Echinococcosis: disease, detection and transmission

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SUMMARY

Echinococcosis is one of the world’s most geographically widespread parasitic zoonoses, with transmission occurring in tropical, temperate and arctic biomes. Most human infections are due to Echinococcus granulosus transmitted between domestic dogs and livestock, but this cosmopolitan species also cycles between wild carnivores (principally canids) and wild ungulates. The other species with significant zoonotic potential is E. multilocularis that occurs naturally in fox definitive hosts and small mammal intermediate hosts. These two species cause human cystic or alveolar echinococcosis respectively, which may be considered serious public health problems in several regions including developed countries. This review provides an introductory overview to the Supplement and summarises the biology and epidemiology of these two related cestodes with an emphasis on applied aspects relating to detection, diagnosis and surveillance in animal and human populations, and includes aspects of transmission ecology, and also considers aspects of community epidemiology and potential for control.

Key words: Echinococcus granulosus, E. multilocularis, diagnosis, epidemiology, transmission.

INTRODUCTION

Echinococcosis is a term used to describe infection of animals and humans with the adult tapeworm or larval metacestode stages of cestode species belonging to the genus Echinococcus. Members of this genus within the family Taeniidae are usually small enteric tapeworms at 1.2–7 mm in length, possessing only a maximum of 7 segments (proglottides). The metacestode develops in the tissues of a wide range of mammalian intermediate hosts and is a fluid-filled cystic or vesicular structure composed of two main layers (often referred to as membranes). The laminated layer is a carbohydrate-rich, acellular structure which is unique to the genus Echinococcus. It is both supportive and also physically encloses the asexually produced protoscoleces which bud off from the living germinal layer and are the infective stage for the definitive host. Asexual reproduction in the metacestode stage is not unique to the genus as it also occurs in a number of other cyclophyllidean genera including Taenia spp., however production of protoscoleces by Echinococcus spp. is prolific ensuring high worm burdens in the carnivore definitive host.

Currently there are four species recognised within the genus Echinococcus i.e. E. granulosus, E. multilocularis, E. vogeli and E. oligarthrus (WHO/OIE, 2001). Formal species recognition of E. granulosus was made in the nineteenth century but for E. multilocularis species recognition occurred as late as 1953 when the parasite and its lifecycle in foxes and rodents was described by Rausch (1954) and Vogel (1957). The discovery and description of E. vogeli and E. oligarthrus were also relatively recent (Thatcher & Sousa, 1966; Rausch, Rausch & D’Alessandro, 1978). In the period 1920–1960, however, at least 10 species or taxa of Echinococcus were proposed but were subsequently reduced to the 4 listed above after revision of the genus (Rausch & Nelson, 1963). The species E. granulosus, nevertheless is now considered to exhibit a number of intraspecific variants (Thompson & Lymbery, 1988; Rausch, 1997). Based on morphological, biological, biochemical and especially molecular biological characteristics the species E. granulosus (which has a cosmopolitan distribution) has now been divided into 8 main genotypes (G1–G8). These include the important sheep strain (G1), two bovid strains (G3, G5), a horse strain (G4), the camelid strain (G6), a pig strain (G7) and the cervid strain (G8) (Bowles, Blair & McManus, 1992; Pearson et al. 2002). A ninth genotype (G9) has also recently been described in Poland (see McManus & Thompson, this supplement). The G1 sheep strain of E. granulosus is the most widespread and important zoonotic genotype, although bovid, cervid, porcine and camelid genotypes also exhibit zoonotic potential. Human infection with the metacestode (hydatid cyst) of E. granulosus is geographically widely distributed, from the sub-arctic to the tropics, with an estimated 2 million cases mostly associated with regions of sheep pastoralism (Craig, Rogan & Allan, 1996). The other three Echinococcus species are also potential zoonoses. E. multilocularis is a species distributed only in the nearctic and palearctic regions and is the cause of substantially more human infections (probably >100 000 cases) than either E. vogeli (approximately 120 cases described) or E. oligarthrus (<5 cases described). The latter two species are largely confined to sylvatic transmission cycles in neotropical forest and wet savannah, and are the subject of only a few epidemiological studies (D’Alessandro, 1997). Within the
species *E. multilocularis*, intraspecific variation appears to be limited in comparison to *E. granulosus*, and based on current assessments nucleic acid analysis can only broadly differentiate *E. multilocularis* regional isolates from Alaska and Eurasia (Rinder *et al.* 1997).

The propensity for *E. granulosus* to form intraspecific variants is in part related to genotypic selection within ‘man-made’ synanthropic animal transmission cycles. Archetypal wild-life transmission cycles involving *E. granulosus* still occur in the holarctic region between wolves and large cervids such as moose (Rausch, this supplement). The sub-species status of the horse, bovid and lion variants of *E. granulosis* is controversial (Macpherson & Craig, 2000) and detailed mitochondrial DNA analysis of the horse ‘strain’ has led to the recent recommendation that it be considered of separate species status (Le *et al.* 2002; McManus & Thompson, this supplement). The estimated time of phylogenetic divergence of the eight or nine current intraspecific forms of *E. granulosus* has surprisingly not yet been determined, but must have been relatively rapid if indeed it occurred since domestication of the dog (approximately 15 000 years ago) or of various ungulate species (3000–8000 years ago). The probability for strains or subspecies (species complexes) of *E. granulosus* existing prior to domestication of mammals cannot however be excluded. A recent molecular taxonomic analysis of two mitochondrial genes in *Taenia solium*, a related tapeworm (that infects humans and pigs), indicates that one genotype restricted to Asia arose at least 40 000 ya, long before the domestication of pigs (Nakao *et al.* 2002).

The epidemiology of endemic human cystic echinococcosis (CE) and alveolar echinococcosis (AE) is complex, and dependent on a number of factors. These include: (1) The presence of susceptible definitive hosts (primarily dogs and foxes), and the biotic potential of the parasite itself. (2) The presence of populations of susceptible mammalian intermediate hosts (domestic or wild ungulates and small mammal species). (3) The degree of immunity within intermediate and definitive mammalian hosts. (4) The occurrence of environmental features that enable egg survival prior to ingestion, and that also provide optimal habitats for small mammals (for *E. multilocularis*) or wild ungulates (for *E. granulosus*). (5) The presence of socio-cultural features conducive to domestic animal involvement (dogs and livestock), and that may lead to increased man–dog contact (Craig, Rogan & Allan, 1996; Macpherson & Craig, 2000).

Risk factors for human CE and AE are not well defined but include age (usually >20 years), sex (females in some endemic areas), ethnicity (e.g. Nilotic, Tibetan or Kazakh), occupation (usually associated with pastoralism or agriculture), dog ownership (duration, number and degree of contact), occurrence of livestock home-slaughter, presence of suitable habitat/landscape features around rural communities, and also possible genetic susceptibility (French, Nelson & Wood, 1982; Giraudoux *et al.* 1998; Shambesh *et al.* 1999; Craig *et al.* 2000; Wang *et al.* 2000; Vuitton, 2002).

There is a similar infective process for the related species *E. granulosus* and *E. multilocularis* that requires the ingestion of the egg which hatches to release an invasive and motile oncosphere that penetrates the gut, enters the circulation and rapidly vesiculates on reaching tissues (primarily liver and lungs). The metacestode grows slowly and initially asymptotically over months/years until eventually pathology may occur. For both these important species the detailed natural history of metacestode growth and pathology in humans requires further characterization, though the basic pathological process is known. Soon after post-oncospheral development, which is similar in CE and AE disease, the early hydatid development begins to differ significantly for human AE. In AE disease abnormal, and eventually prolonged, pathology occurs associated with exogenous multivesicular growth and possible metastases, with subsequent calcification and tissue necrosis. By contrast, in CE disease a univesicular fluid-filled hydatid cyst develops by endogenous growth and may cause pressure effects on surrounding tissues and organs. Cysts of *E. granulosus* can often develop so-called daughter cysts internally which have a similar structure to the parent cyst. There is a general assumption that daughter cyst formation is a natural, proliferative process within normal, healthy hydatid cysts, and that they arise from portions of the germinal layer which bud inwards and produce a laminated layer. This type of development is often shown in schematic text book diagrams (e.g. Cheng, 1981; Schmidt & Roberts, 2000) but may have a number of flaws. Firstly there is no developmental evidence that the germinal layer of intact cysts is capable of forming hydatid cysts internally. Secondly the presence of daughter cysts in human and sheep hydatid infections is almost always associated with changes in the nature of the hydatid fluid of the primary cyst, as shown by aspiration (yellowing or filled with pus/cells/debris) and ultrasound examination (i.e. the fluid in the original cyst cavity has a more echo-dense appearance than that within the daughter cysts or a primary cyst without daughter cysts). Thirdly, there is evidence that daughter cysts of *E. granulosus* arise from protoscolecites, which vesiculate in a similar manner to that which occurs in vitro or in the case of secondary hydatid infection (Rogan, 1988). It is, therefore, unlikely that daughter cysts of *E. granulosus* arise naturally from the germinal layer of intact hydatid cysts, but more likely that daughter cysts are present in hydatids where the primary cyst has.
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become damaged or degenerated in some way (Dew, 1927; Belding, 1965; De Rycke & Pennoit De Cooman, 1978; Rogan, 1988). Daughter cyst formation therefore represents a protective response by the parasite to damage or degeneration of the primary cyst. This may also be an important developmental feature in the natural history of CE infection as discussed later. By contrast, in experimental AE, secondary cystic development, including metastases, is most likely due to germinal cells and microvesicle development and not protoscolex vesiculation (Hemphill et al. 2003). Surgery still remains the main form of treatment for both human CE and AE throughout the world, but is increasingly supported by albendazole drug therapy (WHO/OIE, 2001). The physical nature and location of the cysts however, can influence the effectiveness and choice of treatment.

Epidemiology and transmission ecology studies of echinococcosis still remain a challenge. Such studies require appropriate tools to improve parasite detection and diagnosis of infection for both human and animal definitive and/or intermediate hosts. Diagnostic approaches include the traditional gold-standard parasitological methods of animal necropsy, and improved radiographic/imaging methods for human CE and AE. Significant advances have also occurred in the last 10 years in the development of highly specific coproantigen and serological tests, as well as copro-DNA analysis that have greatly improved ability to detect parasitic infection and exposure and therefore the quality, accuracy and effectiveness of epidemiological and ecological studies. Development of a quantitative modelling approach to transmission dynamics for *E. granulosus* in particular, has also improved the potential for assessment of both rational and cost-effective control programmes for CE (Gemmell & Roberts, 1998; Torgersen & Heath, this supplement).

The combined use of traditional approaches (e.g. use of hospital and abattoir records) and modern approaches (e.g. immunodiagnostic tests and portable ultrasound) to study epidemiology of echinococcosis has resulted in greater recognition of human CE and AE as significant or even re-emergent public health problems in a number of regions. These include AE in western China (with Tibet) (Wang et al. 2000; Wang et al. 2001; Zhou et al. 2000; Ito et al. 2003; Vuitton et al., this supplement), for both AE and CE in western Europe (Lucius & Bilger, 1995; Palmer et al. 1996), CE in eastern Europe (Todorov & Boeva, 1999), and in Kazakhstan (Shaikenov, Vaganov & Torgerson, 1999). Furthermore, geo-political as well as social changes have resulted in an increase in incidence rates of human CE in some areas for example in Israel (Goldsmith et al. 1991; El-On & Hoida, 2002) and in Kyrgyzstan (Torgerson, Shaikenov & Kuttybaev, 2002). Over the last 10 years or so in continental Europe there appears to have been a significant increase in the prevalence of *E. multilocularis* in the common red fox (*Vulpes vulpes*), and also evidence for urban transmission between red foxes and small mammals (Romig et al. 1999a; Hofer et al. 2000). Furthermore, since the introduction around the 1930s of *E. multilocularis* to Hokkaido Island in northern Japan, human AE case rates have increased sufficiently for the whole island to now be considered endemic (Ito et al., this supplement).

Echinococcosis is a classical zoonosis in which parasite transmission may occur in domestic, sylvatic or semi-domestic animal host cycles depending on the *Echinococcus* species (and strain), the nature and lifestyle of the human communities, and the local and regional environment. Knowledge of epidemiology and infection patterns as well as transmission dynamics is therefore important if control intervention is to be considered. Control of echinococcosis is not normally aimed at elimination, (though for *E. granulosus*/CE it maybe achievable) because that is often impractical and uneconomic, but rather at reducing levels of parasite transmission to the extent that human case rates become insignificant in terms of public health. Several hydatid control programmes for CE and *E. granulosus* carried out in particular on islands, have ultimately been very successful especially when regular mass dosing of owned dogs and reduction in dog numbers was efficiently implemented, e.g. in Cyprus, New Zealand, Tasmania and the Falkland Islands (Gemmell & Roberts, 1998). Only a few hydatid control programmes based on continental land masses have achieved this, notably in southern Chile and in patagonian Argentina (WHO/OIE, 2001). The length of period necessary to continue control measures until ovine and human CE rates were negligible in these programmes ranged from 10 to >50 years (Gemmell & Roberts, 1998). For the other main zoonotic species, *E. multilocularis*, knowledge of the transmission ecology of animal host populations will enhance the potential to interrupt the parasite life-cycle and reduce the relative risk of human AE disease. However, programmes to control transmission of *E. multilocularis* will be very difficult and expensive and, probably, unethical when wild-life hosts are involved, though fox baits have had local success (Ito et al., this supplement). When a significant zoonotic role for domestic dogs has been implicated or confirmed in the risk of human AE e.g. for sled dogs in Alaska, then a targeted dog-dosing programme with praziquantel can significantly reduce transmission of *E. multilocularis* at least in the short term (Rausch, Wilson & Schantz, 1990).

**DETECTION AND DIAGNOSTIC METHODS IN INTERMEDIATE HOSTS**

The existence of a two-host life cycle means that detection or diagnosis of infection for epidemiological studies must address both the metacestode in the
intermediate host and the adult worm in the definitive host. The parasite occupies two different locations in these hosts being intestinal in the definitive host and tissue dwelling in the intermediate host, and therefore different approaches to diagnosis must be taken.

Most work on the diagnosis of hydatid disease has been clinically oriented and focused on detection of the parasite in humans. This has been considerably more successful than detection in natural intermediate hosts such as sheep. *Echinococcus* spp. are not typical of many other helminth infections and aspects of their biology create difficulty in developing and interpreting diagnostic approaches. The main problems are: (1) That the parasite infects humans as a microscopic oncosphere that subsequently develops into a much larger metacestode or hydatid cyst which bears no resemblance to the original oncosphere. (2) Differentiation and development of hydatid infections may occur over a long period of time with diagnosis often being made several years after the initial infection. This makes establishing how and where the infection took place, very difficult. (3) The morphology and characteristics of both cystic and alveolar hydatid cysts are variable and may differ considerably within the same individual and between individuals. (4) The location of hydatid cysts within the host is also variable with most occurring in the liver but also occurring in the lungs, heart, kidney, spleen, bones, muscles, brain and other obscure sites. (5) The parasite has the potential to proliferate within the intermediate host, either by an exogenous developmental process in the case of *E. multilocularis* or accidentally through cyst leakage/rupture in *E. granulosus*, thereby causing more serious disease.

Diagnostic approaches based on imaging of the metacestode, such as ultrasound and X-ray can, therefore, be problematic because of the variation in size, morphology and location of the parasitic lesion. Methods based on immunodiagnosis face the problems associated with variable antigen expression between early and late infections; variation in immune responses associated with the presence of the parasite in different locations e.g. lung and liver, and variation between individuals in their ability to recognise potentially diagnostic antigens. The possibility of taking biopsy material to aid in diagnosis is also problematic because of the risk of further dissemination of the disease. Despite the problems associated with these infections, considerable advances have been made in diagnosis and detection of the parasite in humans and often a combined approach can be beneficial.

**Imaging techniques**

Due to the lack of direct parasitological evidence an important diagnostic approach utilizes imaging methods for detection of space occupying lesions. Computerised tomography (CT), magnetic resonance imaging (MRI), ultrasound (US) and radiography (X-ray) are currently the most useful imaging techniques (WHO/OIE, 2001; Reuter et al. 2001). CT scans provide the best overall resolution for CE/AE but are limited to well equipped hospitals and not applicable to field studies. Ultrasound on the other hand is probably the most versatile technique for abdominal cysts, especially hepatic lesions, but is not useful for lung, bone or brain cysts. The development of portable ultrasound units has also been of considerable benefit in field surveys (Macpherson & Milner, 2003).

All imaging techniques rely on the expertise of the operator in differentiating *Echinococcus* infections from other space-occupying lesions. In many cases pathognomonic features such as the presence of a visible laminated layer or daughter cysts makes identification more straightforward. But in other cases the presentation of the image can be confused with other cystic lesions such as tumours, simple epithelial cysts and hepatic abscesses. With *E. granulosus* a number of ultrasound image classifications have been developed such as that of Gharbi et al. (1981) and a recent classification proposed by WHO/OIE (2001) and referred to by Macpherson *et al.* in this supplement. These classifications take account of variations in morphology of the cysts such as calcification or the presence of a detached laminated layer or daughter cysts. Such classifications are useful both in aiding diagnosis and in the clinical management of the disease (Wang *et al.* 2003). In addition, they may also represent a series of developmental stages through which some cysts progress and are therefore also useful in immunological studies (Pawlowski, 1997; Teggi & Di Vico, 2002).

**Immunodiagnosis**

Over the past thirty years the use of applied immunology in the diagnosis of human infections has been very evident, largely through the detection of parasite-specific antibody in serum. In the last 10 years more advanced approaches such as circulating antigen detection, lymphocyte proliferation responses and cytokine analyses and molecular techniques (Siles-Lucas & Gottstein, 2001) have been applied to provide useful information on these infections but it is still antibody detection which is most widely used for confirmation of clinical diagnosis and in epidemiological surveys.

**Cystic echinococcosis.** The main source of antigen for diagnosis has been the hydatid fluid removed from cysts in livestock. In many cases the crude cyst fluid can be used for procedures such as immunoelectrophoresis (detection of arc 5) and the indirect haemaglutination assay with good results but it has now become more frequent to purify components such as Antigen B, a heat-stable lipoprotein, from
the cyst fluid. This appears to be the most specific antigen (>90%) currently available with the major cross-reactions occurring only with the other cestode zoonoses. The sensitivity, however, is variable with an average value of around 80% for ELISA and 70% for recognition of subunits from 8–24 kDa in immunoblotting. Improved performance can sometimes be obtained by detecting specific IgG4 to these antigens (Wen & Craig, 1994; McVie et al. 1997). Recombinant Antigen B proteins have also been used but tend to produce results which are less sensitive than the native Antigen B (Rott et al. 2000). In areas of the world where there are relatively few helminth infections and no other cestode infections, the use of crude hydatid fluid as an antigen in ELISA may be a practical alternative approach producing a higher sensitivity.

**Alveolar echinococcosis.** Antigens involved in the serodiagnosis of human AE are usually derived either from cyst masses or protoscolecis obtained from experimental rodent infections. One of the most useful antigens derived from the cyst is the carbohydrate rich Em2, which is affinity purified, or the commercially available preparation Em2plus which uses a recombinant EM 11/13 protein (Siles-Lucas & Gottstein, 2001). Whole protoscolex homogenate has been used in ELISA with some success but is better when used in immunoblotting as a band with relative molecular mass of 18 kDa has been reported to be highly specific for AE infections (Ito, Schantz & Wilson, 1995). The diagnostic antigens involved in detection of human AE are usually more specific than those involved in CE and sensitivities, in general are >85–90%.

Since there is no immunodiagnostic test which is 100% sensitive and specific for each of these infections, the value of serological screening has been questioned by several workers. The major problems are due to the existence of significant numbers of both false negative and false positive cases. The average sensitivity of ELISA-based diagnostics is around 80% (range 60–95%). Specificities with reference to other, non-cestode infections is usually high (95–100%) but significant levels of cross-reactivity exist within the main larval taeniid infections. In geographical areas where *E. granulosus*, *E. multilocularis* and *Taenia solium* occur together (e.g. western China) serology is more problematic. Serological surveys often indicate a proportion of the population who are antibody-positive but who are asymptomatic and have no ultrasound image indicative of echinococcosis. These are more likely to represent individuals with previous exposure to the parasite without the development of further infection or significant pathology (Cohen et al. 1998; Bartholomot et al. 2002).

The real value of serology is its use in combination with other diagnostic procedures such as imaging. In this respect it can be of benefit in: (1) Confirmation of imaging or clinical evidence, e.g. where an ultrasound image is unclear (Wang et al. 2000). (2) Identification of asymptomatic individuals with no obvious cystic image. For example a study of human CE by Cohen et al. (1998) in Uruguay found 8/1149 individuals (0-5%) with normal ultrasound but who were seropositive for Antigen B ELISA and Arc 5. In follow-up, one patient had a pulmonary cyst confirmed by X-ray and one patient had two peripheral liver cysts confirmed by CT. (3) Providing information on the state of the infection and the immune response against the parasite. Total IgG levels tend to vary with different types of cyst and there is also evidence for variation in IgG sub-class responses, with IgG4 levels being more elevated in symptomatic or actively growing cysts (Gharbi classifications as Types II and III) and low in asymptomatic or Type IV and V cysts (Shambesh et al. 1999; Daeki, Craig & Shambesh, 2000). This is also the case for *E. multilocularis* where high IgG4 activity against Em 18 antigen is associated with active lesions (Ito et al. 1995).

**Detection of parasite antigens in cyst fluid**

Although not a standard diagnostic technique, the detection of *Echinococcus* antigens in samples of fluid taken from suspect cystic lesions can confirm the parasitic nature of the cyst. This is most useful when ultrasound-guided, fine-needle biopsy samples are taken prior to PAIR treatment (puncture, aspiration, injection reaspiration) for human CE. This can be done by ELISA (Craig, Bailey & Nelson, 1986; Paul & Stefaniak, 1997) or dot-ELISA (Wang et al. 2002) using antibodies raised against *E. granulosus* cyst fluid antigens. Use of PCR to amplify DNA from hepatic lesions in rodents and humans has been considered useful (Siles-Lucas & Gottstein, 2001).

**Detection of Echinococcus infections in animal intermediate hosts**

The most frequent approach for the detection of CE in sheep and other domestic livestock is by examination at necropsy (see below). Radiological (X-ray) diagnosis of ovine hydatidosis has been used successfully on a very small scale (Wyn-Jones & Clarkson, 1984) but it is not a suitable system for epidemiological surveys. The potential of ultrasound examination (if any) has yet to be fully assessed; however trials in Kenya and Argentina indicate reasonable sensitivity (>70%) though variable specificity especially regarding false positives caused by *T. hydatigena* cysticercosis (Maxson et al. 1996; Guarnera et al. 2000).

Immunodiagnostic techniques have been employed for ovine hydatidosis although specific serum antibody reactivity tends to be lower than in human
cases making it less useful. A recent study by Kittelberger et al. (2002) evaluated three ELISA tests employing a purified Antigen B subunit, a recombinant oncospherical antigen (EC95) and a crude protoscolex antigen in 249 natural or experimentally infected sheep. The highest diagnostic sensitivity was 63% with the protoscolex antigen but specificities were high at around 96%. Although these sensitivities are relatively low in terms of individual cases, the assay could be beneficial if used on a flock basis.

Techniques for the detection of alveolar echinococcosis in rodents, other than autopsy, are not normally required. The level of E. multilocularis infection in rodent or small mammal communities is usually very low although sufficient to maintain the life cycle. Potential use of PCR to amplify E. multilocularis DNA from wild rodents with small or atypical lesions has been demonstrated in ecological studies (Hofer et al. 2000).

**Survey Methods at Community Level**

Echinococcosis and especially cystic echinococcosis present a number of difficulties in terms of defining the level of infection in particular communities. These largely depend on the fact that they represent zoonotic infections and are often monitored by at least two government departments. On one hand they represent a medical problem, usually dealt with by hospitals and public health authorities, whilst on the other hand they may be considered as veterinary problems, dealt with by ministries of agriculture. In many cases there may be little communication between the two departments and an overall picture is hard to determine.

**Retrospective data**

Initial recognition of hydatid disease as being a problem in different communities often depends on retrospective case finding from sources like surgical case records. Data collected over a 5–10 year period (e.g. Kamhawi, 1995) can be helpful in identifying endemic areas but is must be remembered that these data may be misleading for a number of reasons. Firstly, annual surgical incidences, usually expressed per 100,000 population, are frequently underestimates of the true level of human infection. This is because hospitalised cases are almost always well developed and symptomatic. Individuals who are asymptomatic will therefore, be excluded (Craig, Rogan & Allan, 1996). Secondly, not all infected persons will have the same access to medical care and there may also be a bias for treatment in certain age groups. Thirdly, a particular hospital may serve a large area so that people being treated there may originate from communities some distance away (e.g. French & Nelson, 1982). Record keeping also varies from hospital to hospital and inaccurate identification of parasite lesions may occur; for example, misdiagnosis of alveolar echinococcosis as hepatic carcinoma (Jiang, 1981; Sasaki et al. 1994). Ideally surgical diagnosis should be confirmed by histology but this may not always be the case. Hospital records relating to alveolar echinococcosis may be more difficult to interpret since the diagnosis may simply be recorded as ‘hydatid’ without reference to E. granulosus or E. multilocularis. Surgical records may also show inaccuracies relating to second operations or readmissions and it may be difficult to differentiate these from new cases (Kern et al. 2003). In countries where hydatid disease is notifiable by law, data may be obtained from a central reporting office; however, poor reporting and record keeping may require that investigators obtain these data by active search.

Retrospective data relating to natural intermediate hosts, in the form of livestock, can be one of the most sensitive indicators of the degree of environmental contamination with E. granulosus eggs. The most practical method of collecting data is from animals slaughtered at commercial abattoirs, especially if it is in a location where hydatid disease in livestock is notifiable by law. Information can be incomplete as it tends to be older sheep (6 years or more) or lambs which are most frequently slaughtered. Additional problems include the identification of small cysts during gross examination of livers and lungs and the potential for misidentification with other larval taenid metacestodes such as Taenia hydatigena, or non-parasitic lesions for example due to Corynebacterium infection (Cabrera et al. 1995).

**Radiographic surveys**

Of the imaging techniques described above, ultrasound has proved to be the most versatile because of the availability of portable scanners which can be run from a small generator. Both human CE and AE may be detected in this way. This means that surveys can be carried out in remote locations. Examples of such surveys have been undertaken in Africa (Bahir et al. 1991; Macpherson et al. 1989), South America (Cohen et al. 1998) and China (Wang et al. 2000; Bartholomot et al. 2002). With experienced ultrasonographers this technique can be quickly used to screen several hundred people in a few days. The images obtained can also give some indication of the condition of the cyst(s) or lesion(s). It is now recognised that hydatid cysts of E. granulosus may show a series of developmental states including the collapse of the cyst and the formation of daughter cysts. A number of classification systems have been developed to promote uniformity in cystic description for E. granulosus (Gharbi et al. 1981; WHO/OIE, 2001), and also to describe gross pathology for AE due to E. multilocularis (WHO/OIE, 2001; Bartholomot et al. 2002).
Serosurveys

The use of serological tests in community screening can have a number of benefits. Most tests are relatively inexpensive and easy to perform on large numbers of serum samples. In addition, assays like the ELISA can also be carried out using dried blood samples, on filter paper, if sampling of venous blood or refrigeration is a problem (e.g. Craig et al. 1992). The information obtained from serology is also of use in the following ways.

Confirmation of imaging/clinical evidence: in community surveys for CE and AE where portable ultrasound is used, the results obtained often present a wide variety of cystic lesions. To an experienced ultrasonographer the correct identification of these lesions is often straightforward and an ultrasound classification system of CE cyst types has been produced. In several cases, however, the morphology of a suspected cystic lesion may be unclear and its possible parasitic origin uncertain. The use of an antibody test can therefore provide additional evidence to support the diagnosis. For example Romig et al. (1999b) in southwest Germany, found that 2/2560 people screened for AE had suspicious lesions confirmed by serology. In northwest China a recent survey showed that in addition to 47 CE cases identified by ultrasound, 10/15 suspect images and 16/23 calcified lesions were seropositive for Antigen B (Wang et al. 2000). Such immunological evidence would therefore suggest that these lesions were parasitic in nature.

Identification of asymptomatic, infected individuals with no obvious cystic image: with these zoonotic infections a significant proportion of people may be asymptomatic and have cystic lesions which are not easily detectable. With ultrasound imaging only cysts occurring in the abdominal cavity can be detected. Community-based surveys employing ultrasound cannot therefore detect cysts in other locations such as the lungs, bone or central nervous system. If serology is used, this can allow for the identification of antibody-positive individuals who have no obvious cystic lesion at first screening. The presence of specific antibody alone does not confirm diagnosis as individuals may be seropositive for a number of reasons, such as previous exposure to the parasite without progressive disease or cross-reactivity with other conditions. Identification of antibody positive individuals does, however, allow that a more detailed investigation of these people to be carried out.

DETECTION AND DIAGNOSIS IN DEFINITIVE HOSTS

Accurate determination of Echinococcus spp. prevalence in populations of final hosts (dogs, foxes, cats) is essential for estimating the potential risk for humans and to undertake transmission studies. Diagnosis of Echinococcus spp. in definitive hosts is complicated by the fact that eggs of species in the genera of both Echinococcus and Taenia are morphologically indistinguishable. Therefore parasitological diagnosis relies on direct detection of the tapeworms. The two major parasitological methods are purgation with arecoline salts and examination of the small intestine at necropsy (Deplazes & Eckert, 1996). Both these techniques are time consuming, laborious and require special safety precautions and are not suitable for large-scale epidemiological studies. With the aim of simplifying and improving epidemiological investigations, and to enable diagnosis in living animals, several indirect methods have been described: detection of serum antibodies, coproantigens and parasite DNA in faecal samples.

Detection of serum antibodies

Taeniid cestode antigens (derived from adult worms, juvenile intestinal stages or oncospheres) interact with the immune system of the host and may lead to the production of specific antibodies (Jenkins & Rickard, 1985). Enzyme-linked immunoassays (ELISA) based on detection of these antibodies have been developed for diagnosing Echinococcus spp. infection in final hosts (Jenkins & Rickard, 1986; Gasser et al. 1988). A problem encountered with serum antibody detection is the persistence of antibodies after elimination of the cestodes (Gottstein et al. 1991; Gasser et al. 1993). An ELISA based on the detection of antibodies directed against Em2 antigen was found to have a sensitivity of approximately 60% (Gottstein et al. 1991). Even though no cross-reactions were shown with sera from dogs with various helminths when using this ELISA, animals without a detectable intestinal infection were also positive. The potential value of the Em2-ELISA relates to identification of exposed fox populations rather than an individual diagnosis of intestinal E. multilocularis infection. Serological diagnosis of canine echinococcosis due to E. granulosus has been based on the detection of antibodies (IgG, IgA and IgE) directed against a protoscolex antigen preparation (Gasser et al. 1993). Experimental infection of dogs in Australia showed that IgA antibody levels were detectable after one week post infection (p.i.), while an increase in IgE antibodies was observed after two weeks, and IgG levels peaked after three weeks p.i. (Gasser et al. 1993). Overall specificity ranged from 97–100% but sensitivity varied (53–84%) according to geographic region, though it could be increased by combining the evaluation of various immunoglobulin classes (Gasser et al. 1993; Craig et al. 1995).

Detection of coproantigens

The most important advantage of coproantigen ELISAs over conventional serum antibody assays...
are that they indicate current infection (Allan et al. 1992; Deplazes et al. 1992, 1999; Jenkins et al. 2000). In a given population of dogs exposed to E. granulosus the sensitivity of coproantigen ELISA was superior to use of antibody detection (Craig et al. 1995). Coproantigens are detectable during patent as well as pre-patent periods, and disappear within 2–5 days after the elimination of Echinococcus worms (Deplazes et al. 1999; Jenkins et al. 2000). Coproantigens remain stable for at least 5 days at room temperature and up to 30 °C outside, and can be fixed in 5–10% formalin and then be stored for weeks at 4 °C or at room temperature (Craig et al. 1995; Deplazes et al. 1999; Jenkins et al. 2000). The captured antibodies used in the different ELISA assays can be directed against crude somatic antigens of immature adult proglottides (Allan et al. 1992), or excretory/secretory (E/S) antigens (Deplazes et al. 1999). One of the problems encountered is that faecal material contains desorbing components and factors that could interfere with the ELISA; using 50% foetal bovine serum or heating the faecal sample can reduce the effect of these factors (Viscidi et al. 1984; Allan et al. 1992).

E. multilocularis. Two groups have independently developed coproantigen tests utilising antibodies against E. multilocularis (Kohno et al. 1995; Deplazes et al. 1999). Coproantigens can be detected 6 days p.i. in experimentally infected dogs and foxes, and 11 days p.i. in cats (Nonaka et al. 1996; Deplazes et al. 1999). An overall sensitivity of 83.6% was reported, while in foxes with worm burdens greater than 20–50 worms the sensitivity reached 93.3%. The overall specificity was 99.5%; however cross-reactivity did occur in 16% of 32 dogs experimentally infected with E. granulosus (Deplazes et al. 1999). Coproantigen ELISAs have been used successfully to screen foxes and cats collected in eastern France and to allow evaluation of the distribution of E. multilocularis infected fox populations in the endemic area (Raoul et al. 2001).

E. granulosus. Coproantigen ELISAs developed for E. granulosus have shown differing levels of sensitivity ranging between 50 and 87.5% (Allan et al. 1992; Craig et al. 1995; Moro et al. 1999). As for E. multilocularis the sensitivity of the test is to some extent dependent on the E. granulosus worm burden. A relatively low overall sensitivity of 61.5% was observed by EL-Shehabi et al. (2000) while the same authors obtained sensitivities reaching as high as 87.5% and 100% for dogs with worm burdens greater than 20 and 100 respectively. Variable degrees of genus specificity for this test have been reported to vary between 76.9% to 96.5% with samples from naturally or experimentally infected dogs. Coproantigen tests for E. granulosus have now been applied successfully in epidemiological studies in owned dog populations in Spain (Deplazes et al. 1994), Wales (Palmer et al. 1996), Nepal (Baronet et al. 1994), Uruguay (Cohen et al. 1998) and China (Wang et al. 2000), and for surveillance of canine echinococcosis in a hydatid control programme in Cyprus (Christofi et al. 2002). Echinococcus coproantigen ELISA has also been applied to screen wild carnivore hosts such as lion populations in East Africa (Muller- Graf, 1995), and for wolves in North America (Craig P. S. & Storandt S., unpublished observations) and in Finland (Hirvela-Koski et al. 2003).

Detection of copro DNA

The advantage of detecting Echinococcus DNA by polymerase chain reaction (PCR) in faecal samples is that it has the potential to provide absolute species-specific assays with high sensitivity. Additionally, as the test is aimed at detecting eggs in faecal samples it provides a more accurate estimation of the potential risk for human infection than ELISA-based methods mentioned above. Problems encountered when extracting DNA from faecal samples are that these samples often contain PCR inhibiting substances that create false negatives. Monnier, Deplazes & Eckert (1996) described a protocol that included utilising several different kits to purify DNA, eliminating inhibitory substances. This method is time consuming and additional steps to DNA extraction enhance the possibility of losing the DNA present in the faecal samples. Inclusion of several sieving steps concentrates eggs from the sample to improve sensitivity (Mathis, Deplazes & Eckert, 1996), but may make the protocol complicated and time consuming and may not be so suitable for large epidemiological studies. Use of a commercial kit, the Qiaamp Ministool Kit (Qiagen, UK) combined with a single sieving step provided good results (Abbasi et al. 2003).

E. multilocularis. For the development of an E. multilocularis-specific PCR copro test two different genes have been used: the U1 snRNA gene (Bretagne et al. 1993) and the mitochondrial 12 sRNA gene (Dinkel et al. 1998). The U1 snRNA gene is repeated at least 50 times in the genome and was initially used for a single PCR assay, and the assay was reported to reach a sensitivity of 1 egg in 4 g of faeces and to show a specificity of 100% (Bretagne et al. 1993). To improve sensitivity further, Dinkel et al. (1998) developed a nested PCR with 2 consecutive rounds. This PCR was modified further into a single tube nested PCR retaining the same sensitivity and specificity (Van der Giessen et al. 1999). Copro PCR tests for E. multilocularis have enabled surveys of fox populations in Europe, mainly in confirmation of coproantigen positive faecal samples, but also to provide baseline data for infection in fox populations in low endemic or
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E. granulosus. Until very recently, no equivalent copro DNA test had been developed for E. granulosus. An E. granulosus-specific PCR was described that utilized the mitochondrial CO1 gene with a reported sensitivity equivalent to >200 eggs and no cross-reactions were observed with DNA from E. multilocularis, T. hydatigena, T. ovis or D. caninum cestodes (Cabrera et al. 2002). The reported sensitivity of the PCR was therefore lower than for the E. multilocularis PCR assays that have been described. In the study of Cabrera et al. (2002) no faecal samples were tested. A new repeat sequence (EgG1 Hae III) with 6900 copies has now been identified in the genome of the sheep strain of E. granulosus, and PCR using specific primers detected a single egg with no cross amplification for other taeniid cestode species including E. multilocularis (Abbasi et al. 2003). Furthermore, faecal samples from dogs with burdens of 2–10 000 worms of E. granulosus were all positive while this copro DNA test remained negative with faecal samples from all other infections including E. multilocularis (Abbasi et al. 2003).

While serum antibody ELISAs are not suitable for estimating Echinococcus infection in individual definitive hosts, they may be useful as a pre-screening test. Coproantigen ELISA is the most suitable technique for large-scale epidemiological studies and could even be considered to be an alternative to parasitological examination. While copro PCR is best used as a confirmatory tool (Deplazes et al., this supplement).

Detection of eggs in the environment

In an endemic area for echinococcosis transmission, definitive hosts (dogs, foxes etc.) will contaminate the environment with faeces containing the microscopic eggs of Echinococcus spp. Ultimately transmission to animal and human intermediate hosts will depend on the microenvironmental conditions which allow eggs to remain viable within limits of temperature, humidity and time (Nelson, 1986; Gemmell, 1990; Veit et al. 1995). Risk factors for human CE and AE may also therefore include contact with a contaminated environment, especially through food, water and utensils in underdeveloped conditions. For example, the source of drinking water has been identified as a potential risk factor for both human CE (French et al. 1982; Dowling, Abo-Shehada & Torgerson, 2000), and AE (Yamamoto, Katamura & Miyake, 2001).

Analysis of soil, herbage and water samples for the presence of taeniid eggs has been undertaken, but until the advent of specific probes was limited to confirmation by microscopy for presence of taeniid eggs only – which may be derived from various Taenia or Echinococcus species of human and/or animal origin. An Echinococcus oncosphere-specific monoclonal antibody (Mab4E5) was used in a micro-epidemiological study of environmental contamination in the CE endemic area of Turkana, Kenya (Craig et al. 1988). That study indicated that both topsoil, from within and around dwellings, and drinking water were contaminated with Echinococcus eggs. The development of species-specific DNA probes and PCR copro assays for Echinococcus spp. (Mathis et al. 1996; Abbasi et al. 2003) could enable definitive identification of eggs recovered from the environment (Cabrera et al. 2002).

ECOLOGICAL APPROACHES

The natural transmission cycles of E. granulosus and E. multilocularis depend on the presence of populations of susceptible mammalian definitive and intermediate hosts, and for sustained transmission on the existence of favourable host ecology factors. In particular, host predator–prey interactions will contribute significantly to Echinococcus transmission potential and the distribution of the parasite in ecosystems containing diverse intermediate host populations. Wildlife mammalian host species may interact with each other in predator–prey relationships within habitats in defined landscapes, and trophic transmission of tapeworm species will be facilitated only if the hosts are able meet in the ecosystem and furthermore only if putative hosts are susceptible to the parasite (Combes, 2001).

Host behavioural studies and host population-based sampling approaches are therefore important in understanding the transmission ecology of Echinococcus spp. in both natural sylvatic or peridomestic cycles. This is especially relevant for E. multilocularis which uses fox species as the main definitive hosts and a range of small mammals, predominantly microtine rodent species, as intermediate hosts. Comprehensive public health and ecological studies within the E. multilocularis-endemic areas of eastern France and in south Gansu (China) have been particularly useful in establishing the role of landscape in transmission ecology. Combined studies using animal host ecological and clinical data showed that, despite different species assemblages of potentially susceptible small mammals in France vs. China, risk factors for acquiring human alveolar echinococcosis (AE) at the village or town level were similar for both Regions. Risk was strongly associated with the presence of key microtine rodent species that were prone to seasonal and/or plural annual population fluctuations within agriculturally fragmented upland landscapes (Giraudoux et al. 1996, 1997, 1998, and this supplement). In these studies some of the key data sets comprised accurate village-based human AE prevalence rates (by mass...
ultrasound screening and serological confirmation) or incidence rates (using hospital data), identification of assemblages of rodent species and their population density estimations (use of quantitative trapping rates, activity indices transects), as well as the quantification of landscape cover using defined classes especially the relative proportions of plough and grassland (use of land-use maps and remote-sensed data). Landscapes containing appropriate small mammal habitats can be subject to quantitative-spatial analysis using remote-sensed image data via satellite images, and a predictive model of human AE risk has been developed based on spatial parameters (Danson et al. 2002, and this supplement). Population densities of the main definitive host of *E. multilocularis*, the red fox (*Vulpes vulpes*), and fox prevalence rates were of secondary importance in the Jura (France) AE endemic zone, or unobtainable in south Gansu Province (China), and thus were not vital parameters in developing a landscape-human AE risk models for those regions (Giraudoux et al. 1996; Danson et al. 2002). Of course, fox populations and their spatial distribution is nevertheless fundamental to transmission of *E. multilocularis* in sylvatic cycles and even within urban settings (Hofer et al. 2000; Berke, 2001).

Evidence from south Gansu as well as the eastern Tibetan Plateau (NW Sichuan) in China indicates risk of human AE associated with a history of dog ownership and contact (Craig et al. 2000; Wang et al. 2001). A truly independent cycle of *E. multilocularis* between dogs and small mammals has not yet been unequivocally demonstrated anywhere, though a degree of peri-domestic transmission has been described in Inuit (Eskimo) communities of St Lawrence Island (Alaska), in Han Chinese agricultural communities in south Gansu, and in Tibetan pastoral communities in NW Sichuan (Rausch et al. 1990; Craig et al. 1992; Wang et al. 2001).

In contrast to the transmission patterns for *E. multilocularis*, those that involve *E. granulosus*, at least from a zoonotic viewpoint, are predominantly confined to domestic cycles (or synanthropic cycles) involving dogs and a range of livestock species (e.g. sheep, goats, cattle, camels, horses and pigs) (Macpherson & Craig, 2000). Even so *E. granulosus* in some regions of the northern hemisphere is still actively transmitted wholly within sylvatic cycles as indeed must have occurred prior to domestication of dogs and livestock some 3000–15 000 years ago. Sylvatic transmission of *E. granulosus* readily occurs across the holarctic zone between wolves (*Canis lupus*) and large cervids like the moose (*Alces alces*) and caribou/reindeer (*Rangifer tarandus*) (Messier, Rau & McNeill, 1989, and Rausch, this supplement). The wolf–cervid cycle is also considered to be ancestral to the so-called European domestic form of the parasite (Rausch, 1997). In sub-Saharan Africa transmission of *E. granulosus* between a large felid, the lion (*Panthera leo*), and a range of wild ungulates has also been described (Macpherson & Wachira, 1997, and Jenkins & Macpherson, this supplement). Transmission of *E. granulosus* in wildlife hosts in Australia appears to involve the dingo (*Canis familiaris var dingo*) and a number of macropod species (wallabies and kangaroos). Molecular evidence however points to this being a secondary adaptation of the European form (i.e. sheep G1 genotype) of *E. granulosus* to these wild marsupial hosts after the parasite’s introduction with 18th and 19th Century colonists (Gemmill, 1990; Bowles, Blair & McManus, 1992). The impact of *Echinococcus* infection on wildlife is difficult to assess but in general is probably of little or no importance in terms of pathology especially in the definitive carnivore hosts. In small mammals, although *E. multilocularis* metacestodes grow rapidly, the average lifespan of a typical microtine rodent is less than 1 year. Even so the behaviour of rodents with liver lesions could be altered and thus more prone to predation. This is also the likely case for aged moose (>8–10 years) that are infected with multiple pulmonary hydatid cysts caused by the northern biotype of *E. granulosus*, and they are subsequently more likely to succumb to predation by wolves during a hunt (Messier et al. 1989).

TRANSMISSION DYNAMICS AND CONTROL

At any one time, populations of *Echinococcus* spp. exist as either adult tapeworms in definitive hosts, post-oncosphere or metacestode stages in intermediate-hosts, or as eggs free in the environment. The dynamics of transmission between the environment, intermediate hosts and definitive hosts has been investigated in the classical *in vivo* experimental and field studies of Gemmell and colleagues in New Zealand (reviewed by Gemmell, 1990 and Gemmell & Roberts, 1998). Those studies largely focused on *E. granulosus* and also the dog taeniids *Taenia hydatigena* and *T. ovis* (Gemmell et al. 1986). Relatively little modelling has been achieved to date for *E. multilocularis* transmission, because of the inherent problems relating to quantifying all the parameters in the sylvatic cycle (Roberts & Aubert, 1994). Rather, the behavioural ecology of small mammal and fox hosts is of paramount importance in any study of transmission of *E. multilocularis* in its natural cycles (Giraudoux et al., this supplement). Certain epidemiological features, however, are common to both *E. granulosus* and *E. multilocularis*, that may affect transmission, including the effect of environmental temperature and humidity on egg survival, egg dispersal mechanisms, the basic reproductive rate and biotic potential of the parasite, and the degree of host immunity. Also the role of anthropogenic factors (e.g. husbandry practices) are important in the domestic cycles of *E. granulosus*. 
Human behaviour is also important in relation to zoonotic risks, because of association with susceptible dog and fox (and possibly cat) populations for *E. granulosus* or *E. multilocularis*. Important data that have been collected or experimentally produced that are required to develop transmission models include: the number of eggs produced per worm per day, longevity of eggs, rate and length of immunity acquisition in intermediate hosts, age intensity and prevalence rates for both intermediate and definitive hosts and the degree of aggregation of parasites in hosts (see Torgerson & Heath, this supplement).

The shape of age-intensity curves for ovine CE has been used to define the endemic steady state in a given region, and therefore also helped to ascertain the intervention force required to interrupt transmission (Gemmell, 1990). More recently, age intensity/prevalence data for canine echinococcosis due to *E. granulosus*, have highlighted both the role of immunity in the definitive host in regulating parasite burden, and have also contributed to the modelling of dog-dosing strategies for hydatid control programmes (Torgerson, 2002). The most successful control programmes against cystic echinococcosis have targeted the dog for arecoline or praziquantel treatment over regular intervals (6–8 weeks) using operatives employed by a Ministry of Agriculture (Gemmell & Roberts, 1998). This so-called ‘vertical approach’ contrasts with that of a ‘horizontal approach’ in which general husbandry improvements and health education aspects are upgraded and improved over a much longer period (>25 years). The dog-dosing vertical approach to hydatid control can have a significant impact on ovine CE rates within 3–5 years, and within 10 years may reduce cyst rates in animals and humans below 5% and 1 per 100 000 respectively (Gemmell & Roberts, 1998; WHO/OIE, 2001). Computer modelling will further assist in establishing cost-benefit aspects, the optimal dog-dosing intervals and the proportion of dog population to be treated (Torgerson & Heath, this supplement).

Because *E. multilocularis* is transmitted mainly between sylvatic hosts it is not considered an eradicable disease in the same way as *E. granulosus* (which was eliminated from Iceland by the mid twentieth century, and is close to the point of elimination in New Zealand and Tasmania). However, when domestic dogs are involved as a zoonotic risk for human AE, praziquantel dosing can have a clear impact (Rausch et al. 1990). Use of praziquantel baits in relatively small endemic areas of Germany was able to reduce fox prevalence rates of *E. multilocularis* from 32% to 4% within a 14 month period (Schelling et al. 1997; also Ito et al., this supplement). For *E. multilocularis* specific legislation to treat dogs prior to leaving an endemic region, has been enforced for the UK in the ‘Passport for Pets Scheme’ (MAFF Advisory Group on Quarantine, 1998) and a similar scheme is being considered in northern Japan to prevent extension of the AE focus from Hokkaido to the main island (Takahashi, personal communication).

Potential for developing vaccines against *Echinococcus* spp. has been realised with the development of the recombinant sub-unit EG95 vaccine for *E. granulosus* in sheep (Lightowlers & Gauci, 2001), and also experimentally for *E. multilocularis* in rodents e.g. the 14-3-1 vaccine (Siles-Lucas et al. 2003). The EG95 vaccine which has a 95% efficacy is close to commercial development and should help significantly to reduce the length of time required to deliver the costly ‘attack phase’ in CE control programmes. However, the impact of any vaccination programme against *E. granulosus* can only be truly evaluated if appropriate tools are utilised for epidemiological studies relating to sheep, dog and human infections.

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