to sediment the red blood cells. Clean sera were then decanted into clean plastic bottles.

For preservation, 0.01% sodium azide (BDH, Poole, England) was added to the sera which were then stored at -20°C.

After each bleeding the antisera harvested were tested by immunodiffusion using homologous and heterologous antigens described in Part A.

B:7 Antisera obtained from other laboratories

Samples of sera coded RS1 and RS6 were obtained from animals reared free of C. bovis, while sera coded RS8 and RS10 were from animals orally infected with T. saginata eggs. All these samples were obtained from Edinburgh, Scotland, through the courtesy of Dr. P. Stevenson. Serum samples labelled M1 were collected at weekly intervals from a calf which had been reared at Muguga (Kenya Agricultural Research Institute, KARI). The calf had been orally infected with 50,000 eggs of T. saginata and a total of 8,630 C. bovis cysts were recovered from the animal at slaughter. These samples were obtained through the courtesy of Dr. J. Onyango-Abuje.

C: Analysis of the antigens

C:1 Immunodiffusion

The microtechnique of Ouchterlony double diffusion as described by Crowle (1973) was used with slight modifications. Briefly, the method was as follows: 1% (w/v) of purified Oxoid agar (Oxoid Ltd., England) in phosphate buffered saline pH 7.4 was used in the preparation of the media for double diffusion; 0.1% sodium azide was added as a preservative. Three millilitres of molten agar was poured on

the microscope glass slides (25mm x 75mm) to give approximately a depth of 2mm. With a gel-puncher (Gelman Instrument Co., Ann Arbor Michigan, U.S.A.) wells of 4.0mm diameter and 5 mm apart were cut in hexagonal pattern with a central well. The agar in the well was removed by suction. The central well was filled with 15 ul of antiserum and each of the peripheral wells contained 15 ul of different antigens. This pattern was sometimes changed by placing the antigen in the centre well and the specific antisera in the peripheral wells.

The slides were then placed in a humid chamber and diffusion allowed to proceed for a maximum time of 48 hours at room temperature (25^{o}C). The precipitin bands were read before and after staining as described in section C:3 for the crossed immunoelectrophoresis. Sometimes the plates were stained with Ponceau 'S'.

C:2 Immunoelectrophoresis

The presence of host components in C. bovis and C. tenuicollis antigen preparations was determined by the classical immunoelectrophoresis microtechnique of Grabar and Williams (1953) following the method described by Scheidegger (1955), with the following modifications: 15 mls of 1% (w/v) of Litex agarose (type HSA-Litex Glostrup, Denmark) in sodium barbital-calcium lactate buffer pH 8.6 and ionic strength 0.02 was used.

10 ul of the antigen was filled into the wells, and electrophoresis carried out at 10 v/cm for 60 minutes. The relevant antiserum was placed in the trough and diffusion allowed to take place for 48 hours. The precipitated bands were read after pressing, drying and staining as described for the crossed immunoelectrophoresis.

C:3 Crossed immunoelectrophoresis

The two dimensional immunoelectrophoresis was used in the characterisation of the various C. bovis antigens. The technique followed was that described by Weeke (1973) with slight modifications as follows: 1% (w/v) of Litex agarose in sodium barbital-calcium lactate buffer into which 0.05% Triton X100 (see appendix) was added was used in the first and second dimension electrophoresis. The gel was 1.5mm thick in the first dimension electrophoresis. The antigen well contained 15 ul of antigen. The first dimension electrophoresis was carried out for 60 minutes at 10 v/cm. The second dimension electrophoresis was carried on 50 x 50 mm glass plates using the same agarose type but mixed with 0.5 mls of antiserum to the final volume of 3 mls. The electrophoresis was carried out at 2 v/cm for 20 hours. The gel was pressed as described by Weeke (1973) and then washed in 3% (w/v) trisodium citrate (Koch Light Laboratories Colonbrook, Buckshire, England), pH 8.5 for 24 hours in order to remove the non-precipitated proteins. After washing, the plates were rinsed in distilled water, pressed, dried and stained with Coomassie brilliant blue for 40

minutes and destained until the background was clear.

C:4 Crossed immunoelectrophoresis with intermediate gel

This method was used to demonstrate the monospecificity of the antisera prepared (described in Section B3:1:4). The technique followed was that described by Axelsen (1973) with the following modifications:- 0.2 mls of specific antiserum or normal serum in control plates was mixed with the same agarose type used in crossed immunoelectrophoresis to make the final volume of 1 ml. This was poured to make the intermediate gel. 0.5 mls of the reference antiserum was mixed with the same type of agarose to a final volume of 2 mls. This was poured onto the plate as described by Axelsen (1973).

The second dimension electrophoresis was performed as described for the crossed immunoelectrophoresis. Pressing, washing, drying and staining was done in the same way as described for crossed immunoelectrophoresis.

D: Isolation of the antigen used in solid-phase radioimmunoassay

The immunodiffusion results (Section 4) show that antigen 11 of C. bovis is common to T. saginata, T. hydatigena C. cellulosae and C. tenuicollis. Thus any of these parasites might be used as a source of the antigen.

Antigen 11 was prepared from C. tenuicollis cyst fluid obtained from goats, since it was easy to obtain this fluid in large quantities.

D:1:1 Isolation of Antigen 11 from C. tenuicollis cyst fluid

The fluid was collected as described in Section A:3:3. One litre of the fluid was dialysed against 10 liters of 0.15M phosphate buffered saline (PBS) pH 7.4 for 18 hours. The dialysed fluid was centrifuged at 2,000 xg and the supernatant was concentrated 50 times by ultrafiltration through DIAFLO PM10 ultrafilter. An aliquot of the concentrated fluid was examined for the presence of Antigen 11 by immunodiffusion using the monospecific antiserum No.879.

The fluid was then circulated through the anti-whole goat serum immunoadsorbent column (Appendix 2) in order to remove the host components present in the cyst fluid. Aliquots of absorbed fluid were then tested for the presence of Antigen 11 as described above.

D:2 Further purification of Antigen 11

After the removal of host components the fluid was purified further by affinity chromatography using IgG fraction from the monospecific antiserum (antiserum 879).

D:2:1 Isolation of the IgG fraction from specific antiserum against Antigen 11 (antiserum No.879)

An IgG fraction was prepared following the method described by Fey et al. (1976) with slight modifications as follows: An equal volume of 100% saturated ammonium sulphate was added slowly while stirring to an equal volume of antiserum. The mixture was left standing at room

temperature (25°C) for 15 minutes to equilibrate and centrifuged at 3,000Xg for 30 minutes. The precipitate was washed twice with 35% saturated ammonium sulphate and dissolved in normal saline. The gammaglobulin fraction was dialysed against distilled water containing 0.02% sodium azide for 18 hours at 4°C and against 0.005M phosphate buffer pH 8 for 72 hours at 4°C. The dialysed gammaglobulin fraction was then passed through a diethylaminoethyl cellulose column (BioRad Laboratories, Richmond, California, U.S.A.) pre-equilibrated with 0.005M phosphate buffer pH 8. A stepwise elution was performed and the fraction obtained with 0.01M phosphate buffer pH 8 was collected. This fraction was concentrated by ultrafiltration using DIAFLO PM30 ultrafilter with a cut off point of 30,000 daltons.

The protein concentration of this fraction was determined following the method described by Lowry et al. (1951). The fraction was shown to contain only IgG by immunoelectrophoresis using rabbit anti-whole goat serum (antiserum No. 539).

D:2:2: Prepration of the affinity chromatography column

The concentrated IgG fraction obtained from the monospecific antiserum No.879 contained 12.86 mg protein per millilitre. This was coupled to CNBr-Sepharose to prepared an immunoadsorbent column as described in Section B:5:1.

The immunosorbent was washed with excess (10x volumes) 0.2M glycine/HCl buffer pH 2.5 into which 10% 1,4-dioxane

(diethylene dioxide, Fisher Scientific Company, Fair Lawn, New Jersey) was added. The immunosorbent was then equilibrated with 0.1M borrate buffer pH 8 containing 0.5M sodium chloride and 0.02% sodium azide.

One hundred and twenty millilitre amounts of C. tenuicollis cyst fluid prepared as described in Section D:1:1 was mixed with the immunosorbent for 18 hours at 4°C using an end over end mixer. The uncoupled proteins were washed out with excess borate buffer and then with saline. The bound antigen was eluted with 0.2M glycine/HCl buffer pH 2.5 containing 10% dioxane into tubes containing 1M phosphate buffer pH 8 to neutralize the acid. The eluate was then extensively (10x volumes) dialysed against PBS pH 7.4 at 4°C. The eluted antigen was concentrated 50 times by ultrafiltration through DIAFLO PM10 ultrafilter and the protein contents was determined by the method of Lowry et al. (1951).

The eluted antigen was examined by immunodiffusion using specific antiserum against Antigen 11 (antiserum No. 879) as well as antisera against C. tenuicollis, T. saginata and C. bovis (Antisera Nos. 888, 824, 154 and 140).

The absence of host components in the eluted antigen was determined by immunodiffusion using anti-whole goat serum (Antiserum No.539).

E. Analysis and characterization of the antigen eluted from the affinity column

Analysis and characterization of the eluted antigen

was done by polyacrylamide gel electrophoresis in the presence of the detergent sodium dodecyl sulphate (SDS-PAGE), and by isoelectric focusing.

SDS-PAGE can be used in the determination of molecular weights of proteins while isoelectric focusing is a useful preparative and analytical method for isolation of proteins based on their different isoelectric points (Weber and Osborn 1969; Dunker and Rucker 1969; Sargent and George 1975).

E.1. Preparation of SDS-PAGE

The method described by Weber and Osborn (1969) was used. Briefly, the polyacrylamide gel in which sodium dodecyl sulphate was added was prepared in the proportion recommended by Weber and Osborn (1969-Appendix 5).

The gel was allowed to polymerize for 15 minutes at room temperature and pre-equilibrated at 5mA per tube for 30 minutes. The samples and standards were prepared according to the method of Weber and Osborn (1969). These were applied to separate gels and electrophoresis performed at 8mA per tube for 4 hours. After electrophoresis the gels were removed from the tubes by squirting water from a syringe between the gel and glass wall. The gels were placed in test tubes containing a Coomassie brilliant blue staining solution at room temperature. The gels were destained after 18 hours in a destaining solution containing 7% acetic acid and 20% methanol (v/v) in distilled water.

E:2 Further analysis of eluted antigen by isoelectric focusing

The eluted antigen showed five bands in SDS-PAGE analysis. It was therefore decided to further analyse the antigen by isoelectric focusing.

The isoelectric focusing was performed in the 110 mls (LKB 8100-1) column according to the manufacturer's recommendations (LKB Bromma, Sweden). Ampholine with a pH range between 3.5 to 10 was used to make the pH gradient (Appendix 6).

The sample of concentrated eluted antigen (7.2 mg protein per ml) was equilibrated by dialysis against 1% glycine (w/v,Merck Darmstadt, Germany) for 18 hours at 4°C. An equal volume of the antigen was then placed in the light and dense gradient solutions and the electrofocusing was performed at 1600v and 14mA for 18 hours at 4°C.

The different protein fractions were eluted and dialysed against excess (10 x volume) PBS pH 7.4 for 24 hours. These fractions were then concentrated to the original volume by ultrafiltration using DIAFLO PM10 ultrafilter and tested by immunodiffusion using Antigen 11 specific antiserum(No.879)and polyspecific antisera (Nos. 140, 154, 824 and 888). The inner membrane-scolex of C. bovis was used as the reference antigen.

The fractions which showed reactivity were those in the pH range between 4.14 and 6.22. These fractions were again analysed in SDS-PAGE, and two common bands were found to be present in all the fractions. It was,therefore,

decided to perform a narrow range isoelectric focusing on these fractions.

Ampholine with pH range between 4 and 6 was used to make the pH gradient and the rest of the procedure was as described for the wide range isoelectric focusing.

F. Detection of antibody response to Antigen 11 in cattle experimentally infected with C. bovis

A two step solid-phase radioimmunoassay was used in the evaluation of the usefulness of the antigen obtained from the isoelectric focusing column in the detection of antibodies in sera collected from experimentally infected animals.

F.1 Determination of optimum dilution of antigen for the radioimmunoassay

The protein content of the antigen (isolated, purified and concentrated in section D and E) as determined by the method of Lowry et al. (1951) was 1.6 mg protein per ml.

A chequer board titration according to the method described by Kabat and Meyer (1967) was used to find the most suitable dilution of antigen for coating the plates. The preinfection and known positive sera (section B) were used in the titration. The dilution of antigen that gave the lowest titratable counts was 1:160 (10 ug protein/ml) but for subsequent assays the dilution chosen was 1:100 (16 ug protein/ml).

F:2 The assay

The solid-phase radioimmunoassay was performed as described by Tsu and Herzenberg (1980). Briefly, the method was as follows: Aliquots of 50 ul of diluted antigen (1:100) were added to each well of microtitre plate (Flow Laboratories, Rockville, U.S.A.) except those which contained the controls for the assay system. The plate was kept at room temperature for 1 hour and was then washed three times with radioimmunoassay buffer (Appendix 7). The plate was then filled with 200 ul of radioimmunoassay buffer and incubated for 1 hour at room temperature to saturate the free-binding sites in the wells. Thereafter, the buffer was drained off, 50 ul of test and control serum (diluted in tenfold dilutions from 10^{-1} to 10^{-6} in radioimmunoassay buffer) was added to each well and the incubation process repeated. The wells were then drained and washed as described above before the addition of 50 ul ^{125}I-labelled goat anti-bovine IgM or IgG_1, containing 20,000 cpm (a gift from Dr. R.A. Masake of ILRAD). The plates were incubated for another hour at room temperature washed 3 times with radioimmunoassay buffer and dried by "throwing" the plate face down onto several layers of absorbent paper. The wells were cut apart with a hot wire, placed into the tubes and the bound radioactivity measured in a Packard 5360 Auto gamma Scintillation Spectrometer (Amanan Refrigeration Inc. Iowa, U.S.A.).

The controls for the assay are shown in Table 3.

Table 3: The controls for the radioimmunoassay

Control	Contents in the wells
Buffer controls (Background counts "B")	50 ul radioimmunoassay buffer + 50 ul of ^{125}I-labelled goat anti-bovine IgM or IgG_1
Serum controls	50 ul serum sample + 50 ul of ^{125}I-labelled antiserum
Antigen controls	50 ul antigen + 50 ul ^{125}I-labelled antiserum
Test samples	50 ul antigen + 50 ul test serum + 50 ul ^{125}I-labelled antiserum

F:3 Calculation of titres of antibodies in serum samples

All the tests were carried out in duplicate. The mean counts per minute were calculated and plotted against the dilutions of the serum samples.

The difference between the points of intercept of the background line by the titration curves of the controls and test serum gave the titre of the antibody for that sample (Fig.18).

RESULTS

4: RESULTS

4:1 Analysis of C. bovis antigens for the presence of host components

Immunoelectrophoretic analyses (Figure 5 and 6) show that the C. bovis inner fluid and outer fluid fractions contain host components which migrate in the albumin, beta and gamma regions. Figure 7 shows that the host components present in the inner membrane-scolex of C. bovis mainly migrate in the albumin and gamma regions.

The presence of host components in C. tenuicollis fluid from both sheep and goats was also confirmed. Figure 8 shows that the host component in C. tenuicollis fluid (goat) have an electrophoretic mobility similar to those of C. bovis fractions, inner fluid and outer fluid; viz, the host components migrate in albumin beta, and gamma regions.

Using specific antiserum, the slow-migrating host component was shown to be gamma globulins belonging to immunoglobulin class IgG, while the fast-migrating host components belonged to albumin class since it had the same migration mobility as the reference bovine serum albumin.

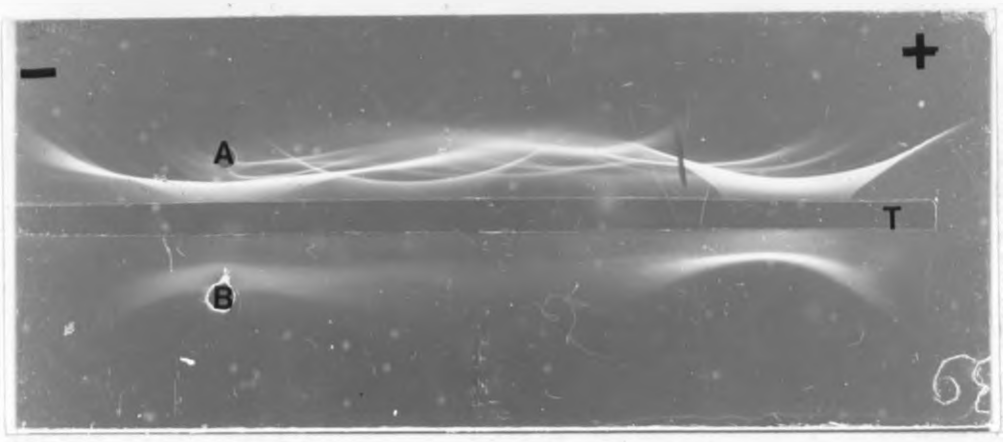


Figure 5: Well A: Bovine serum

Well B: Inner fluid (C. bovis)

Trough T: Rabbit anti-bovine serum (Number 87).

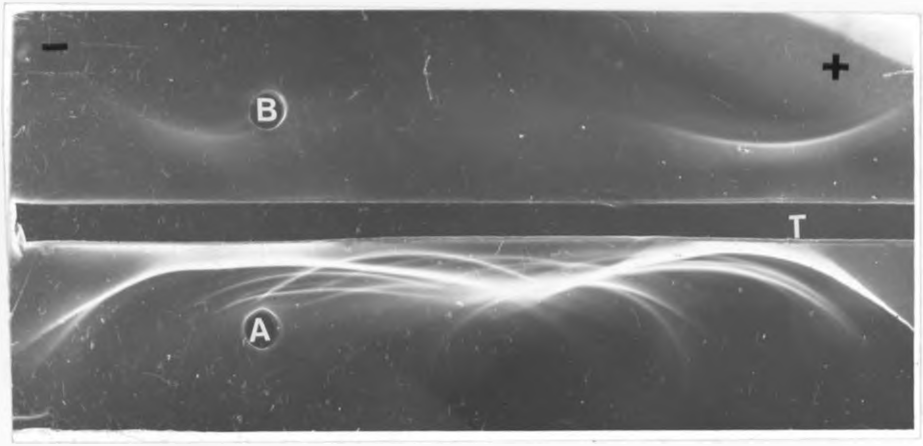


Figure 6: Well A: Bovine serum

Well B: Outer fluid (C. bovis)

Trough T: Rabbit anti-bovine serum (Number 87)

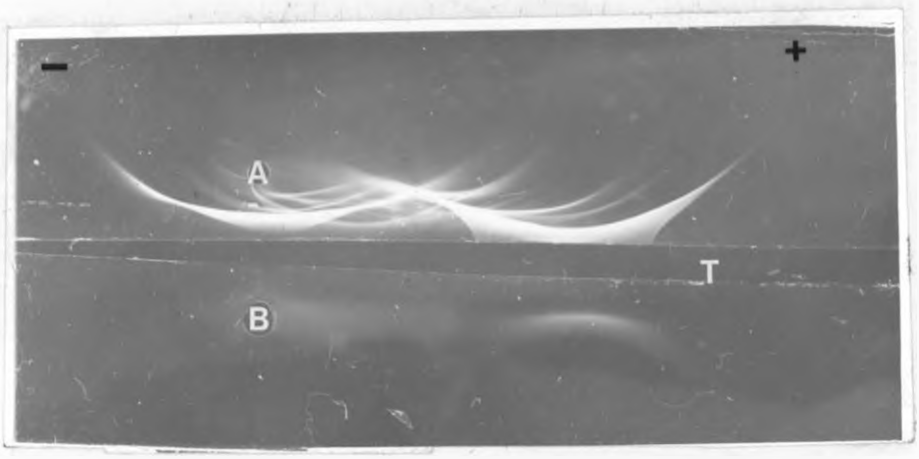


Figure 7: Well A : Bovine serum

Well B : Inner membrane scolex (C. bovis)

Trough T : Rabbit anti bovine serum (Number 87)

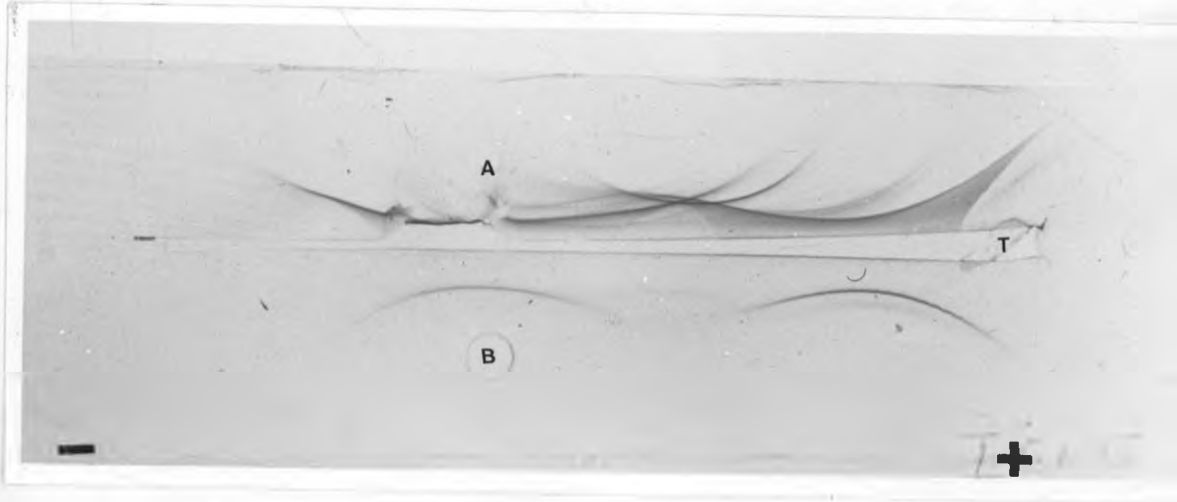


Figure 8: Well A : Goat serum

Well B : C. tenuicollis (goat)

Trough T : Rabbit anti goat serum

4:2 Characterisation of various antisera and antigens

The reactions between the antisera prepared in this study and the various antigens were examined using the Ouchterlony double diffusion technique. All antisera were absorbed prior to use as shown in Appendix 2.

The following relationships were studied (i) The relationship between antigenic extracts of C. bovis and T. saginata. (ii) The relationship between C.bovis, T. saginata and various parasites described.

These reactions are summarised for each antiserum in Tables 4 to 14. The precipitin lines were given arbitrary numbers, starting with the one nearest to the well containing the antiserum. For each set of tables the number given for a precipitin line represents the same antigenic component.

Antiserum against the precipitin line formed between anti-T. saginata (Antiserum No.824) and C.bovis inner membrane-scolex (see Section B:4:1) was characterized by two dimensional immunoelectrophoresis. The antigenic component detected by this antiserum was defined as component number 11, using a reference pattern described in Section 4:3:1. This antiserum reacted only with one antigenic component (Antigen 11) in extracts from T. saginata, C. bovis, T. hydatigena, C. tenuicollis and C. cellulosae and did not react with extracts from the rest of the parasites tested (Table Nos. 4 to 14).

4:2:1 Immunodiffusion results obtained with rabbit anti-*C. bovis* Inner membrane-scolex (Antiserum 140/158)

The precipitin reactions observed with this antiserum are summarized in Tables 4a and 4b.

The antiserum (Table 4a) detected five components components in the homologous antigen (inner membrane-scolex). Of these only three (1,4 and 11) were found to be present in the inner and outer fluid of *C. bovis*. Two of these components (1 and 11) showed complete identity with antigenic components of *T. saginata* whole worm extract. The third component (4) present in the inner membrane-scolex extract showed identity with the inner and outer fluid of *C. bovis* only:

T. saginata extracts also showed one component (No.2) which was shared with the inner membrane-scolex extracts only and was not present in the inner and outer fluid of *C. bovis* There was another component which was present only in the inner membrane-scolex.

Figure 9 shows the antigenic relationship between *C. bovis* and *T. saginata* antigenic extracts.

Table 4a: Precipitin reactions observed with Antisera Nos. 140/158 and 879.

Antigens	Antiserum	
	Number 140/158	Number 879
Inner membrane-scolex (*C. bovis*)	1, 2, 4, 5, 11	11

Table 4a (Cont'd)

ANTIGENS	ANTISERUM	
	Number 140/158	Number 879
Inner fluid (C.bovis)	1, 4, 11	11
Outer fluid (C.bovis)	1, 4, 11	11
Outer membrane(C.bovis)	-	-
T. saginata	1, 2, 11	11

- = No precipitin lines observed.

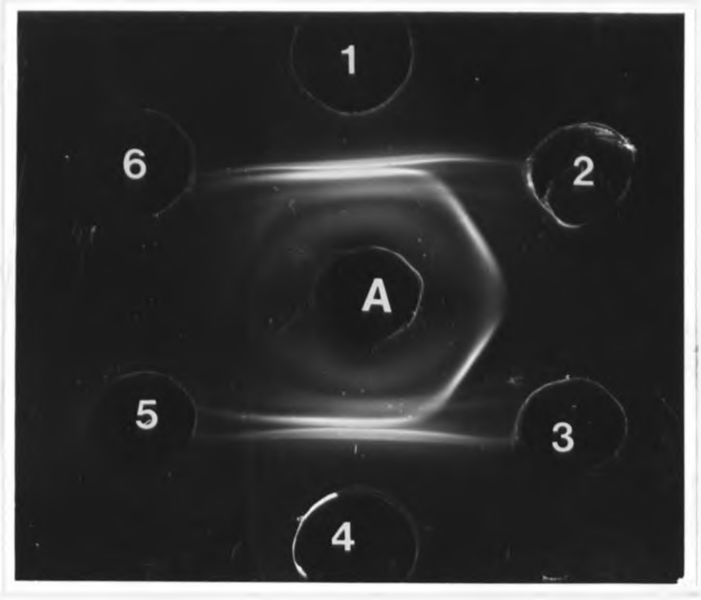


Figure 9: Well A: Pooled rabbit anti-C. bovis (antiserum 140/158)

Well 1: Inner membrane-scolex (C. bovis)

Well 2: Inner fluid (C. bovis)

Well 3: Outer fluid (C. bovis)

Well 4: Inner membrane-scolex (C. bovis)

Well 5: Outer membrane (C. bovis)

Well 6: T. saginata

Table 4b (cont'd) Precipitin reactions with other parasite extracts

ANTIGENS	ANTISERUM	
	No. 879	No.140/158
F. gigantica (cattle)	-	-
Paramphistomum spp. (cattle)	-	2
Paramphistomum spp. (goat)	-	2
S. mansoni	-	-
H. contortus (cattle)	-	-
Trichuris spp. (cattle)	-	-
Oesophagostomum spp. (cattle)	-	2
T. brucei	-	-
A. suum (pig)	-	-
C. cellulosae	11	2, 11

- = No precipitin lines observed.

4.2.2. Immunodiffusion results obtained with rabbit anti-C.bovis scolices (Antiserum No.79)

Table 5a shows the precipitin reactions obtained with Antiserum No.79. This antiserum detected five antigenic components in the inner membrane-scolex as illustrated in Figure 10. Three of these components (1,2, and 11) showed identity with the inner and outer fluid of C. bovis

Four of the five components of C. bovis were also present in T. saginata extracts including Antigen 11.

Table 5a Precipitin reactions observed with rabbit anti-C. bovis scolices (Antiserum 79) as compared to Antiserum No.879.

This table shows precipitin reactions with C. bovis and T. saginata extracts.

ANTIGENS	ANTISERUM	
	Number 79	Number 879
Inner membrane-scolex (C. bovis)	1,2,4,5,11	11
Inner fluid (C. bovis)	1,2,11	11
Outer fluid (C. bovis)	1,2,11	11
Outer membrane (C.bovis)	-	-
T. saginata	1,2,4,11	11

- = No precipitin lines observed.

Figure 10: Well A: Pooled rabbit anti-C.bovis scolices (Antiserum No.79).

Well 1: Inner membrane scolex (C. bovis)

Well 2: Inner fluid (C. bovis)

Well 3: Outer fluid (C. bovis)

Well 4: Inner membrane scolex (C. bovis)

Well 5: Outer membrane(C. bovis)

Well 6: T. saginata

Table 5b shows reactions between Antiserum Number 79 and extracts of various parasites.

Antiserum No.79 was able to detect Antigen 11 in T.hydatigena, C. tenuicollis and C. cellulosae. This antiserum also identified another antigen (Number 2) which was present in C. bovis and the following parasites: C. tenuicollis, C. cerebralis, hydatid cyst fluid, S. hepatica, F. gigantica, Paramphistomum spp, Oesophagostomum spp and C. cellulosae.

This antiserum also detected Antigen No.1 in C. cellulosae.

Table 5b: Precipitin reactions between Antisera Nos. 879 and 79 with other parasite extracts

ANTIGENS	ANTISERUM	
	No.879	No. 79
T. hydatigena	11	11
C. tenuicollis fluid (goats)	11	2, 11
C. tenuicollis fluid (sheep)	11	2, 11
C. tenuicollis membrane-scolex (goat)	11	2, 11
C. tenuicollis membrane-scolex (sheep)	11	2, 11
C. cerebralis fluid (goat)	-	2
C. cerebralis (membrane & scolices)	-	2
Hydatid cyst fluid (cattle)	-	2
Moniezia spp (cattle)	-	2

- = No precipitin lines observed.

Table 5b (cont'd) Precipitin reactions observed between Antisera Nos. 879 and 79 with other parasite extracts

ANTIGENS	ANTISERUM	
	No.879	No.79
Moniezia spp (goat/sheep)	-	2
S. hepatica (goat)	-	2
C. fasciolaris (mice)	-	-
F. gigantica (cattle)	-	2
Paramphistomum spp. (cattle)	-	2
Paramphistomum spp. (goat)	-	-
S. mansoni	-	-
H. contortus (cattle)	-	-
Trichuris spp. (cattle)	-	-
Oesophagostomum spp. (cattle)	-	-
T. brucei	-	-
A. suum (pig)	-	-
C. cellulosae	11	1,2,11

- = No precipitin lines observed.

Table 6b: Precipitin reactions observed between Antisera No. 879 and 846 with other parasite extracts.

ANTIGENS	ANTISERUM	
	No. 879	No.346
T. hydatigena	11	11
C. tenuicollis fluid (goat)	11	11
C. tenuicollis fluid (sheep)	11	11
C. tenuicollis (membrane-scolex) (goat)	11	11
C. tenuicollis membrane-scolex (sheep)	11	11
C. cerebralis fluid (goat)	-	-
C. cerebralis (goat membrane & scolices)	-	-
Hydatid cyst fluid (cattle)	-	-
Hydatid cyst fluid (sheep)	-	-
Moniezia spp (cattle)	-	-
Moniezia spp (goat/sheep)	-	-
S. hepatica (goat)		
C. fasciolaris (mice)	-	-
F. gigantica (cattle)	-	-

- = No precipitin lines observed.

Table 6b (cont'd) Precipitin reactions observed between Antisera Nos. 879 and 846 with other parasite extracts

ANTIGENS	ANTISERUM	
	No.879	No.846
Paraphistomum spp (cattle)	-	-
Paramphistomum spp (goat)	-	-
S. mansoni	-	-
H. contortus (cattle)	-	-
Trichuris spp. (cattle)	-	-
Oesophagostomum spp. (cattle)	-	-
T. brucei	-	-
A. suum (pig)	-	-
C. cellulosae	11	11

4.2.4. Immunodiffusion results obtained with rabbit anti-Inner fluid C. bovis (Antiserum No. 146).

Antiserum No. 146 shows four antigenic components with the homologous antigen, the inner fluid of C. bovis.

Two of these components are shared with inner membrane scolex, Outer fluid and T. saginata. One of these components is Antigen 11 and shows complete identity.

There is another component (No.3) which is shared between Inner membrane-scolex and Inner fluid, while the fourth component (No.4) is present only in the Inner fluid (Table 7a).

Table 7a: Precipitin reactions observed with rabbit anti Inner fluid C. bovis (Antiserum 146) as compared to Antiserum No.879.

This table shows precipitin reactions with C. bovis and T. saginata extracts.

ANTIGENS	ANTISERUM	
	No. 146	No.879
Inner fluid (C.bovis)	1,3,4,11	11
Inner membrane-scolex (C. bovis)	1,3,11	11
Outer fluid (C. bovis)	1,11	11
Outer membrane (C. bovis)	-	-
T. saginata	1, 11	11

- = No precipitin line observed.

This antiserum was also able to detect Antigen Number 3 which was shared between C. bovis and the following parasite extracts: T. hydatigena, C. tenuicollis, C. cerebralis, Oesophagostomum spp. and C. cellulosae. Antigen 11 was detected only in C. cellulosae, T. hydatigena and C. tenuicollis (Table 7b).

Table 7b: Precipitin reactions observed between Antisera Nos.879 and 146 with other parasite extracts.

ANTIGENS	ANTISERUM	
	No.879	No.146
T. hydatigena	11	3, 11

Table 7b (cont'd) Precipitin reaction observed between Antisera Nos.879 and 146 with other Parasite extracts

ANTIGENS	ANTISERUM	
	No.879	No.146
C. tenuicollis fluid (goat)	11	3, 11
C. tenuicollis fluid (sheep)	11	3, 11
C. tenuicollis membrane-scolex (goat)	11	3, 11
C. tenuicollis membrane-scolex (sheep)	11	3, 11
C. cerebralis fluid (goat)	-	3
C. cerebrallis (membrane-scolices)	-	3
Hydatid cyst fluid (cattle)	-	-
Hydatid cyst fluid (goat/sheep)	-	-
Moniezia spp. (cattle	-	-
Moniezia spp. (goat/sheep)	-	-
S. hepatica (goat)	-	-
C. fasciolaris (mice)	-	-

- = No precipitin lines observed.

Table 7b (cont'd): Precipitin reactions observed between Antisera Nos.879 and 146 with other parasite extracts

ANTIGENS	ANTISERUM	
	No.879	No.146
F. gigantica (cattle)	-	-
Paramphistomum spp. (cattle)	-	-
Paramphistomum spp. (goat)	-	-
S. mansoni	-	-
H. contortus (cattle)	-	-
Trichuris spp. (cattle)	-	-
Oesophagostomum spp. (cattle)	-	3
T. brucei	-	-
A. suum (pig)	-	-
C. cellulosae	11	11

- = No precipitin lines observed.

4:2.5: Immunodiffusion results obtained with calf anti-Inner fluid of C. bovis (Antiserum No.835)

This antiserum detected two antigenic components in the homologous antigen(s). One of these components was Antigen 11 which was common to Outer fluid, Inner membrane-scolex and T. saginata extracts.

The other component (Number 2) was common to Inner membrane-scolex and Inner fluid (Table 8a).

Table 8a: Precipitin reactions observed with calf anti-Inner fluid (Antiserum No.835) as compared To Antiserum No.879.

This table shows the precipitin reactions with C. bovis and T. saginata extracts.

ANTIGENS	ANTISERUM	
	No.835	No.879
Inner fluid (C. bovis)	2, 11	11
Inner membrane scolex (C. bovis)	2, 11	11
Outer fluid (C. bovis)	11	11
Outer membrane (C. bovis)	-	-
T. saginata	11	11

- = No precipitin lines observed.

On further examination, antiserum No.835 was found to detect Antigen Number 2 in the following parasite extracts: C. cerebralis, Oesophagostomum spp. and Moniezia spp.

The antiserum was also able to detect Antigen 11 in C. cellulosae, C. tenuicollis and T. hydatigena.

Table 8b: Precipitin reactions observed between Antisera Nos. 835 and 879 with other parasite extracts

ANTIGENS	ANTISERUM	
	No.835	No.879
T. hydatigena	11	11
C. tenuicollis fluid (goat)	11	11
C. tenuicollis fluid (sheep)	11	11
C. tenuicollis membrane-scolex (goat)	11	11
C. tenuicollis membrane-scolex (sheep)	11	11
C. cerebralis fluid (goat)	2	-
C. cerebralis membrane and Scolices	2	-
Hydatid cyst fluid (cattle)	-	-
Hydatid cyst fluid (goat/sheep)	-	-
Moniezia spp. (cattle)	2	-
Moniezia spp. (goat/sheep)	2	-
S. hepatica (goat)	-	-
C. fasciolaris (mice)	-	-

- = No precipitin lines observed.

Table 8b (cont'd) Precipitin reactions observed between Antisera Nos. 835 and 879 with other parasite extracts

ANTIGEN	ANTISERUM	
	No.835	No.879
F. gigantica (cattle	-	-
Paramphistomum spp. (cattle)	-	-
Paramphistomum spp. (goat)	-	-
S. mansoni	-	-
H. contortus (cattle)	-	-
Trichuris spp. (cattle)	-	-
Oesophagostomum spp. (cattle)	-	-
A. suum (pig)	-	-
C. cellulosae	11	11

- = No precipitin lines observed.

4:2:6 Immunodiffusion results obtained with calf anti-Outer fluid C. bovis (Antiserum Number 118)

Antiserum number 118 shows three antigenic components in the homologous antigen, the Outer fluid (C. bovis). Two of these components (Number 4 and 11) are shared with T. saginata extracts, Inner membrane-scolex and Inner fluid (C. bovis). Component Number 1 was also common to other fractions of C. bovis (Table 9a).

Table 9a: Precipitin reactions observed with calf anti-Outer fluid C. bovis (Antiserum No.118) as compared to Antiserum No.879

This table shows precipitin reactions with C. bovis and T. saginata.

ANTIGEN	ANTISERUM	
	No.118	No.879
Outer fluid (C. bovis)	1, 4, 11	11
Outer membrane (C. bovis)	-	-
Inner fluid (C. bovis)	1, 4,11	11
Inner membrane scolex (C. bovis)	2, 4,11	11
T. saginata	4, 11	11

- = No precipitin lines observed.

Antiserum No.118 was also able to detect component No.4 in the following parasite extracts: C. cerebralis, hydatid cyst fluid, Oesophagostomum spp., C. tenuicollis and T.hydatigena (Table 9b)

This antiserum was also able to detect component number 11 in T. hydatigena, C. tenuicollis and C. cellulosae.

Table 9b: Precipitin reactions observed between Antisera Nos. 118 and 879 with other parasite extracts

ANTIGENS	ANTISERUM	
	No.118	No.879
T. hydatigena	11, 4	11
C. tenuicollis fluid (goat)	4, 11	11
C. tenuicollis fluid (sheep)	4, 11	11
C. tenuicollis membrane-scolex (goat)	4, 11	11
C. tenuicollis membrane-scolex (sheep)	4, 11	11
C. cerebralis fluid (goat)	4	-
C. cerebralis membrane scolices	4	-
Hydatid cyst fluid (cattle)	4	-
Hydatid cyst fluid (Sheep/goat)	4	-
Moniezia spp. (cattle)	-	-
Moniezia spp. (goat/sheep)	-	-
S.hepatica (goat)	-	-
C. fasciolaris	-	-

- = No precipitin lines observed.

Table 9b (cont'd) Precipitin reactions observed between Antisera Nos.118 and 879 with other parasite extracts

ANTIGENS	ANTISERUM	
	No.118	No.879
F. gigantica (cattle)	-	-
Paramphistomum spp. (cattle)	-	-
Paramphistomum spp. (goat)	-	-
S. mansoni	-	-
H. contortus (cattle)	-	-
Trichuris spp. (cattle)	-	-
Oesophagostomum spp. (cattle)	4	-
T. brucei	-	-
A. suum (pig)	-	-
C. cellulosae	11	11

- = No precipitin lines observed.

4:2:7 Immunodiffusion results obtained with calf anti-T. saginata (Antiserum number 900)

Antiserum 900 detected two antigenic components in the homologous antigen T. saginata whole worm extract. One of this component was Antigen 11 and this was common to Inner membrane-scolex, Inner and Outer fluid C. bovis.

The other component(No.2) was common to Inner membrane-scolex and T. saginata (Table 10a)

Table 10a: Precipitin reactions observed with calf anti-T. saginata (Antiserum No.900) as compared to Antiserum No.879

This table shows precipitin reaction with C. bovis and T. saginata extracts.

ANTIGENS	ANTISERUM	
	No.900	No.879
T. saginata	2, 11	11
Inner membrane scolex (C. bovis)	2, 11	11
Inner fluid (C. bovis)	11	11
Outer fluid (C. bovis)	11	11
Outer membrane (C. bovis)	-	-

- = No precipitin lines observed.

Antiserum Number 900 was also able to detect antigen Number 2, in the following parasite extracts: C. tenuicollis T. hydatigena, C. cerebralis, Hydatid cyst fluid, moniezia spp., S. hepatica, Oesophagostomum spp. and C. cellulosae.

This antiserum was also able to detect component Number 11 in T. hydatigena, C. tenuicollis and C. cellulosae (Table 10b).

Table 10b. Precipitin reactions observed between Antisera Nos. 900 and 879 with other parasite extracts

ANTIGENS	ANTISERUM	
	No.900	No.879
T. hydatigena	2, 11	11
C. tenuicollis fluid (goat)	2, 11	11
C. tenuicollis fluid (sheep)	2, 11	11
C. cerebralis fluid (goat)	2	-
C. tenuicollis membrane-scolex (goat)	2, 11	11
C. tenuicollis membrane-scolex (sheep)	2, 11	11
C. cerebralis membrane scolices	2	-
Hydatid cyst fluid (cattle)	2	-
Hydatid cyst fluid (sheep/goat)	2	-
Moniezia spp. (cattle)	2	-
Moniezia spp. (goat/sheep)	2	-
S. hepatica spp. (goat)	2	-
C. fasciolaris (mice)	-	-

- = No precipitin line observed.

Table 10b (continued) Precipitin reactions observed between Antisera Nos.900 and 879 with other extracts.

ANTIGENS	ANTISERUM	
	No. 900	No.879
F. gigantica (cattle)	-	-
Paramphistomum spp. (cattle)	-	-
Paramphistomum spp. (goat)	-	-
S. mansoni	-	-
H. contortus	—	—
Trichuris spp. (cattle)	—	—
Oesophagostomum spp. (cattle)	2	-
T. brucei	-	-
A. suum (pig)	—	—
C. cellulosae	2, 11	11

- = No precipitin lines observed.

4:2:8 Immunodiffusion results obtained with goat anti-T. saginata (Antiserum Number 824)

This antiserum shows three antigenic components in the homologous antigens. One of these components is Antigen 11 and it shows complete identity with antigenic components in the Inner fluid, Outer fluid and Inner membrane-scolex C. bovis (Table 11a).

Another component (Number 2) shows partial identity with Inner membrane-scolex, Inner and Outer fluid (Figure 11).

Table 11a: Precipitin reactions observed with goat anti-T.saginata(Antiserum No.824)as compared to Antiserum No.879.

This table shows precipitin reactions with C. bovis and T. saginata extracts.

ANTIGENS	ANTISERUM	
	No.824	No.879
T. saginata	1,2,11	11
Inner membrane scolex (C.bovis)	2, 11	11
Inner fluid (C. bovis)	2, 11	11
Outer fluid (C. bovis)	2, 11	11
Outer membrane (C. bovis)	-	-

- = No preciptin lines observed.

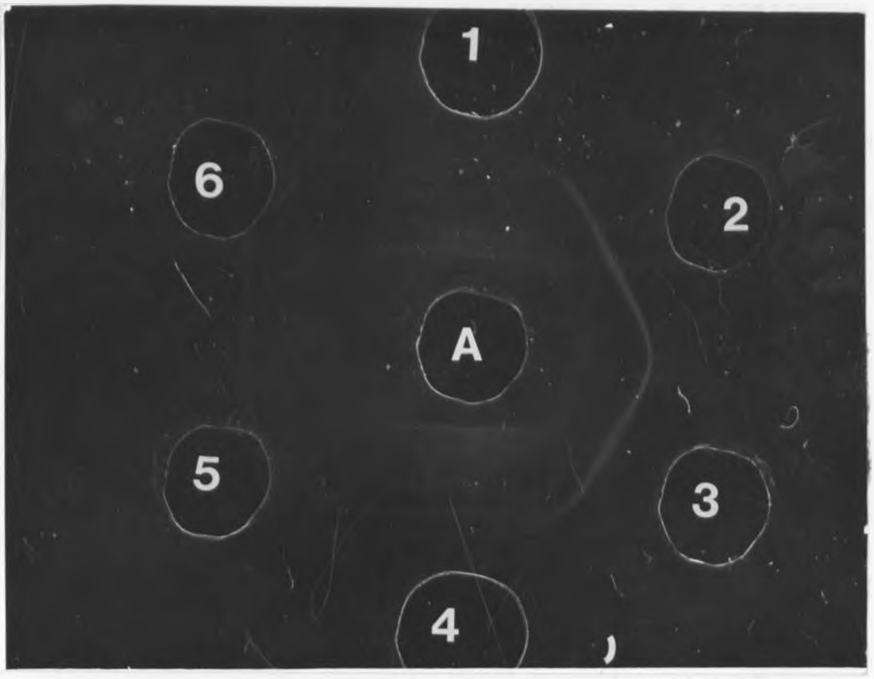


Figure 11: Well A: Pooled goat anti-T. saginata (Antiserum No. 824).

Well 1: T. saginata.

Well 2: Inner fluid. (C. bovis)

Well 3: Inner membrane scolex. (C. bovis)

Well 4: T. saginata.

Well 5: Outer fluid. (C. bovis)

Well 6: Outer membrane (C. bovis)

This antiserum could also detect component number 1, which was common to the following parasite extracts:- C. cerebralis, Moniezia spp., S. hepatica, H. contortus, Trichuris spp., Oesophagostomum spp. and C. cellulosae.

Antigen 11 could also be detected by this antiserum in T. hydatigena, C. tenuicollis and C. cellulosae. There was another component (Number 2) which could be detected in C. celluosae (Table 11b;).

Table 11 b: Precipitin reactions observed between Antisera Nos. 824 and 879 with other parasite extracts

ANTIGEN	ANTISERUM	
	No.824	No.879
T.hydatigena	11	11
C. tenuicollis fluid (goat)	11	11
C. tenuicollis fluid (sheep)	11	11
C. tenuicollis membrane-scolex (goat)	11	11
C. tenuicollis membrane-scolex (sheep)	11	11
C. cerebralis fluid (goat)	1	-
C. cerebralis membrane scolices	1	-
Hydatid cyst fluid (cattle)	-	-
Hydatid cyst fluid (sheep/goat)	-	-
Moniezia spp. (cattle)	1	-

- = No precipitin lines observed.

Table 11b (cont'd) Precipitin reactions observed with Antisera Nos.824 and 879 with other parasite extracts

ANTIGEN	ANTISERUM	
	No.824	No.879
Moniezia spp. (goat/sheep)	1	-
S. hepatica	-	-
C. fasciolaris (mice)	-	-
F. gigantica (cattle)	-	-
Paramphistomum spp. (cattle	-	-
Paramphistomum spp. (goat)	-	-
S. mansoni	-	-
H. contortus (cattle	1	-
Trichuris spp. (cattle)	1	-
Oesophagostomum spp. (cattle)	1	-
T. brucei	-	-
A. suum (pig)	-	-
C. cellulosae	1,2,11	11

— = No precipitin lines observed.

4:2:9 Immunodiffusion results obtained with rabbit anti-T. saginata (Antiserum number 154)

The preciptin reactions observed with this antiserum are summarised in Tables 12a and 12b.

This antiserum showed four antigenic components in the homologous antigen (T. saginata). Two of these components show complete identity with C. bovis Inner membrane-scolex, Inner and Outer fluid. The other two components (number 3 and 4) showed partial identity with Inner membrane-scolex (Fig.12).

Using monospecific antiserum (Number 879) one of the components showing complete identity was found to be Antigen 11.

Table 12a: Precipitin reactions observed with rabbit anti-T.saginata (Antiserum No.154) as compared to Antiserum No.879

This Table shows precipitin reactions with C. bovis and T. saginata extracts.

ANTIGENS	ANTISERUM	
	No.154	No.879
T. saginata	1,3,4,11	11
Inner membrane scolex (C. bovis)	1,3,4,11	11
Inner fluid (C. bovis)	1, 11	11
Outer fluid (C. bovis)	11	11
Outer membrane (C. bovis)	-	-

- = No precipitin lines observed.

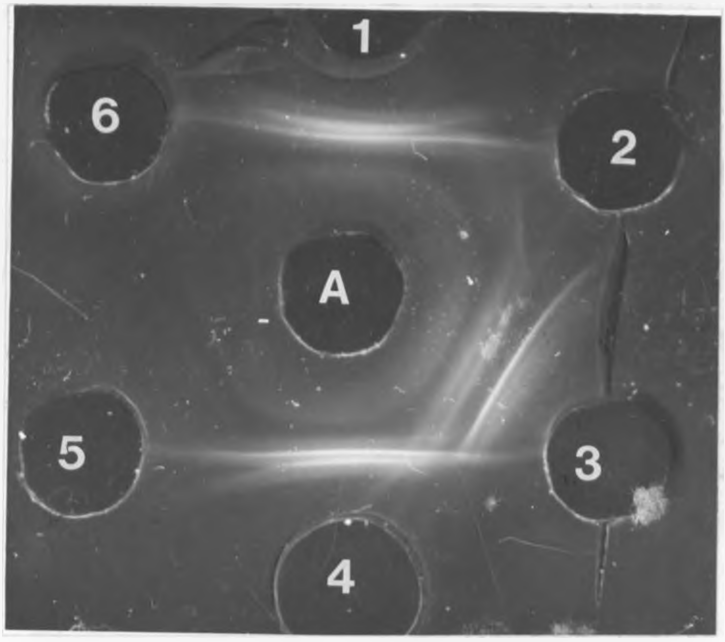


Figure 12: Well A: Pooled rabbit anti-T. saginata (Antiserum 154).

Well 1: T. saginata

Well 2: Inner fluid (C. bovis)

Well 3: Inner membrane-scolex (C. bovis)

Well 4: T. saginata

Well 5: Outer fluid (C. bovis)

Well 6: Outer membrane (C. bovis)

Table 12b shows the summary of the precipitin reactions obtained between Antiserum Number 154 and the other parasite extracts. It can be seen that the following parasites share antigenic components with T. saginata: T.hydatigena, C. tenuicollis, Oesophagostomum spp. H. contortus, F.gigantica, Paramphistomum spp., Moniezia spp., S. hepatica, C. cerebralis and C. cellulosae.

Figure 13 shows that one component(Number 4)in T. saginata is shared with F. gigantica and C. cerebralis fluid. On further examination this component was found to be present in Moniezia,spp, C. cellulosae, S. hepatica and Paramphistomum spp.

Using monospecific Antiserum to Antigen 11, this antigen was identified in C. cellulosae,T. hydatigena and C. tenuicollis.

Table 12b. Precipitin reactions observed between Antisera Nos.154 and 879 with other parasite extracts

ANTIGENS	ANTISERUM	
	No. 154	No. 879
T. hydatigena	4,3,11	11
C. tenuicollis fluid (goat)	4,3,11	11
C.tenuicollis fluid (sheep)	4,3,11	11
C. tenuicollis membrane-scolex (sheep)	4, 11	11
C. tenuicollis membrane-scolex (goat)	4, 11	11

Table 12(b) (Cont'd) Precipitin reactions observed between Antisera Nos.154 and 879 with other parasite extracts

ANTIGEN	ANTISERUM	
	No. 154	No. 879
C. cerebralis fluid (goat)	4	-
C. cerebralis membrane & scolices	4,3	-
Hydatid cyst fluid (cattle)	-	-
Hydatid cyst fluid (sheep/goat)	-	-
Moniezia spp. (cattle)	4,3	-
Moniezia spp. (goat/sheep)	4,3	-
S. hepatica (goat)	4	-
C. fasciolaris (mice)	-	-
F. gigantica (cattle)	4	-
Paramphistomum spp. (cattle)	4	-
Paramphistomum spp. (goat)	4	-
S. mansoni	-	-
H. contortus (cattle)	3	-
Trichuris spp. (cattle)	-	-
Oesophagostomum spp (cattle	3	-
T. brucei	-	-
A. suum (pig)	-	-
C. cellolosae	3,4,11	11

— = No precipitin lines observed.

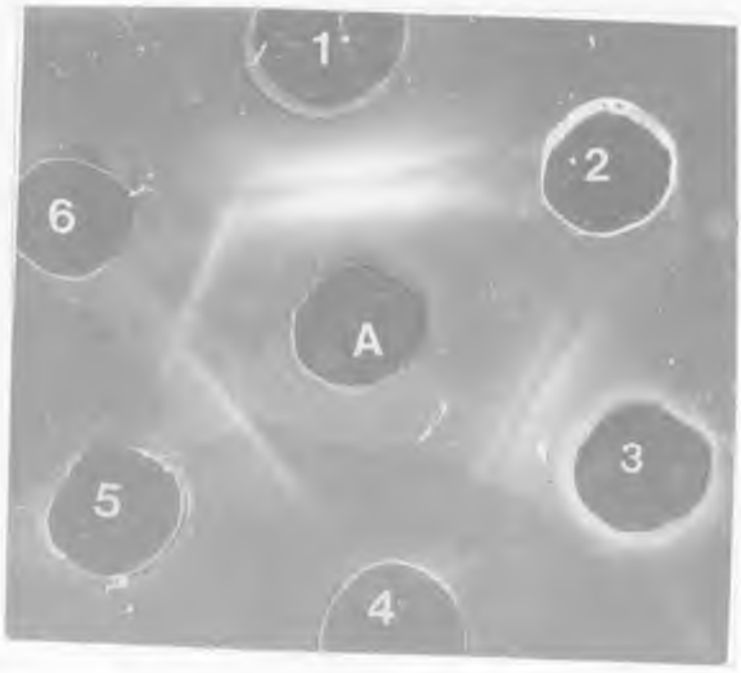


Figure 13: Well A: Pooled rabbit anti-T. saginata (Antiserum 154)

Well 1: T. saginata

Well 2: C. fasciolaris

Well 3: C. tenuicollis

Well 4: Hydatid cyst fluid

Well 5: C. cerebralis (fluid)

Well 6: F. gigantica

4.2.10. Immunodiffusion results obtained with goat anti-C. tenuicollis (Antiserum Number 888)

This antiserum shows four antigenic components in the homologous antigen (Table 13a).

Two of these components were shared with the Inner membrane-scolex of C. bovis. One of the components was identified as Antigen 11 and was found to be present in T. saginata, Inner and Outer fluid of C. bovis (Table 13a).

C. tenuicollis antigens also shared components 3 and 4 with the Inner fluid of C. bovis.

Table 13a: Precipitin reactions observed with goat anti-C. tenuicollis (antiserum No.888) as compared to Antiserum No.879.

This table shows precipitin reactions with C. bovis and T. saginata extracts.

ANTIGENS	ANTISERUM	
	No.888	No.879
C. tenuicollis (goat)	1,3,4,11	11
C. tenuicollis (sheep)	1 3,4,11	11
Inner fluid (C.bovis)	1,3,4 11	11
Inner membrane scolex (C.bovis)	1, 11	11
Outer fluid (C. bovis)	11	11
T. saginata	11	11

- = No precipitin lines observed.

Using this antiserum the following parasite extracts were shown to share antigenic components with C. tenuicollis:- C. cerebralis, Hydatid cyst fluid Moniezia spp, S. hepatica, F. gigantica, Paramphistomum spp. S. mansoni, H. contortus, Oesophagostomum spp and C. cellulosae.

None of these parasites except C. cellulosae showed Antigen 11 reactivity when the monospecific antiserum (No.879) was used. Antigen number 1 and 3 showed reactivity only with cestodes while Antigen number 4 was common to a wide variety of parasites (Table 13b).

Table 13b: Precipitin reactions observed between Antisera Nos. 888 and 879 with other parasite extracts

ANTIGENS	ANTISERUM	
	No. 888	No.879
C. cerebralis fluid (goat)	1	-
C. cerebralis membrane scolices	1, 3	-
Hydatid cyst fluid (cattle)	1	-
Hydatid cyst fluid (goat/sheep)	1	-
Moniezia spp. (cattle)	3	-
Moniezia spp. (goat/sheep)	3	-
S. hepatica (goat)	3	-
C. fasciolaris (mice)	-	-
F. gigantica	4	-

- = No precipitin lines observed.

Table 13b (cont'd) Precipitin reactions observed between Antisera Nos.888 and 879 with other parasite extracts

ANTIGENS	ANTISERUM	
	No.888	No. 879
Paramphistomum spp. (cattle)	3	-
Paramphistomum spp (goat)	3	-
S. mansoni	3	-
H. contortus (cattle)	4	-
Oesophagostomum spp (cattle)	4	-
Trichuris spp. (cattle)	-	-
T. brucei	-	-
A. suum (pig)	-	-
C. cellulosae	4, 11	11

- = No precipitin lines observed.

4.2.11. Synopsis of the antigenic relationship between C. bovis, T. saginata and other parasites.

The results of the immunodiffusion tests have been summarised in Tables 14a and 14b. A positive sign (+) has been used to indicate one precipitin line (i.e. a single antigenic component) as it has not been possible to assign a specific number to all precipitin lines. Reactions of identity have been recorded wherever possible with the use of several antisera in the same immunodiffusion test (Section C).

Table 14a summarises the precipitin reactions observed with C. bovis and T. saginata using various antisera prepared in the course of this study.

Antisera prepared in rabbits gave more precipitin lines than those prepared in calves. Rabbit antisera showed at least three common components between C.bovis and T. saginata One of these components was common to a wide range of parasites (Table 14b), while the other two components showed restricted cross reactions. One of these two components was identified as Antigen 11 using an antiserum prepared specifically against this antigen.

Antigen 11 of C. bovis was shown to be present in C. cellulosae, C. tenuicollis, T. hydatigena and T. saginata (Figure 14). The third component was shown to be restricted to C. bovis and T. saginata only. and may therefore be species specific.

Antisera prepared in calves and goats showed two components common to C. bovis and T. saginata. One of these two components was shown to be Antigen 11 using

Antiserum No.879. The other component was shown to be identical to the widely occurring cross-reacting components detected by rabbit antisera (Table 14b). It was noted that calf anti-Inner membrane-scolex of C. bovis (Antiserum No.846) detected only Antigen 11, despite the fact that this calf had been extensively immunized over a period of 18 months with the whole homogenate of Inner membrane-scolex of C. bovis. This may suggest that Antigen 11 is the principal antigen in C. bovis which might be capable of eliciting an immune response also in naturally infected animals. This is important since the choice of an antigen suitable for immunodiagnostic purposes will depend on whether the antigen chosen elicits an immune response in animals during natural infection.

Table 14a: A summary of precipitin reactions observed with all the antisera

Precipitin reactions observed with C. bovis and T. saginata extracts are summarised in this table.

ANTISERUM / ANTIGENS	Inner membrane scolex (C.bovis)	Inner fluid (C. bovis)	Outer fluid (C.bovis)	Outer membrane (C. bovis)	T. saginata
Pooled rabbit anti C.bovis (inner membrane scolex-antiserum No.140/158)	+ + + + +	+ + +	+ + +	-	+ + +
Pooled rabbit anti C.bovis scolices (No.79)	+ + + + +	+ + +	+ + +	-	+ + + +
Pooled calf anti C.bovis (inner membrane scolex No.846)	+	+	+	-	+
Pooled calf anti inner fluid C. bovis (No.835)	+ +	+ +	+	-	+ +
Pooled rabbit anti inner fluid (No. 146)	+ + +	+ + + +	+ +	-	+ +
Pooled calf anti outer fluid (No. 118)	+ + + +	+ + +	+ + +	-	+ + +
Pooled rabbit anti T. saginata (No.154)	+ + + +	+ +	+	-	+ + + +
Pooled calf anti T.saginata (No.900)	+ +	+	+	-	+ +

\+ = The number of positive signs corresponds to the number of precipitin lines observed in immunodiffusion.

\- = No precipitin line observed in immunodiffusion

Table 14a: (cont'd) Precipitin reactions with C. bovis and T. saginata extracts

ANTISERUM / ANTIGENS	Inner membrane scolex (C.bovis)	Inner fluid (C. bovis)	Outer fluid (C. bovis)	Outer membrane (C. bovis)	T. saginata
Pooled goat anti T. saginata (antiserum No.824)	+ +	+ +	+ +	-	+ + +
Pooled goat anti-serum (No.879) specific for component No.11	+	+	+	-	+

\+ = The number of positive signs corresponds to the number of precipitin lines observed in immunodiffusion

\- = No precipition line observed in immunodiffusion

Figure 14: Well A: Goat anti-Antigen 11 Serum (No.879).

Well 1: C. cellulosae.

Well 2: C. tenuicollis

Well 3: T. saginata

Well 4: C. cellulosae

Well 5: Inner fluid (C. bovis)

Well 6: Inner membrane-scolex (C. bovis)

Table 14b summarises the precipitin reactions observed between all antisera and various other parasites.

It is important to note that antisera prepared against T. saginata and C. bovis showed precipitin reactions not only with closely related cestodes like Moniezia spp and hydatid cyst antigens but also with trematodes such as F. gigantica and nematodes such as Oesophagostomum spp. This is an important point in the choice of antigen for immunodiagnosis since the most reactive antigen may be one which occurs widely in other parasites. Therefore, the specificity of tests employing such an antigen must necessarily be poor.

Antigen 11 of C. bovis was shown to occur only in T. saginata, C. cellulosae, C. tenuicollis and T. hydatigena. Thirteen other parasites gave negative reactions for Antigen 11 in immunodiffusion tests. Antigen 11 was, therefore, analysed further, and used in the solid-phase radioimmunoassay to investigate the potential of this antigen to distinguish between known infected and non-infected animals.

Table 14b Precipitin reactions with other parasite extracts using various antisera

ANTIGENS \ ANTISERUM	Pooled rabbit anti C.bovis (inner membrane scolex (Antiserum No.140/158)	Pooled rabbit anti C. bovis scolices (Antiserum No.79)	Pooled calf anti C. bovis (inner membrane scolex Antiserum No.846)	Pooled calf anti inner fluid (Antiserum No.835)	Pooled rabbit anti inner fluid (Antiserum No.146)	Pooled calf anti outer fluid (Antiserum No.118)	Pooled rabbit anti T. saginata (Antiserum No.154)	Pooled calf anti T. saginata (Antiserum No.900)	Pooled goat anti T. saginata (Antiserum No.824)	Pooled goat anti C. tenuicollis (Antiserum No. 838)	Pooled goat antiserum to Antigen 11 (Antiserum No.879)
T.hydatigena(cattle)	+	+	+	++	++	++	++	++	+	++	+
C.tenuicollis fluid (goat)	++	++	+	+	++	+	+	+	+	+++	+
C. tenuicollis fluid (sheep)	++	++	+	+	+++	++	+++	++	+	++	+
C. cerebralis fluid (goat)	-	+	-	+	+	+	+	+	+	+	-
C. cerebralis membrane scolices	-	+	-	+	+	+	++	+	+	++	-
HCF (cattle	-	+	-	-	-	+	-	+	-	+	-
HCF (goat/sheep)	-	+	-	-	-	+	-	+	-	+	-
Moniezia spp (cattle)	+	+	-	+	-	-	++	+	+	+	-
Moniezia spp (goat/sheep)	+	+	-	+	-	-	++	+	+	+	-
S. hepatica (goat)	-	+	-	-	-	-	+	-	-	+	-
C. fasciolaris (mice)	-	-	-	-	-	-	-	-	-	-	-

\+ = The number of positive signs corresponds to number of precipitin lines observed in immunodiffusion.

\- = No precipitin lines observed in immunodiffusion.

Table 14b (cont'd): Precipitin reactions with other parasite extracts using various antisera

ANTIGENS \ ANTISERUM	Pooled rabbit anti C.bovis (inner membrane scolex (Antiserum No. 140/158)	Pooled rabbit anti C.bovis scolices (Antiserum No. 79)	Pooled calf anti C. bovis (inner membrane scolex Antiserum No. 846)	Pooled calf anti-inner fluid (Antiserum No.835)	Pooled rabbit anti-inner fluid (Antiserum No.146)	Pooled calf anti cuter fluid (Antiserum No.118)	Pooled rabbit anti T. saginata (Antiserum No.154)	Pooled calf anti T. saginata (Antiserum No.900)	Pooled goat anti T. saginata (Antiserum No.824	Pooled goat anti C. tenuicollis (Antiserum No.888)	Pooled goat antiserum to Antigen 11 (Antiserum No.879)
F. gigantica (cattle)	-	+	-	-	-	-	+	-	-	+	-
Paramphistomum spp (cattle)	+	+	-	-	-	-	+	-	-	+	-
Paramphistomum spp (goat)	+	+	-	-	-	-	+	-	-	+	-
S. mansoni	-	-	-	-	-	-	-	-	-	-	-
H. contortus	-	-	-	-	-	-	+	-	+	+	-
Trichuris spp.	-	-	-	-	-	-	-	-	+	-	-
Oesophagostomum spp (cattle)	+	+	-	+	+	+	+	+	+	+	-
T. brucei	-	-	-	-	-	-	-	-	-	-	-
A. suum	-	-	-	-	-	-	-	-	-	-	-
C. cellulosae	++	+++	+	+	+	+	+	++	+++	++	+

\+ = The number of positive signs corresponds to number of precipitin lines observed in immunodiffusion.

\- = No precipitin line observed in immunodiffusion.

4.3. The establishment of a reference system for antigenic constituents of C. bovis using crossed immunoelectrophoresis

In order to identify and recognise the various antigenic components of C. bovis, the Inner membrane-scolex extract was considered as the reference antigen. The antiserum used was a pool of sera from 3 rabbits (number 140, 158 and 79) as described in Materials and Methods.

The results of crossed immunoelectrophoresis are shown as a tracing of the enlarged stained pattern and in a colour photograph of the same (figure 15a and b). The precipitin peaks were numbered beginning from the anodic side. Fifteen precipitin peaks could be distinguished.

4.3.1. Identification of antigen shared between C. bovis T. saginata, C. tenuicollis, T. hydatigena and C. cellulosae:

In this study it has been established by crossed immunoelectrophoresis that there are 15 C. bovis antigenic components, using the Inner membrane-scolex extract as the reference antigen and antiserum No.140/158/79. It was,therefore, also important to establish which one of these antigens was shared between C. bovis, T. saginata, T. hydatigena, C. tenuicollis and C. cellulosae.

Using an intermediate gel containing the monospecific antiserum No.879, if was shown that the antigen shared by C. bovis, T. saginata, T. hydatigena, C. tenuicollis and C. cellulosae was Antigen Number 11 (Figure 16a and b). Since

incoporation of antiserum in an intermediate gel is a very sensitive method for demonstration of antibody activity (Axelsen 1973, Axelsen et al., 1974) and only one peak on the reference pattern showed a significant change in position, this is a strong indication that the monospecific antisera contains antibody activity to this antigenic component.

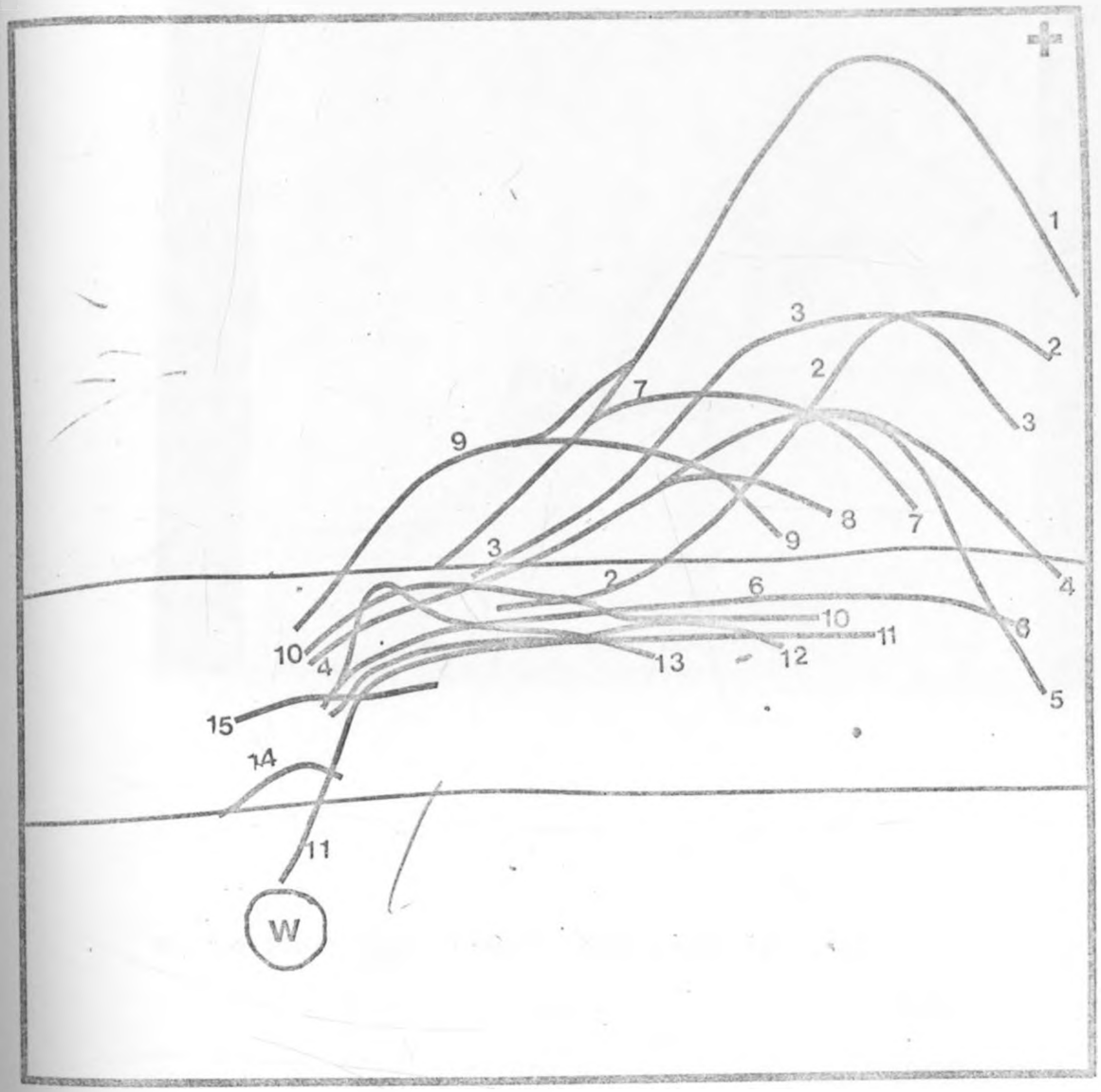


Figure 15a: A tracing of the reference pattern for the antigenic constituents of C.bovis using crossed immunoelectrophoresis. Antigen well (w) contains inner membrane-scolex of C.bovis while the intermediate gel contains 0.2 mls of preimmune antisera No.79/140/158. The top gel contains pooled absorbed rabbit anti-inner membrane-scolex No.79/140/158. The anode is to the right in the first dimension and at the top in the second dimension electrophoresis. The precipitin peaks are arbitrarily numbered beginning with the most anodic component.

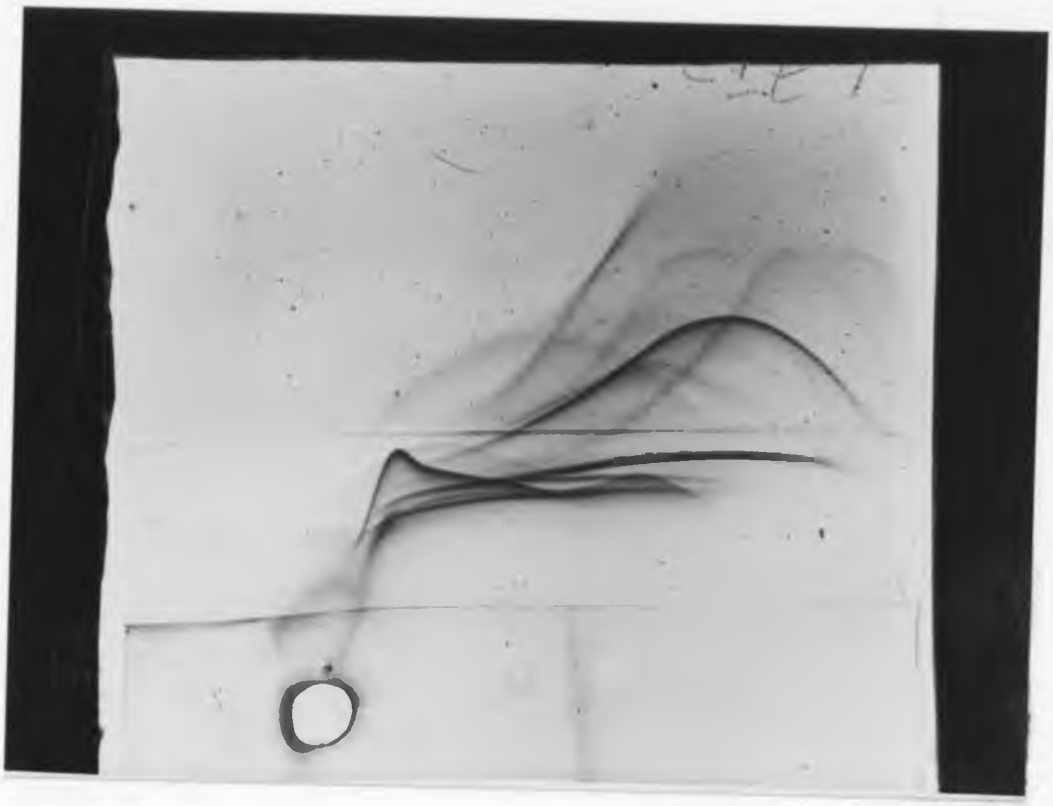


Figure 15b: The crossed immunoelectrophoresis with intermediate gel, pattern showing the position of Antigen 11 (Photograph of the gel illustrated in Fig. 15a

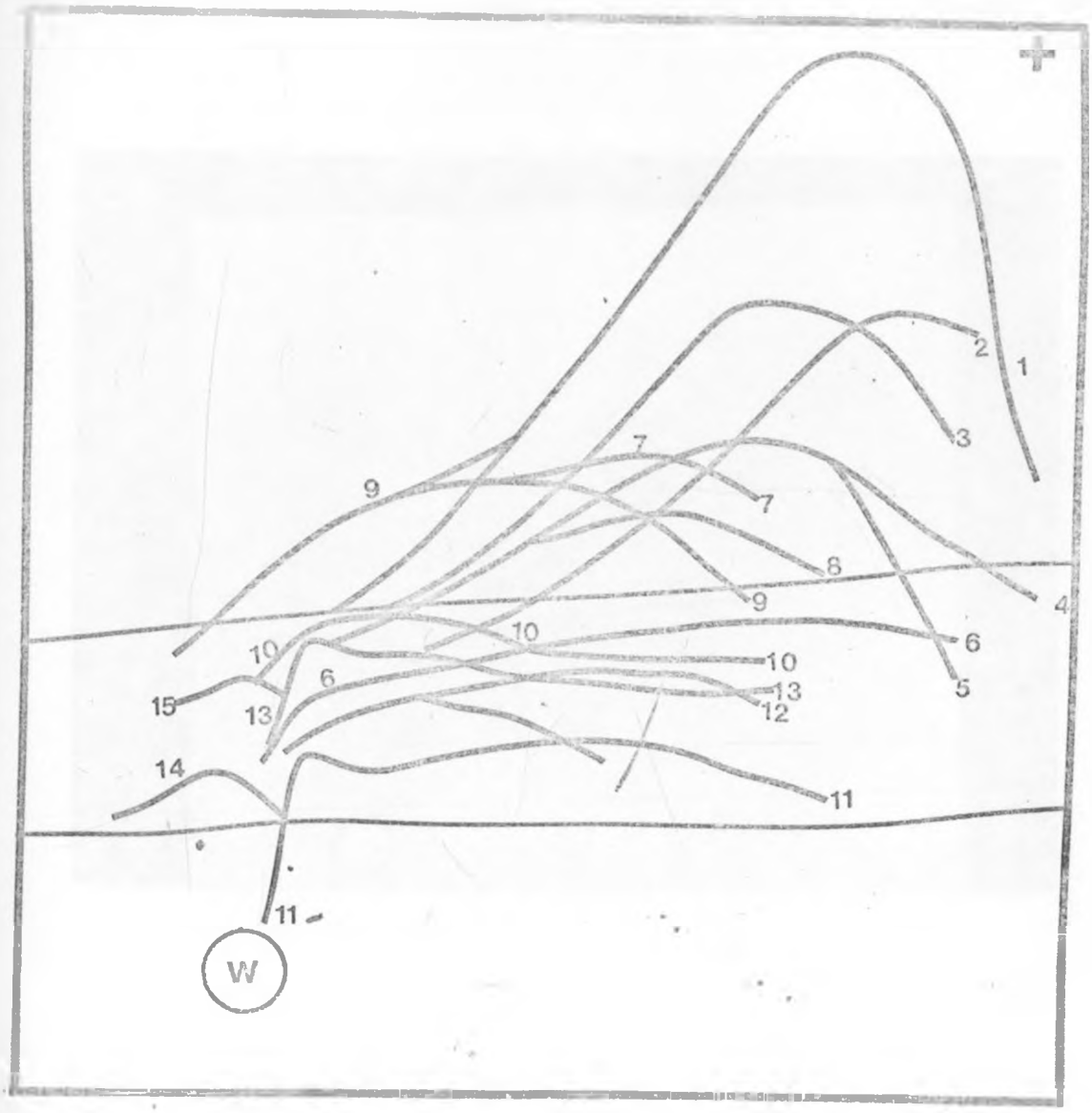


Figure 16a: A tracing of the crossed immunoelectrophoresis with intermediate gel, pattern showing the position of Antigen 11. Antigenic well(W) contains inner membrane-scolex of C.bovis while intermediate gel contains 0.2 mls of monospecific antiserum No.879. The top gel contains pooled absorbed rabbit anti-inner membrane-scolex No.79/140/158. The anode is to the right in the first dimension and at the top in the second dimension electrophoresis.

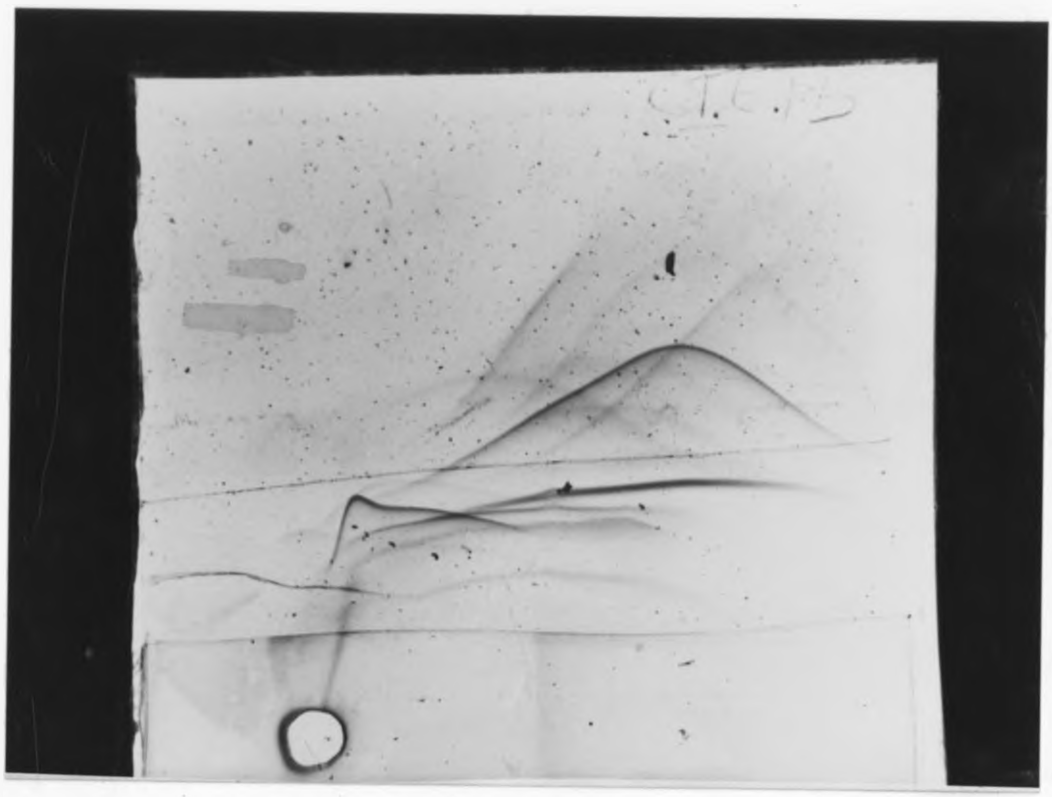


Figure 16b: The crossed immunoelectrophoresis with intermediate gel, pattern showing the position of Antigen 11 (photograph of the gel illustrated in Fig. 16a

4.4. Charcterisation of Antigen 11

Antigen 11 eluted from affinity column gave a reaction of complete identity with an antigen present in T. saginata, C. bovis and C. tenuicollis by immunodiffusion using the monospecific antiserum to Antigen 11 (Antiserum No.879) as shown in Figure 17. The presence of this shared antigen in the eluted antigen, was also confirmed with polyspecific antisera to C. bovis, T. saginata and C. tenuicollis (Antisera Nos. 140, 154, 824 and 888).

The eluted antigen was further characterised in SDS-PAGE. Five bands were obtained corresponding to molecular weights between 15,000 to 100,000 daltons. Since it was difficult to determine which of these bands corresponded to Antigen 11, analytical isoelectric focusing was subsequently performed.

The results of wide range isoelectric focusing (pH 3.5-10) gave three fractions with isoelectric points between 4.14 to 6.22. These fractions reacted in immunodiffusion tests with the monospecific antiserum (No.879) and gave a single line with the polyspecific antisera to C. bovis, T. saginata and C. tenuicollis (Nos.140, 154, 824, 888).

Analysis of the three fractions on SDS-PAGE showed that they contained two common bands. On further isoelectric focusing of these fractions on a narrow pH-range, two fractions with isoelectric points of pH 4.47 and 5.50 were obtained.

SDS-PAGE analysis of each fraction under both reducing and non-reducing conditions, showed two bands which correspond to molecular weights of 63,000 and 78,000 daltons.

Both of these two fractions reacted in immunodiffusion with the monospecific antiserum (No.879) and gave a single line with polyspecific antisera Nos.888, 824,154 and 140.

From these immunodiffusion results it was concluded that these two fractions contained Antigen 11 reactivity.

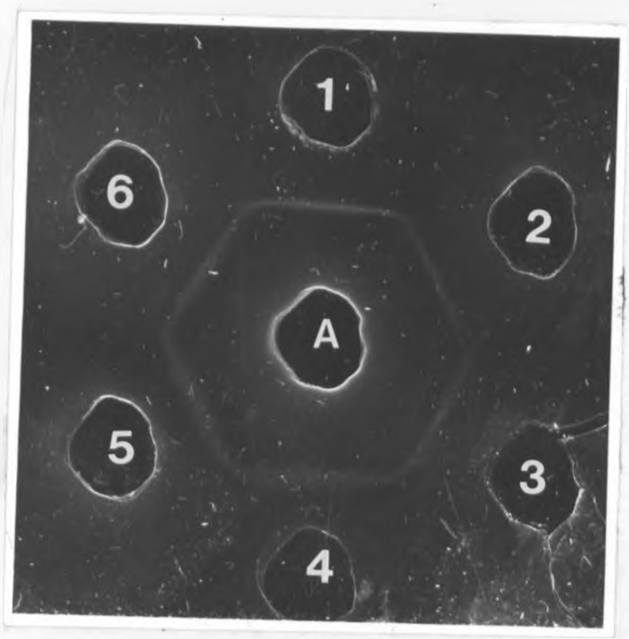


Figure 17: Well A: Goat anti-Antigen 11 (Antiserum No 879)

Well 1: Inner membrane-scolex (C. bovis)

Well 2: C. tenuicollis

Well 3: T. saginata

Well 4: Inner fluid (C. bovis)

Well 5: Outer fluid (C. bovis)

Well 6: 50X concentrated eluted Antigen 11.

4.5. The antibody response to Antigen 11 in animals infected with C. bovis

The two fractions of Antigen 11 obtained after isoelectric focusing in a narrow range pH (Section 4.3) were pooled and used in the solid-phase radioimmunoassay to determine whether or not C. bovis infected cattle produce antibodies against the antigen.

The results are shown in Table 15. It was found that antibodies of IgG1 and IgM classes were produced in infected animals. IgG1 antibodies were detected after 5 weeks of infection and highest titres of antibody were detected 18 to 28 weeks after infection. By contrast, IgM antibody titres were a thousand-fold lower and they were not detected until 36 weeks after infection. High antibody levels were also demonstrated in sera from two other infected animals, whereas sera from non-infected animals RS1 and RS6 were negative (Table 15 and Figure 18).

Table 15: Antibody response to Antigen 11 in experimental cysticercosis

Identification of the Samples	Week Post-Infection	Reciprocal Titres IgM	IgG1
	0	-	-
	1	0	0
	2	0	0
	5	0	170
	10	0	175
	18	0	2500
	23	0	26000
	24	0	28000
	28	0	14000
	34	0	180
	36	10	170
	42	10	160
	43	10	150
	45	10	150
	64	10	150
Rs8(infected control)	-	150	1000000
Rs10(infected control)	-	16800	1000000
RS1(Non infected control)	-	0	0
RS6(Non infected control)	-	0	0

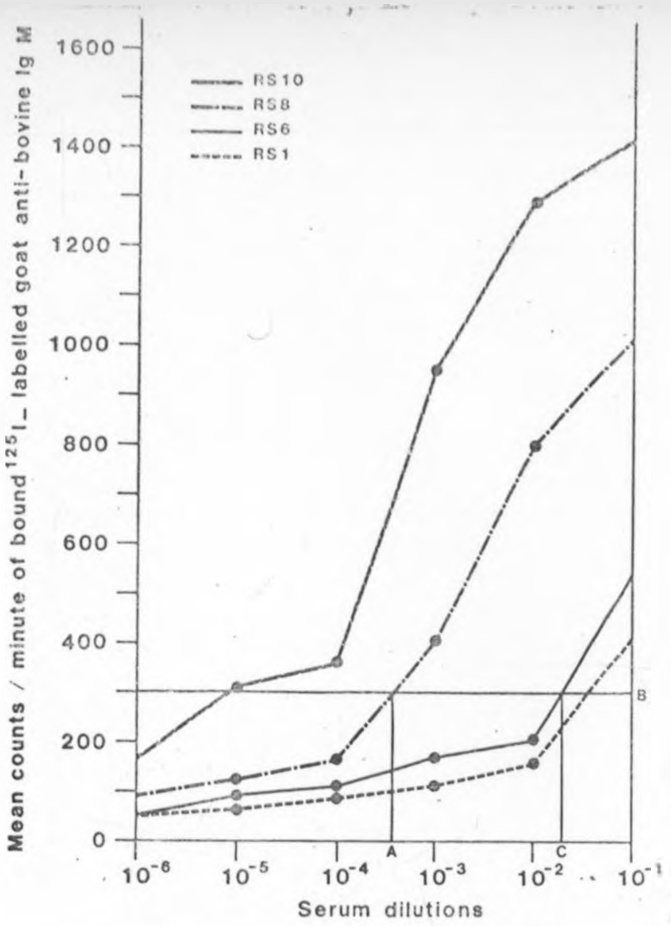


Figure 18: A graph showing an example of calculations of antibody titres:

The results plotted are the mean counts per minute of duplicate assays of samples against the dilution of serum.

Key: RS1, RS6, RS8 and RS10 are the samples.

B = Background counts

C = The serum dilution belonging to the "negative control".

Titre of RS8 is given by A-C.

DISCUSSION

5: DISCUSSION

Bovine cysticercosis is an important economic problem in developing countries which are seeking to establish an export market for their cattle industries. Despite the availability of highly effective preventive measures and effective drugs for the treatment of taeniasis in man, these measures have had little or no effect on the incidence of bovine cysticercosis. Since the present meat inspection procedure is inadequate in detecting the lightly infected animals, reliable antemortem diagnostic procedures would represent a considerable advantage.

The major drawback of the present immunodiagnostic procedures for bovine cysticercosis is the lack of a well defined antigen(s) which possesses the necessary specificity together with a diagnostic test which possesses the required sensitivity (Greet et al., 1979; Gathuma and Waiyaki, 1980). The problem is further aggravated by the possibility that infected animals may respond poorly to cysticercus antigens. This has been shown in human cysticercosis due to C. cellulosae where only 50% of patients produce antibodies detectable by the methods in common use (Flisser et al., 1980).

The immune response to parasites is regulated by many factors of both host and parasite origin. Genetically determined differences and previous contact with the antigen or a cross reacting antigen may considerably influence the host immune responses. The potency of the immunogen and its

concentration are also of great importance (McDevitt and Benacerraff, 1969; Amsbaugh et al., 1972; Di-Pauli, 1972; Salk and Salk, 1976).

In this study calves were found to be poor antibody responders to antigens of C. bovis despite the extremely heavy immunizing schedule, using adjuvants, over a period of about 18 months. It was also noted that a calf immunized with the outer fibrous capsule (membrane) of C. bovis did not respond to this component. Failure to respond to the outer fibrous capsule of C. bovis may be due to the possibility that this membrane is derived from the host tissues as suggested by Thornton (1968a & b). Thus it would appear that the mechanism by which C. bovis is able to persist in the host may have its explanation in the outer membrane, since the membrane is generally in contact with the host tissues. In this regard it is pertinent to note that Gemmel and McNamara (1971) have suggested that cestodes may indeed evade the host immunological attack by formation or acquisation of host or host-like antigens which mask the inner or deeply situated parasite antigens.

There were two main reasons for using calves to produce antisera against C. bovis antigens. Firstly, calves being natural hosts of the intermediate stages of the parasite it was thought that responses in immunized animals might give an insight into the immune responses expected in natural infections. Secondly, the presence of host components (i.e. of cattle origin) in the antigenic preparation used for immunization would not be recognised if the immunization

took place in the same species.

The presence of the host components in the cyst fluid of some cestodes has been thoroughly studied, especially in hydatid cyst fluid (Chordi and Kagan, 1965; Norman and Kagan, 1966; Oriol et al., 1971; Varela-Diaz and Cortorti .1974; Coltorti and Varela-Diaz 1974, 1975). The host components present in antigenic preparations used in the serodiagnosis of various helminthic infections have been known to cause difficulties in standardization and also lower the specificity of the antigen used (Kagan, 1968; Oriol et al., 1971). Several workers have attempted to use the cysticercus as their source of antigens for the immunodiagnosis of cysticercosis in humans, pigs and cattle (Bugyaki, 1961; Froyd, 1963; Proctor and Elsdon-Dew, 1966; Flisser et al., 1980). However, the literature on the presence of host components in C. bovis is very limited (Sviridenko-Stepankovskya et al. 1972).

In the present study, the host components have been detected in the cyst fluids of C.bovis and C. tenuicollis They were found to migrate in the albumin, beta and gamma regions in immunoelectrophoresis while those found in the inner membrane-scolex of C. bovis were found mainly in the albumin and the gamma regions. Using specific antisera, these components were identified as host albumin and immunoglobulin G. Host IgG_1, IgM and albumin have been found in hydatid cyst fluid (Chordi and Kagan, 1965; Kagan, 1968; Kagan and Agosin, 1968; Kassis and Tanner, 1977). Other host components in cyst fluid are blood group antigens

such as P1 and ABO(H) (Cameron and Stavely, 1957; Enyenihi, 1974). Ben-Ismail et al. (1980), described major cross-reactions between patients with hydatidosis and fascioliasis due to Fasciola hepatica. They showed that the presence of anti-P1 antibodies in patients with fascioliasis was the main cause of the cross reactions. A search for the presence of blood group substances in the cyst fluids of C. bovis or antibodies to these substances in naturally infected animals should therefore be undertaken because the results of such a study could be of considerable importance in the evaluation of cross-reactions in serodiagnostic tests for C. bovis.

Owing to these foreseeable problems, all the antisera prepared in this study were absorbed with insoluble immunosorbents prepared from host serum proteins and red cells of appropriate blood groups assumed to be present in the antigenic preparations used for immunizations. These absorptions were carried out routinely, even with those antisera which had not been shown to react with host antigens. All antisera were examined and shown not to react with host antigens after absorptions. All the reactions observed in subsequent assays were, therefore, considered to be due to antigenic determinants of the parasites themselves. It should be noted, however, that certain blood group-like antigens may indeed be of parasite origin, but for the purpose of this investigation these antigens have been intentionally avoided by prior absorption of antisera.

Although the calves gave relatively poor precipitating antibody responses to the antigens used for immunization, they did respond to a common antigen found in C. bovis and T. saginata (Table 14a). However, several animals of the same species should have been used in the production of antisera, since it is known that animals respond differently to the same immunizing agent (Crowle, 1973). Constraints in finances and facilities for keeping a large number of animals prevented the use of more than one calf for each antigenic preparation of C. bovis.

In general, rabbits were found to be better responders Three precipitin lines were found to be common to C. bovis and T. saginata as detected by double immunodiffusion tests in gel (Table 14a). One precipitin line was detected early (after 3 months of immunization), while the other two precipitin lines appeared late (after 16 months). The early precipitin line may correspond to the antigenic components described by Machnicka (1974).

Antisera to C. bovis and T. saginata, prepared in goats and calves, showed 1 to 2 precipitin lines (Table 14a). One precipitin line showed a reaction of identity between C. bovis and T. saginata. On further examination, using a specific antiserum (Antiserum No.879), this common antigen was identified as Antigen 11. It should be emphasised that calf No.846 immunised with whole homogenate of Inner membrane-scolex of C. bovis produced antibody only to Antigen 11. This suggests that Antigen 11 is one of the most highly

immunogenic components of C. bovis.

The existence of antigenic relationships between the larval stages and adult tapeworms has been known for many years. Campbell (1936) reported the presence of antigens common to T.taeniaeformis and C. fasciolaris and showed that a protein fraction of T.taeniaeformis elicited immunity in rats to subsequent infections with C. fasciolaris. Later, Nemeth (1971) demonstrated antigenic identity between a component of T. pisiformis and C. pisiformis. In other studies oncospheres from adult cestodes have been used to induce immunity to metacestodes. Gemmell (1964, 1965) infected sheep intramuscularly with hatched oncospheres of T. hydatigena and T. ovis and induced immunity to both homologous and heterologous infections. Wilkerhauser et al. (1971) immunized calves against C. bovis by intramuscular injection of artificially hatched oncospheres of T. saginata and T. hydatigena and obtained results similar to those of Gemmell (1964, 1965).

In the present study, it was, therefore, decided to investigate in considerable detail the antigenic relationship between the larval stage and the adult stage of T. saginata. The finding that there are common antigens in C. bovis and T. saginata, may indicate that the selected antigen(s) from either the larval stage or the adult stage may be used in the serodiagnosis of bovine cysticercosis or taeniasis in man.

The antigenic relationship between C. bovis, T. saginata, adult and larval stages of other cestodes, as well as other unrelated parasites, were also examined. The results in Table

14b show that the antisera prepared against C. bovis and T. saginata gave precipitin reactions with several parasites, both in the Phylum Platyhelminthes and in the Phylum Nemathelminthes. This finding is similar to that of Greets et al., (1979) who found that hyperimmune rabbit antiserum to T. saginata (non gravid segments) also reacted with cestodes and trematodes in immunoelectrophoresis. In the choice of antigen for serodiagnosis of bovine cysticercosis this factor must be considered.

The antigenic components of C. bovis were also studied using two dimensional electrophoresis (crossed immunoelectrophoresis). This was done with the intention of establishing a pattern for antigenic components of C. bovis, which could be used for reference purposes by identifying each antigenic component with a number. The reference pattern was established using one pool of antiserum (Rabbit Nos.79, 140 and 158) and one batch of antigen (Homogenate of a large number of C. bovis inner membrane and scolices). Fifteen precipitin lines were defined, each representing a single antigenic component. The high resolving power of crossed immunoelectrophoresis was confirmed, and the incorporation of TritonX-100$^{(R)}$ in the agarose gel for crossed immunoelectrophoresis resulted in an increased resolving power of the method.

Such a reference pattern for the antigenic constituents of C. bovis is invaluable, since it provides a means for

the selection of antigens unique to C. bovis as well as for the elucidation of cross-reactions with other parasites. Through exchange of antisera and antigensbetween various laboratories a two dimensional immunoelectrophoresis reference pattern would enable research workers to compare results and identify the antigens of interest.

The established reference pattern showing 15 precipitin arcs was used to examine one particular antigen which had been chosen because of its limited cross reactivity with other parasites. By incorporating monospecific antiserum (goat antiserum No.879) in the intermediate gel, the antigen was identified as the precipitin line which had been given the number 11 in the reference pattern. The presence of an antigen identical or similar to Antigen 11 in T. saginata, T.hydatigena, C. tenuicollis and C. cellulosae was therefore confirmed by the crossed immunoelectrophoresis method. All the antisera prepared against various constituents of C. bovis and homogenate of T. saginata reacted with Antigen 11

The complexity of shared antigenic determinants between a number of adult and larval stages of parasites which taxonomically are far apart, precludes an easy choice of antigen(s) for a specific test. A logical source of starting material for the preparation of an artigen suitable for an immunodiagnostic test, would be the form of the parasite which develops in that particular host. Even though antigens which are specific for this stage might be purified, however,

two major issues still remain, namely, the quantity available for processing and purification, and the natural immune response of the host to these specific antigen(s). Clearly, highly specific antigens would be of no use whatsoever if the host cannot respond to this antigen in a reasonably consistent way. Secondly, the antigen must be of such nature that it can be utilised in an immunodiagnostic test.

The finding that Antigen 11 had a restricted cross reactivity and was also one of the antigens which consistently elicited antibody production in all animals immunized with C. bovis and T. saginata antigens, led to the conclusion that this may be the antigen of choice for the serodiagnosis of bovine cysticercosis. Furthermore, Antigen 11 was isolated from C. tenuicollis cyst fluid, an easily obtainable source of antigen.

The antigen was further characterised by isoelectric focusing and SDS-PAGE analysis. Antigen 11 was found to have molecular weight between 63,000 and 78,000 daltons and isoelectric at pH 4.47 to 5.50. These values are given as two figures since it was not possible to purify this antigen further at this time to obtain a single band in SDS-PAGE. The molecular weights as well as the isoelectric points can, therefore, only be referred to as estimates. This "semi-purified" antigenic preparation, nevertheless, reacted with monospecific antiserum to Antigen 11. Poly-

specific antisera against C. bovis, C. tenuicollis and T. saginata showed only one component, which was found to give a reaction of identity with the monospecific antiserum to Antigen 11 (Antiserum No.879). Furthermore, antisera to host components (goat) from which the C. tenuicollis fluid was harvested, gave no reaction with the semipurified antigen. It was, therefore, concluded that, this antigenic preparation could be considered pure for further investigations.

Antigen 11 was subsequently used in solid-phase radioimmunoassay to detect antibody responses in calves experimentally infected with C. bovis. The results showed that IgG antibody levels were generally higher than IgM levels. These findings are similar to those of Flisser et al., (1980) who found that IgG levels were higher than IgM in human cysticercosis caused by C. cellulosae.

Significant rises in IgG titres were detected 5 weeks after infection. The detection of serum antibodies from week 5 post-infection is in general agreement with Nemeth (1971) who observed a primary antibody response to C. pisiformis in rabbits between 2 and 5 weeks after infection.

The IgG antibody levels reached a peak on week 24. A gradual fall of titres was then recorded up to week 64 post-infection.

The fall in antibody levels from week 24 to week 64 may be due to a decrease of C. bovis antigens, possibly due to degeration and calcification of the cysts. The calci-

fication of C. bovis cysts has been recorded as early as 3 months post-infection (Smyth, 1963; Froyd,1964).

In view of the unexpected fall in titres, the possibility that the samples after week 24 were wrongly labelled, was also considered. However, the results of samples from non-infected calves (RS1 and RS6) and infected calves (RS8 and RS10) corresponded very well with the results recorded in other laboratories using the enzyme linked immunosorbent assay (Dr. Stevenson personal communication). These results show that Antigen 11 is capable of distinguishing experimentally infected animals from non-infected controls.

Thus the results obtained so far, strongly indicate that the component of C. bovis labelled Antigen 11 according to the place it occupies in a crossed immunoelectrophoresis reference pattern, might be useful in serodiagnostic tests for bovine cysticercosis. Although the antigen shows a restricted cross-reactivity with other parasites, out of 16 parasites investigated, only its presence in C. tenuicollis may possibly give rise to false positive reactions in cattle exposed to eggs of T. hydatigena. Since there may be a possible development of C. tenuicollis in cattle, a subsequent immune response to Antigen 11 is to be expected. There may be also other parasites of cattle not yet examined which share antigenic determinants with Antigen 11.

In view of the finding that Antigen 11 is present in T. saginata and C. cellulosae, it would be of great interest to investigate the immune responses to Antigen 11 in man infected with T. saginata or in cysticercosis due to

C. cellulosae.

In the course of this investigation, another potentially more useful antigen of *C. bovis* was detected. The antigen appears to be shared only by *T. saginata* and *C. bovis*. The specificity of this as yet unlabelled antigen, would make it a *priori* the antigen of choice. At present, information regarding its availability and ability to induce measurable immune responses is urgently needed. Until promising results are obtained with this antigen, Antigen 11 appears to be the antigen of choice for the diagnosis of bovine cysticercosis.

CONCLUSIONS

6. CONCLUSION

In this study, 15 antigenic components of C. bovis have been established using the crossed immunoelectrophoresis method. One of these components identified as Antigen 11 was found to be present in C. bovis, T. saginata, T. hydatigena, C. tenuicollis and C. cellulosae.

An attempt has been made to isclate,characterise and use this antigen in the serodiagnosis of experimental cysticercosis using the solid-phase radioimmunoassay. The results have shown that this antigen can clearly differentiate between experimentally infected and non infected animals.

The use of this antigen in other serodiagnostic tests for bovine cysticercosis may substantially reduce the occurrence of non-specific reactions. However, further studies are required for the identification and characterization of other antigens which may be strictly specific to C. bovis.

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APPENDIX

APPENDIX 1a: Immunoadsorbents used in this study:

	Antigen coupled to CNBr-Sepharose	Amount of protein (determined by the method of Lowry et al., (1951)	Percentage coupled to Sepharose %
1.	Whole bovine serum (pool of randomly selected normal sera)	80 mg protein per ml	90
2.	Whole goat serum (pool of randomly selected normal sera)	80 mg protein per ml	90
3.	Preimmune goat serum (No.824)	80 mg protein -"-	-"-
4.	Anti-whole goat serum (antisera No.539)	70 mg protein per ml	90
5.	Fasciola gigantica	2.70 mg protein per ml	82
6.	Stilesia hepatica	8.84 mg protein per ml	75
7.	Moniezia spp (ccw origin)	2.30 mg protein per ml	85

CNBr Sepharose 4B (Batch No. DF 6993 Pharmacia Fine Chemical AB Uppsala, Sweden) was used in the preparation of all immunoadsorbants.

APPENDIX 1b: Immunoadsorbents used in this study

Red blood cells (rbc's) treated with gluteraldehyde		Number of animals from which the pool was made	Place of origin
1.	Sheep rbcs	20	Ongata Rongai
2.	Goat rbcs	30	"
3.	Cattle rbcs	20	Athi River
4*	Human P1 red cells	Obtained from Prof. K. Lindqvist	Kabete

* Human P1 red cells were not treated with glutaraldehyde.

APPENDIX 2a. SCHEME OF ABSORPTION OF ANTISERA

IMMUNOADSORBENT / ANTISERA	Whole bovine serum	Whole goat serum	Pre-immune goat serum No. 824	T. gigantica	S. hepatica	Moniezia spp
Antiserum No.10	x	-	-	-	-	-
Antiserum No.118	x	-	-	-	-	-
Antiserum No.835	x	-	-	-	-	-
Antiserum No.846	x	-	-	-	-	-
Antiserum No.900	-	-	-	-	-	-
Antiserum No.824	-	-	-	-	-	-
Antiserum No.888	-	x	-	-	-	-
*Antiserum No 879	x	x	x	x	x	x
Antiserum No.140	x	-	-	-	-	-
Antiserum No.154	x	-	-	-	-	-
Antiserum No.158	X	-	-	-	-	-
Antiserum No.79	x	-	-	-	-	-
Antiserum No.146	x	-	-	-	-	-
Antiserum No.878	-	-	-	x	x	x

X = Absorbed with the immunoadsorbent

- = not absorbed with that particular immunoadsorbent.

* = Before antiserum 879 was absorbed with parasite extracts it was tested in immunodiffusion with the same parasites

APPENDIX 2a (Cont'd)

SCHEME OF ABSORPTION OF ANTISERA

IMMUNOADSORLENT ANTISERA	Sheep rbcs	Goat rbcs	Cattle rbcs	Human rbcs (P1)	REMARKS OF THE ABSORPTION SCHEME
Antiserum No.10	x	x	x	x	Absorption of anti-bovine components and anti-blood group substances
Antiserum No.118	x	x	x	x	"
Antiserum No.835	x	x	x	x	"
Antiserum No.846	x	x	x	x	"
Antiserum No.900	x	x	x	x	Absorption for anti- blood-group substances
Antiserum No.824	x	x	x	x	"
Antiserum No.888	x	x	x	x	Absorption of anti-goat components and anti-blood group substances
Antiserum No.879	x	x	x	x	Absorption for anti-allotypes, anti-bovine components and other antibodies which might be present
Antiserum No.140	x	x	x	x	Absorption for anti-bovine components and anti-blood group substances
Antiserum No.154	x	x	x	x	Absorption for anti-blood group substances
Antiserum No.158	x	x	x	x	Absorption for anti-bovine and anti-blood group substances
Antiserum No.79	x	x	x	x	"
Antiserum No.146	x	x	x	x	"
Antiserum No.878	x	x	x	x	Absorption for anti-blood group substances

Appendix 2b: Scheme of absorption of C. tenuicollis fluid.

C. tenuicollis fluid obtained from goat was absorbed with rabbit anti-whole goat serum immunoadsorbent to remove the host components present in the fluid.

Appendix 3: Buffers and solutions used in the preparation of insoluble immunoadsorbent

3.1 1mM hydrochloric acid solution for washing the freeze dried cyanogen bromide activated sepharose 4B.

1mM hydrochloric acid solution was prepared by dilution 36% concentrated acid sp. gr. 1.18.

3.2 0.1M sodium bicarbonate (NaHCO3) pH 8.3 with 0.5M sodium chloride (protein coupling buffer).

Sodium bicarbonate.....8.40g.

Sodium chloride.....29.22g.

8.4g of sodium bicarbonate was dissolved into 200 mls of distilled water, the pH was adjusted to 8.3 with 1M Sodium hydroxide solution under magnetic stirring,29.22g of Sodium chloride was added and stirring continued until the salt had dissolved the pH was checked again. The buffer was then transferred into the volumetric flask and the volume made to 1000 mls.

3.3. 1M Diethanolamine pure pH 9.0 (blocking agent)

Diethanolamine pure 105.14g/l

3.4. 0.1M acetate buffer pH 4 containing 0.5M sodium chloride (to wash away excess blocking agent)

Acetic acid.....5.77g.

Sodium acetate.....8.2g.

Sodium chloride.....29.22g.

Dissolved in 1000 mls distilled water.

APPENDIX 4: Reagents and solutions used in the Folin Ciocalteu reaction

4.1. Solution 1

2% sodium carbonate in 0.1N sodium hydroxide.

Solution 2:

0.5% Copper sulphate ($CuSO_4.5H_2O$) in 1% sodium tartrate was prepared freshly each day by mixing equal volume of double strength reagents.

Solution 3:

Alkaline copper solution was prepared by mixing 50 parts of solution 1 to one part of solution 2.

4.2. Phenol reagent

Commercial Phenol reagent (Fisher Scientific Company, New York) was diluted in the ratio of 5 parts reagent to 4 parts of distilled water (v/v).

4.3. Bovine serum Albumin (BSA)

BSA (No.A-4503, lot No.107C-0307 Fraction V powder Sigma Chemical Company St. Louis USA) was used to prepare the standard curve for determination of protein.

APPENDIX 5: SDS-PAGE reagents

5.1. Gel buffer (0.2M phosphate pH 7.0 with 0.2% SDS)

Sodium hydrogen phosphate 1 hydrate

($NaH_2PO_4 \cdot H_2O$).....17.64g

di-sodium hydrogen phosphate

(Na_2HPO_4 anhydrous).....40.89g

Sodium dodecyl sulphate (SDS).....4.00g

Dissolve the salts in 1 litre distilled water and adjust the pH to 7.0 (with 0.1N acid or alkali) then make up to 2 L in distilled water. For use in electrophoresis dilute the buffer 1:2 (Buffer : Distilled water).

5.2. 7.5% Acrylamide stock solution

Acrylamide.....42.2g

Bis-acrylamide..... 1.125g

Dissolve the polyacrylamide reagents into 250 mls of distilled water then filter through Whatman No.1 filter paper to remove the insoluble material.

5.3. 70% Glycerol

Glycerol (Spgr 1.26) (BDH-Poole England) - 55.6ml

Distilled water.....44.4 ml

Glycerol was mixed with distilled water in the volumes shown above.

5.4. 0.05% Bromophenol blue

Dissolve 0.01g Bromophenol blue dye into 20 mls distilled water.

5.5. 44 mM phenlyl methyl sulphonyl fluoride (PMSF)

C.038 (PMSF) was dissolved into 5ml acetone/ethanol to make a 44mM solution.

5.6 Coomassie Brilliant blue 250R stain

Coomassie Brilliant blue 250R.....1.0g

Methanol.....452.mls

Distilled water.....452 mls

Glacial acetic acid ..... 96 mls

The stain was filtered through Whatman No.1 filter paper to remove the insoluble material.

5.7. Gel destaining solution

Glacial acetic acid.....70 mls

Methanol.....200 mls

Distilled water.....730 mls

5.8. 0.02% Ammonium per sulphate analytical grade (Fisher Scientific Company, New York, USA).

Dossolve 0.12g of ammonium per sulphate into 8 mls of distilled water.

5.9. N,N,N,'N- tetramethylenediamine (TEMED)

TEMED (Merck, Darmstadt) was used as accelerator of the polymerisation reaction.

5.10. Preparation of SDS-PAGE (gel)

The following proportion of the above mentioned reagents were used

15 mls of gel buffer

13.5 mls of stock acrylamide solution

1.5 mls of ammonium per sulphate

20 ul of TEMED

5.11 Preparation of sample mixture for SDS-PAGE

In the preparation of the samples to be loaded on SDS-PAGE (gels) the following proportion of reagents were used.

	70% glycerol	40 ul
	5% SDS	20 ul
	50mml phosphate buffer pH 8.0	50 ul
*	B-mercaptoethanol	5 ul
	PMSF	5 ul
	0.05% Bromophenol blue dye	10 ul
	Sample	100 ul
	Total volume	230.0 ul

* If the sample is to be reduced and denatured B-mercaptoethanol is added in the proportion shown above the whole mixture (230 ul) is then boiled in waterbath for 2 minutes.

APPENDIX 6: Isoelectricfocusing solutions and reagents

6.1. Density gradient solutions for use with 1% Ampholine pH range 3.5-10

All the solutions were prepared according to the manufacturers recommendations (LKB-Bromma Sweden) LKB 8100-1(110 ml) column was used for all the electrofocusing experiments.

Density Gradient Solutions prepared.	Weight and volumes of solutions used
(a) Dense gradient solution	
Sucrose	27g
Distilled water	31 ml
1% Ampholine(R)	2 ml
Sample	4 ml
Total volume	54 ml
Concentration of sucrose	50% (w/v)
(b) Light Gradient Solution	
Sucrose	2.7 g
Distilled water	48.3 ml
1% Ampholine (R)	0.7 ml
Sample	4 ml
Total volume	54 ml
Concentration of sucrose	5% (w/v)
(c) Electrode Solutions	
(i) Cathode at the top of the column	
Cathode Solution	
1M sodium hydroxide	2.5 ml
Distilled water	7.5 ml
Total volume	10 ml
(ii) Anode Solutions	
1M phosphoric acid(H_3PO_4)	4 ml
Distilled water	12 ml
Sucrose	15 g
Total volume	25 ml
Concentration of sucrose	60% (w/v)

6.2. Density gradient solutions for use with 1% Ampholine pH 4-6

Density Gradient Solutions prepared	Weights and Volumes of Solutions
(a) Density Gradient Sulutions	
Sucrose	27 g
Distilled water	34 ml
1% Ampholine	2.0 ml
Sample	1 ml
Total volume	54 mls
Concentration of Sucrose	50% (w/v)
(b) Light Gradient Solutions	
Sucrose	2.7 g
Distilled Water	51.3 ml
1% Ampholine	0.7 ml
Sample	1 ml
Total volume	54 ml
Concentration of Sucrose	5% (w/v)
(c) Electrode Solutions	
(i) Cathode at the top of the column	
Cathode Solution	
1M sodium hydroxide	2.5 ml
Distilled water	7.5 ml
Total volume	10 ml
Anode Solution	
1M H_3PO_4	14 ml
Distilled water	12 ml
Sucrose	15 g
Total volume	25 ml
Concentration of sucrose	60% (w/v)

APPENDIX 7. DULBECCO'S PBS USED IN THE RADIOIMMUNOASSAY(RIA)

7.1. Solutions for preparing Dulbecco's PBS

Solution A

KCL	2.0 g
KH_2PO4	2.0 g
NaCl	80.0 g
Na_2HPO4	11.5 g

Solution B

	$CaCl_2$	1 g
or	$CaCl_2.2H_2O$	1.3 g

Solution C

	$MgCl_2$	0.4 g
or	$MgCl_2.6H_2O$	2.5 g

These chemicals were used to make 10 litres of Dulbeco's PBS.

7.2. Radioimmunoassay buffer with 1% normal rabbit serum

The radioimmunoassay buffer was made by adding the reagents shown below:

Normal rabbit serum	10 ml
Dulbecco's PBS	990 ml
0.1% Sodium azide	1 g.

7.3. Buffer for dilution of antigen used in RIA

Dulbecco's PBS was used to dilute the Antigen 11.

APPENDIX 8: List of buffers and solutions used in immunoelectrophoresis and immunodiffusion

8.1. Barbital Calcium lactate buffer for immunoelectrophoresis

8.1.1. For electrophoresis chambers

Sodium barbital (Sodium 5.5-diethylbarbiturate)-105.1g.

Barbital(5.5 diethylbarbituric acid) - 16.6g.

Calcium lactate - 15.2g.

This was made into 10 l. of distilled water the pH was adjusted to 8.4 or 8.6.

8.1.2 For preparation of agarose

The above buffer was diluted with distilled water in the ratio 2:1 (2 parts buffer and 1 part water (v/v)

8.2. Phosphate buffered saline for preparation of agar gel for immunodiffusion and other uses.

8.2.1 Phosphate buffer 0.15M pH 7.4.

di Sodium hydrogen phosphate (anhydrous) 21.2g. This was dissolved in about 800 mls of distilled water; the pH was adjusted to 7.4 by adding dilute hydrochloric acid (1N HCl), the volume was then filled up to 1000 ml in the volumetric flask.

8.2.2 Phosphate buffered saline (PBS) pH 7.4

1 volume of 0.15M phosphate buffer pH 7.4 was added to 9 volumes of saline. (0.9% sodium chloride in distilled water).

APPENDIX 9: Agar and agarose used in the Immunodiffusion and immunoelectrophoresis

9.1. 1% Agar in phosphate buffered saline pH 7.4 for immunodiffusion.

Purified Oxoid Agar	2g.
Phosphate buffered saline	50 ml
Distilled water	150 ml
Sodium azide (NaN_3)	0.02 g

0.1% (w/v) sodium azide was added as preservative to to prevent microbial growth on the agar during immunodiffusion.

9.2. 1% Agarose in barbital lactate buffer with Triton X-100 (p-Isooctylphenoxypolyethoxyethanol) for crossed immunoelectrophoresis and immunoelectrophoresis.

Litex agarose (Tpye HSA)	2g
Barbital lactate buffer	50 ml
Distilled water	150 ml
Triton X-100	100 ul

0.5% Triton X-100 was added to the agarose and the antigen before electrophoresis. Triton X-100 is non ionic detergent.

APPENDIX 10: Protein staining solutions and destaining solutions

10.1 Coomassie Brilliant Blue 250 R solution

Coomassie Brilliant Blue 250R	10g.
Ethanol	900 ml
Glacial acetic acid	200 ml
Distilled water	900 ml

10.1 Destaining solution/procedure for Coomassie Blue staining solution

Ethanol	900 ml
Glacial acetic acid	200 ml
Distilled water	900 ml

10.3 Ponceau S solution

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

10.4. Destaining solution for ponceau S staining procedure

2% (v/v) glacial acetic acid in distilled water.

APPENDIX 11: The amount of protein as determined by the method of Lowry et al., (1951). In different batches of parasites.

11.1. Cysticercus bovis

Place of origin (Where infected meat was brought from)	Batch Number	Number of cysts pooled together	Amount of protein in mg/ml			
			Outer fluid	Outer membrane	Inner fluid	Inner membrane
Athi River	1	500	1.62	10.09	0.587	12.39
Athi River	2	"	1.60	11.00	0.572	12.30
Athi River	3	"	0.690	11.00	0.550	12.40
Athi River	4	"	1.590	10.00	0.580	12.00
Athi River	5	"	1.560	9.09	0.577	14.01
Ongata Rongai	6	"	1.500	9.98	0.550	12.11
Ongata Rongai	7	"	2.090	9.88	0.557	12.12
Ongata Rongai	8	"	1.590	9.00	0.555	12.14
Ongata Rongai	9	"	1.580	10.00	0.550	12.15
Ongata Rongai	10	"	1.670	10.00	0.580	12.16
Athi River	11	"	1.500	10.00	0.582	12.10
Athi River	12	"	1.670	10.00	0.581	12.20
Athi River	13	"	1.575	9.82	0.580	12.40
Athi River	14	"	1.500	9.85	0.580	12.50
Athi River	15	"	1.620	9.91	0.580	12.78
Athi River	16	"	1.620	10.09	0.580	12.00
Athi River	17	"	1.500	10.02	0.579	12.82
Athi River	18	"	1.600	10.07	0.575	12.82
Athi River	19	"	1.570	9.08	0.578	12.00
Athi River	20	"	1.575	9.80	0.580	12.02
Athi River	21	"	1.578	10.00	0.560	12.04
Athi River	22	"	1.700	10.72	0.580	12.05
Dagoretti	23	"	1.590	10.07	0.582	12.16
Dagoretti	24	"	1.550	10.18	0.583	12.17
Kabete	25	"	1.700	10.20	0.580	12.20

APPENDIX 11: The amount of protein as determined by the method of Lowry et al., (1951). In different batches of parasites

11.1 Cysticercus bovis (Cont'd)

Place of origin (where infected meat was brought from	Batch Number	Number of cysts pooled together	Amount of protein in mg/ml			
			Outer fluid	Outer membrane	Inner fluid	Inner membrane
Ongata Rongai	26	500	1.700	10.10	0.590	12.18
Ongata Rongai	27	"	1.600	10.20	0.550	12.20
Ongata Rongai	28	"	1.556	10.00	0.575	12.40
Ongata Rongai	29	"	1.500	10.11	0.581	12.15
Ongata Rongai	30	"	1.542	10.32	0.580	12.18
Ongata Rongai	31	"	1.550	10.30	0.580	12.16
Athi River	32	"	1.200	10.30	0.581	12.17
Athi River	33	"	1.500	10.00	0.581	12.40
Athi River	34	"	1.500	10.00	0.580	12.00
Athi River	35	"	1.600	10.00	0.580	12.39
MEAN			1.56	10.03	0.57	12.29
STANDARD DEVIATION		±	0.20	0.41	0.01	0.37

11.2. Taenia saginata (Crude saline extract)

Place of origin	Batch Number	Amount of Protein in mg/ml
Kajiado District	1	19.350
Nairobi(Kariobangi)	2	19.347
"	3	20.002
"	4	20.000
"	5	19.000
"	6	19.450
"	7	20.003
"	8	20.004
"	9	20.005
"	10	20.000
"	11	20.000
"	12	19.340
"	13	19.000
"	14	19.045
"	15	19.000
"	16	19.200
"	17	19.450
"	18	19.354
"	19	19.340
"	20	19.356
	MEAN	19.51
	STANDARD DEVIATION $\pm$	0.39

11.3 C. tenuicollis

Place of origin	Batch Number	Source	No.of cysts pooled	Amount of protein in mg/ml	
				Fluid	Membrane scolex
Ongata Rongai	1	goat	50	1.144	9.79
"	2	goat	"	0.898	9.70
"	3	goat	"	1.056	9.75
"	4	goat	"	1.03	9.00
"	5	goat	"	1.35	9.65
Dagoretti	6	goat	"	1.60	9.50
"	7	goat	"	1.70	10.00
Dandora	8	goat	"	1.89	9.00
"	9	sheep	"	0.789	9.65
"	10	sheep	"	0.730	9.00
"	11	goat	"	1.000	9.65
Ongata Rongai	12	"	"	1.000	9.50
"	13	"	"	1.000	9.75
"	14	"	"	1.800	9.00
"	15	"	"	1.900	9.00
"	16	"	"	1.700	9.75
"	17	sheep	"	0.72	9.80
"	18	"	"	0.74	9.00
"	19	"	"	1.73	9.75
"	20	"	"	1.76	9.50
Goat		MEAN		1.347	9.449
		STANDARD DEVIATION	±	0.367	0.368
Sheep		MEAN		1.078	9.45
		STANDARD DEVIATION	±	0.517	0.363

11.4. Other parasite extracts

Place of Origin	Parasite	Source	Amount of protein in mg/ml		
			mg/ml	sd	$\bar{X} \pm sd$
Ongata Rongai	F.gigantica	Goat	2.72		2.71±0.01
"	"		2.70		
"	"	sheep	2.71		2.71 ± 0.01
"	"	"	2.70		
"	"	cattle	2.81		2.76 ± 0.08
"	"	"	2.70		
Ongata Rongai	S.hepatica	goat	8.874		
"	"	"	8.810		
"	"	"	8.860		8.84±0.04
"	"	"	8.800		
Dandora	"	sheep	8.700		8.58±0.18
Athi River	"	"	8.750		
Ongata Rongai	Monieza pp	cattle	2.304		2.30±0.001
"	"	"	2.305		
"	"	sheep	8.91		8.91
"	"	goat	8.16		8.16
Ongata Rongai	Hydatid cyst fluid	goat	1.76		1.755±0.007
"	"	"	1.75		
Athi River	"	cattle	4.124		4.13 ± 0.008
"	"	"	4.136		
Dandora	"	sheep	1.755		1.756±0.001
"	"	"	1.756		
Ongata Rongai	Paramphistomum pp	goat	12.48		12.48±0.007
"	"	"	12.47		
"	"	sheep	10.67		10.67
"	"	cattle	12.48		12.48
Kabete	C.fasciolaris	Mice	0.0502		0.0502

11.4 Other parasite extracts

(cont'd)

Place of origin	Parasite	Source	Amount of protein in mg/ml	
			mg/ml	$\bar{X}$-Sd
Turkana	C.cerebralis fluid	goat	2.75	2.45±0.007
"	"	"	2.74	
"	Membrane scolex	"	3.96	3.96
Kabete	Trichuris spp	cattle	4.311	3.81±0.71
Kabete	"	"	4.312	
Ongata Rongai	Oesophagostomum spp	cattle	8.913	
"	"	"	8.900	
"	"	"	9.000	
Athi River	"	"	9.200	
"	"	"	8.920	
"	"	"	8.800	
Ongata Rongai	H.contortus	cattle	0.193	
"	"	"	0.205	0.20±0.01
"	"	"	0.211	
Turkana	T.hydatigena	Dog	9.273	9.27
"	"	"	9.273	
Kabete(ILRAD)	T.brucei 221	cattle	7.699	7.70±0.001
"	"	"	7.698	
Kenyatta National Hospital(Nairobi)	S.mansoni	Man	13.658	13.65±0.01
"	"	"	13.650	
Uplands	A. suum	Pig	10.837	
(Limuru)	"	"	10.840	
"	"	"	10.850	10.84±0.01
"	"	"	10.831	