Prevention and control of cystic echinococcosis

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Human cystic echinococcosis (hydatid disease) continues to be a substantial cause of morbidity and mortality in many parts of the world. Elimination is difficult to obtain and it is estimated that, using current control options, achieving such a goal will take around 20 years of sustained efforts. Since the introduction of current (and past) hydatid control campaigns, there have been clear technological improvements made in the diagnosis and treatment of human and animal cystic echinococcosis, the diagnosis of canine echinococcosis, and the genetic characterisation of strains and vaccination against *Echinococcus granulosus* in animals. Incorporation of these new measures could increase the efficiency of hydatid control programmes, potentially reducing the time required to achieve effective prevention of disease transmission to as little as 5–10 years.

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Introduction

Cystic and alveolar echinococcosis (hydatid disease) are a cause of substantial morbidity and mortality in most of the world, including parts of Europe, North America, and South America (figure 1). Recent technological advances could facilitate the implementation of improved control programmes and reduce the time period required for elimination. In this Review, we summarise and update information presented and discussed at a workshop on the control of cystic hydatid disease held in May, 2005, in Lima, Peru, under the sponsorship of the Office of Rare Diseases, National Institutes of Health (Bethesda, MD, USA) and the Universidad Peruana Cayetano Heredia (Lima, Peru). Reflecting the presented material, this paper mainly focuses on *Echinococcus granulosus* infections, with some discussion of specific areas relating to *Echinococcus multilocularis* and alveolar hydatid disease.

The parasites

Hydatidosis is a chronic cyst-forming parasitic helminthic disease of human beings as well as domestic and wild ungulates. It is caused by infection with the larval (metacestode) stages of dog tapeworms belonging to the genus *Echinococcus* (family Taeniidae) and is also referred to as echinococcosis. Three broad morphological forms of echinococcosis are recognised clinically: cystic echinococcosis caused by *E granulosus*, alveolar echinococcosis caused by *E multilocularis*, and polycystic echinococcosis caused by *Echinococcus vogeli* or *Echinococcus oligarthrus* (figure 2).[1–5] Human cystic echinococcosis is the most common presentation and probably accounts for more than 95% of the estimated 2–3 million global cases,[6,7] with human alveolar echinococcosis causing around 0·3–0·5 million cases (all in the northern hemisphere);[8] fewer than 150 cases of polycystic echinococcosis have been described, all in Central and South America.[2,3,5,9,10] The global burden (disability-adjusted life years [DALYS]) for human cystic echinococcosis was recently estimated to be more than that for onchocerciasis and almost the same as that for African trypanosomiasis.[7] Until 2005, only four *Echinococcus* species were recognised, but a fifth species, *Echinococcus shiquicus*, has now been described in small mammals from the Tibetan Plateau, although its zoonotic potential is unknown.[11]

Echinococcus spp diagnosis, detection, and pathology

Diagnosis of human echinococcosis remains highly dependent on imaging techniques (eg, computed tomography scan, magnetic resonance imaging, ultrasound, and radiography) to detect the space-occupying cysts or lesions caused by the developing, dying, or dead metacestode(s) of *Echinococcus* spp.[1,3,9,12–15] (figure 2 and figure 3). The WHO expert group on echinococcosis has produced an international classification of ultrasound images of cystic echinococcosis which, in principle, should be used whenever ultrasound diagnosis is done (figure 3).[14] Additionally, laboratory-based diagnosis can provide a useful confirmation of clinical infection and can also be applied to aid epidemiological surveys of cystic and alveolar echinococcosis in endemic regions. Such methods mainly depend on detection of specific serum antibodies

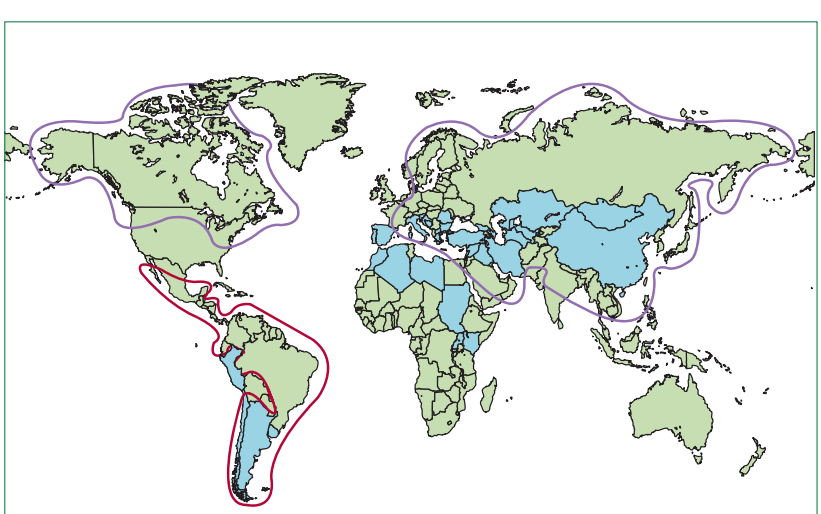

Figure 1: **Geographical distribution of cystic, alveolar, and polycystic echinococcosis (hydatid disease)**
Blue=countries endemic for cystic hydatid disease. Purple line=endemic areas for alveolar echinococcosis. Red line=areas where polycystic echinococcosis has been reported.

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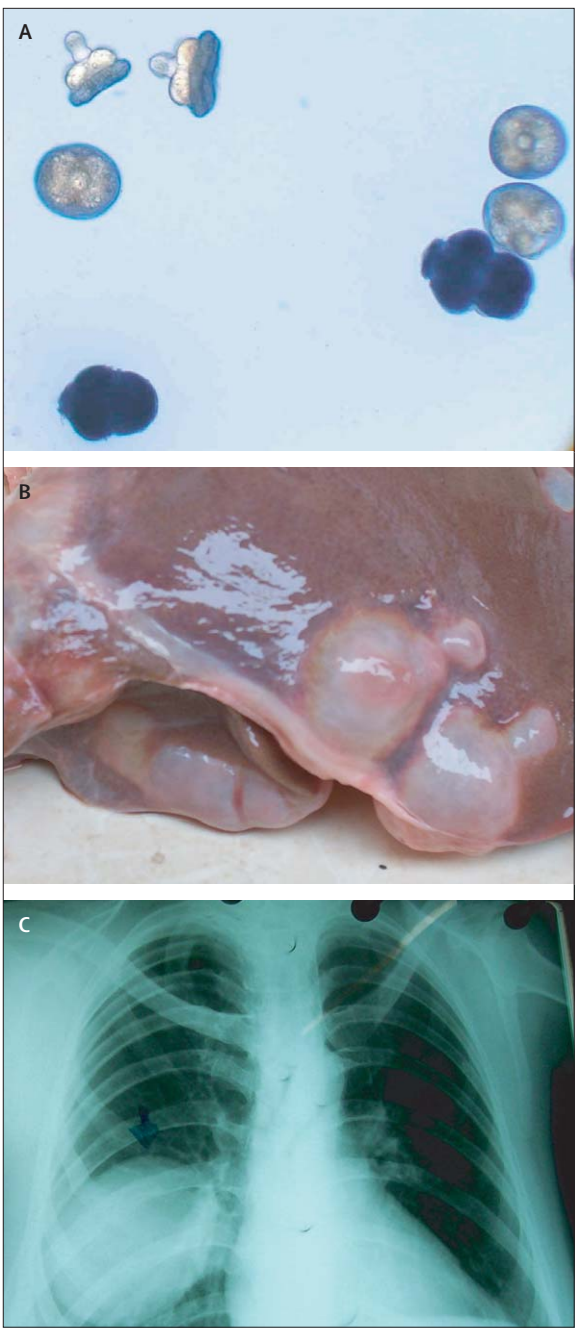

***Figure 2*: Cystic echinococcosis: the parasite**
(A) Light microscopy showing protoscolices from *E granulosus*. (B) Macroscopic appearance of cysts in liver tissue. (C) Lung cyst on chest radiograph.

in suspected cases or in people enrolled in mass screening programmes.[1,16,17] Serological techniques are never 100% sensitive and specific, and some cystic echinococcosis patients might not produce a marked antibody response.[17–19] Specificity problems for cystic echinococcosis serology may become important because inter-taeniid species cross-reactions with alveolar echinococcosis or cysticercosis patients are not infrequent.[5,16,20–23]

Current gold standard serology for human cystic echinococcosis is based on detection of IgG antibodies to hydatid cyst fluid-derived native or recombinant antigen B subunits, either in ELISA or in immunoblot formats.[9,16,19,20,24–31] A novel 32 kDa calcium-binding *E granulosus* recombinant antigen, EpC1, has recently been cloned from a protoscolex cDNA library, which detected antibodies in 92·2% (107 out of 116) of pre-surgical cystic echinococcosis cases compared with 84·5% (98 of 116) cases detected by native antigen B. Furthermore, only 4·5% (4 out of 89) of alveolar echinococcosis cases and 9·3% (16 out of 172) of cysticercosis cases cross-reacted with EpC1, compared with more than 14% with antigen B.[32,33] The EpC1 antigen appears to be located in the germinal layer of the hydatid cyst as well as the early protoscolex.

Other studies on the hydatid cyst of *E granulosus* indicate that high levels of host IgG heavy chain occur in the germinal layer of non-fertile cysts and suggest that the host immune response might be a cause of destruction of protoscolex production by inducing apoptosis of the germinal membrane, possibly opening up an avenue for vaccination against established cysts.[34–38]

Mitochondrial DNA-based detection of *Echinococcus* species has been shown to be an excellent tool for analysis of strain/genotypic variation in the genus, determining phylogenetic relationships, and informing taxonomic species questions.[4,39] DNA amplification has also shown great potential for diagnosis of canine and vulpine echinococcoses by development of stool-based PCR (copro-PCR) tests for both species-specific and strain-specific pre-patent and patent detection of adult *E granulosus* infections.[3,9,40–45]

Human disease in cystic echinococcosis relates to the development and growth of fluid-filled cysts mainly in the liver and the lungs, although it can affect the abdominal cavity, heart, bone, muscle, nervous system, or other locations.[1,5] Growth of cystic larvae is slow and well tolerated by the host, occasionally leading to large parasitic masses.[1,46] A proportion of cases detected by surveys in field conditions has been reported to spontaneously regress.[46] By contrast, alveolar echinococcosis does not have well-defined external limits and infiltrates the surrounding parenchyma.[1–3,9] Polycystic echinococcosis has characteristics intermediate between cystic echinococcosis and alveolar echinococcosis.

Treatment for human hydatidosis is difficult because most cysts or cystic lesions develop in the liver, lungs, or other organs.[1,2] Surgery still remains the main treatment, but medicosurgical approaches are becoming more widespread, along with percutaneous drainage for hepatic cystic echinococcosis. Albendazole, mebendazole, and praziquantel drugs have cure rates (from chemotherapy alone) of approximately 30% and another 10–20% of patients will demonstrate substantial regression of cyst size and symptom alleviation.[1–3,5,9,47–66]

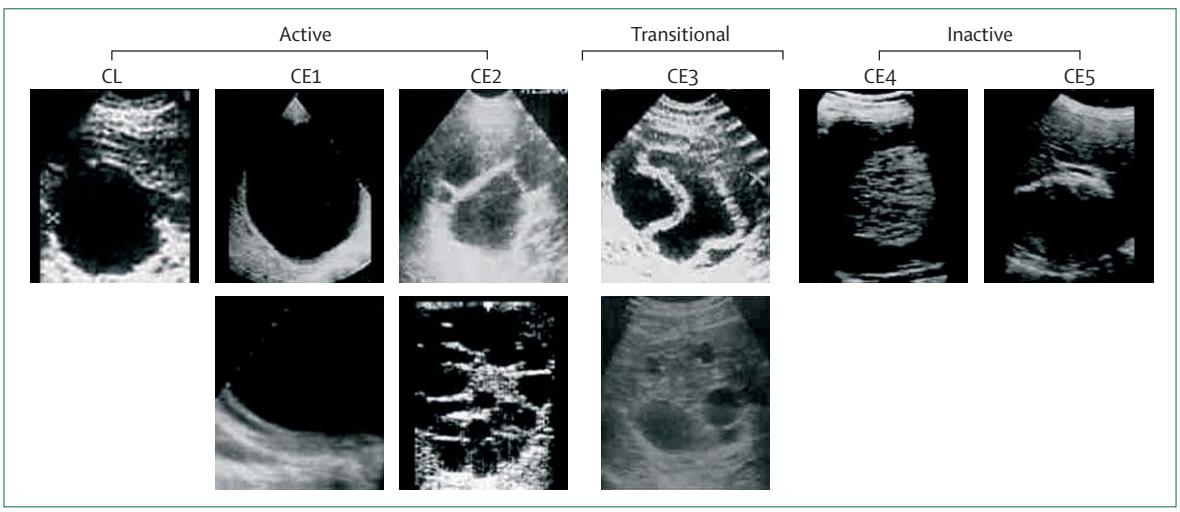

***Figure 3*: Ultrasound findings in cystic echinococcosis (WHO classification)**
Cysts are classified as active, transitional, or inactive according to their imaging characteristics. Reproduced from reference 14, with permission from Elsevier.

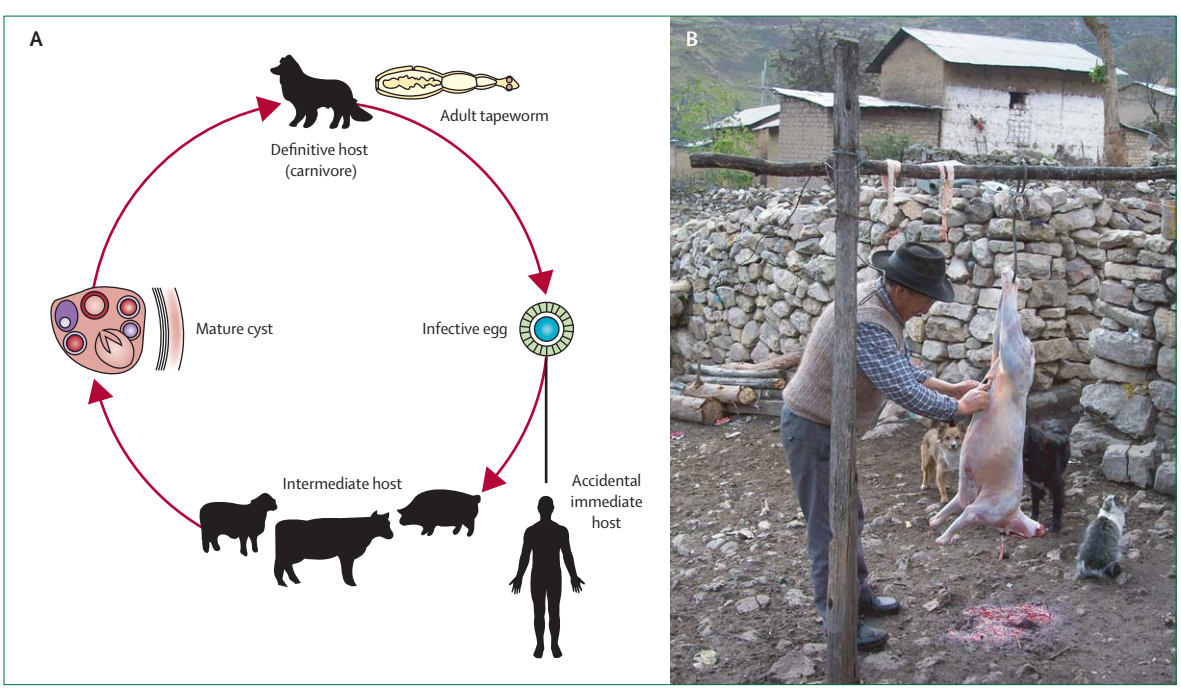

***Figure 4*: Transmission of cystic hydatid disease**
(A) Life cycle of *E granulosus* (adapted from reference 69, with permission from the United States Animal Health Association). (B) Sheep slaughtering close to free-ranging dogs.

Adverse reactions including neutropenia, liver toxicity, and hair loss—reversible on cessation of treatment—have been reported. Outlook for individual patients is rarely predictable and long-term imaging follow-up is required.[48,50,67] In the absence of active mass screening (using abdominal ultrasound scans with serological confirmation), the average time from infection to diagnosis/treatment is around 5–10 years, often resulting in large debilitating cyst or lesion growth over a variable asymptomatic period. Alveolar echinococcosis is associated with progressive disease and poor response to therapy.[1,2,5,9] There is only scarce non-controlled evidence of the usefulness of antiparasitic drugs in polycystic echinococcosis.

Epidemiology and control

Epidemiologically, human cystic echinococcosis occurs predominantly in poor pastoral communities that raise sheep and other livestock, and keep dogs for guarding and/or herding animals. *E granulosus* is mainly transmitted in a cycle between dog definitive hosts that harbour the small intestinal tapeworm, and livestock

(especially sheep) after the latter ingest the microscopic eggs while grazing pastures that are contaminated with dog faeces.[1] Dogs usually acquire infection from hydatid-carrying livestock as a result of their deliberate feeding of infested offal (liver and lungs) by owners who practise home-slaughter.[1,68] Thus, human behaviour helps to perpetuate the domestic cycle of *E granulosus* (figure 4). Human beings become exposed to the eggs of the tapeworm after close contact with an infected dog or its contaminated environment.[1,3,9,68,70,71] In endemic regions, human incidence rates can reach more than 50 per 100 000 person-years and prevalences as high as 5–10% may occur, as in parts of Peru, Argentina, east Africa, central Asia, and China.[46,68,72,73] Risk factors for human cystic echinococcosis include a pastoral occupation, a history of dog ownership, poor education, age, sex, and drinking water source.[1]

Since the 1860s when the Icelandic government embarked on a health education programme to eradicate hydatidosis, effective approaches for control of cystic echinococcosis and the transmission of the causative organism, *E granulosus*, have been understood.[3,5,9,74] By 2002 there had been seven successful hydatid control programmes, five on island states/nations including two (New Zealand and Tasmania) where the region/country declared themselves to have eliminated the parasite following more than 30 years of dog-targeted control. Supervised regular arecoline purge testing then quarantine, or anthelmintic dosing (using praziquantel) of dogs coupled with effective management/logistics of the control authority and education have been key factors in the reduction in human and ovine cystic echinococcosis rates in the successful programmes. However, at least five other national or regional control programmes (mostly continental rather than islands) had only a limited effect or were unsuccessful.[1,3,9]

New approaches to control and prevention of hydatidosis, including an effective livestock vaccine, potential dog vaccines against the tapeworm stage of *E granulosus*, tailored educational programmes, development of better diagnostics for definitive hosts and human beings (including dog coproantigen detection), more effective antiparasitic treatments (ie, oxfendazole), and the use of mathematical models to simulate best possible cost-effective interventions, will be expected to shorten the time of the attack phase and improve surveillance in the consolidation phase of hydatid control programmes.[1,75–83] Some of these measures have already been proven effective[1,3,38,42–45,75,76,84–102] and initial data have been produced for dog vaccination.[38,75,103–105] Research on mathematical models of cystic echinococcosis control indicate that vaccination of sheep would be an effective control strategy, provided that over 90% vaccine coverage of the sheep population was achieved. However, the most effective intervention that was revealed by the modelling was a combination of vaccinating sheep and dog anthelmintic treatment. If about 75% vaccine coverage of the sheep population is achieved, anthelmintic treatment of dogs could be reduced to 6-month intervals while still achieving a high level of control of disease transmission, thereby greatly reducing the cost of a control programme and probably also increasing compliance from dog owners.[76] Important additional gains should be expected if the efficacy of the dog vaccine is confirmed and it is incorporated into control options.

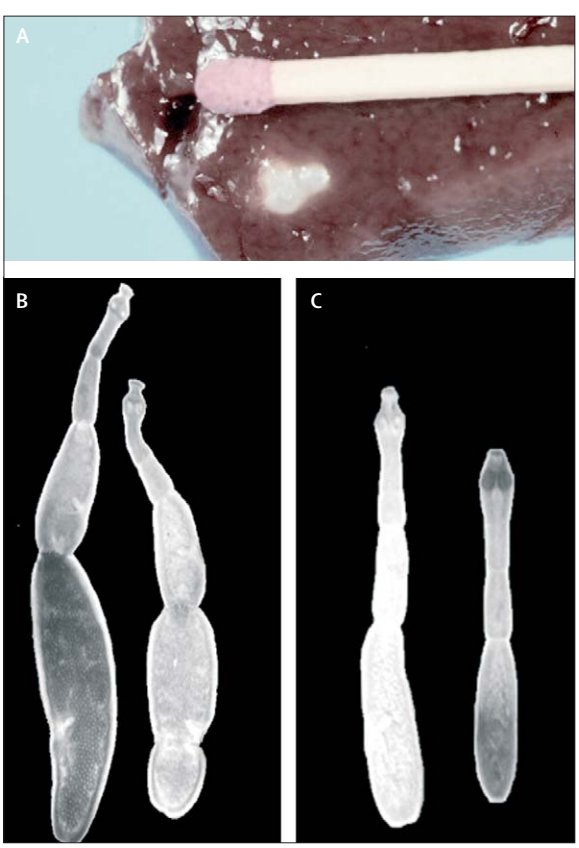

Figure 5: **Echinococcosis vaccine evaluation**
(A) Early cyst at 12 weeks after experimental infection with *E granulosus* protoscolices in non-vaccinated sheep. (B) *E granulosus* obtained from dogs vaccinated with an adjuvant-only control group compared with (C) *E granulosus* obtained from dogs vaccinated with soluble native proteins isolated from protoscolices. (B) and (C) reproduced from reference 105, with permission from the University of Chicago Press.

Relevance of species and genotype of *E granulosus*

E granulosus comprises a number of genetic variants and, to date, analyses of mitochondrial DNA sequences have identified ten distinct genetic types (genotypes G1–10). This categorisation follows closely the pattern of strain variation emerging based on biological characteristics. The extensive variation in nominal *E granulosus* may influence life-cycle patterns, host specificity, development rate, antigenicity, transmission dynamics, sensitivity to chemotherapeutic agents, and pathology. It might therefore have implications for the design and development of vaccines, diagnostic reagents, and drugs affecting control, although no concrete evidence of this effect has yet been demonstrated.

Dog coproantigen detection

The diagnosis of *E granulosus* in dogs using coproantigen-detection ELISA method has a number of advantages over the use of arecoline purgation as a diagnostic test. Coproantigen-detection ELISA has easier sample collection, is faster to do, and requires less personnel, all of which make it suitable for surveillance of large dog populations.[43] Unlike arecoline purgation (which requires taking dogs to purge sites and concentration of dogs in specific places), faecal samples for coproantigen testing can be collected in the field for some of the dogs, eliminating the need of transporting them to a specific location.[106] Coproantigen may be detectable early in the course of infection[107,108] both for *E granulosus* and *E multilocularis*.[44]

Vaccines against *E granulosus*

Control programmes against cystic echinococcosis have traditionally relied on anthelmintic dosing of dogs, improved slaughter hygiene and surveillance, and instigated health education relating to human–dog behaviour.[1,9,81,84,85] Echinococcus vaccines would ideally prevent oncosphere development to hydatid cysts in sheep, and thus stop the development of adult gravid tapeworms in dogs.[40,86–89] (figure 5).

A defined recombinant vaccine for ovine cystic echinococcosis (called EG95) was developed in 1996 by the groups of Marshall Lightowlers and David Heath in Australia and New Zealand. The native molecule is 24·5 kDa and cloned as a 16·5 cDNA fusion peptide of 155 aminoacids with a fibronectin-like motif under the control of seven closely related genes.[40,87,90–92] Linear synthetic peptides spanning the full EG95 sequence were antigenic in sheep but were not protective, indicating that the three-dimensional conformational structure of the molecule is functionally essential.[91,93–96] Field trials in Australia, New Zealand, Argentina, Italy, and China over the next 8–10 years demonstrated more than 95% protection for at least 12 months in sheep (with colostral transfer of immunity) following two injections in QuilA adjuvant[9,92,97–100] (table). Research into improved delivery is focused on the use of heterologous vectors (eg, *Corynebacterium* spp, salmonella, and adenovirus), and also delivery as a DNA vaccine, although the latter produced equivocal results. Perhaps the most attractive option for this kind of zoonoses vaccine in view of the low disease/pathology status of infected sheep is the incorporation of EG95 with an existing commercial livestock vaccine such as tetanus, leptospirosis, or sheep orf, or even as a dual vaccine in combination with a new *Taenia multiceps* vaccine for ovine gid-cysticercosis hepatitis.[97]

Although the EG95 vaccine against ovine hydatidosis is a reality that now requires innovative delivery strategies, no similarly effective vaccine exists against canine echinococcosis. This is not because of the lack of potential for application, because such a vaccine for dogs would be of enormous benefit in further reducing the effective period (currently more than 10 years) required to stop transmission of *E granulosus* to people and their livestock. Rather, the scarcity of vaccine candidates for immune protection against adult tapeworm infection reflects the lack of research reports on specific immune correlates, the lack of evidence until recently of natural immunity in dogs, and the difficulties and cost in maintaining experimental canids.[103,104] Serum antibody responses and specific gut lymphoid tissue activation have been observed in the past but correlation of these responses to systemic and mucosal immunity is lacking.[109] More recently, a study in experimentally infected dogs showed that although IgG responses were similar among infected dogs, IgE and IgA responses differed widely. IgA responses were not related to final parasite burden, but antiparasite IgE seemed inversely associated to parasite load.[110] Studies in experimentally infected dogs in Uruguay indicate that the cytokines interleukin 4 and interferon γ are differentially expressed in the peripheral circulation during infection. Furthermore, recombinant cytokines delivered in vivo by a salmonella vector can modify the outcome of parasite secondary infection in infected mice.[111] Studies undertaken in China on dogs vaccinated with recombinant proteins from mature adult worms of *E granulosus* resulted in substantial repression of worm growth and suppression of egg production compared with parasites from adjuvant control dogs.[105] These encouraging results auger well for future development of an effective dog vaccine against *E granulosus*.

	Number of viable cysts in individual sheep	Mean number of cysts	Protection
Trial 1: New Zealand			
Controls	85, 49, 39, 11, 0	36·8	100%
EG95-vaccinated	0, 0, 0, 0, 0	0·0	
Trial 2: Australia			
Controls	16, 9, 9, 2, 2, 2, 1, 1, 0	4·7	96%
EG95-vaccinated	1, 1, 0, 0, 0, 0, 0, 0, 0, 0	0·2	
Trial 3: Argentina			
Controls	64, 62, 51, 23, 11, 7, 4, 4, 3, 2	23·1	99%
EG95-vaccinated	1, 0, 0, 0, 0, 0, 0	0·1	

Detailed information on immunisation schemes and other methods can be found in references 97 and 98. Protection was calculated as the percentage reduction in the mean number of parasites found in vaccinated animals compared with the mean number found in controls.

Table: **Efficacy of sheep immunisation with EG95 vaccine**

Reasons for failure of *E granulosus* control programmes

Until now, only the five island-based hydatid control programmes (Iceland, New Zealand, Tasmania, Falkland Islands, and Cyprus) have been successful, mostly based on health education, control, or elimination of home slaughter of sheep. A decline in canine infection was followed by a drop in the prevalence of infection in sheep

***Panel*: Research needs and recommendations**

- Measure the real burden of cystic echinococcosis disease in South America similar to that recently characterised for some other regions
- Undertake a multicentre comparative standardised study of native, recombinant, and peptide antigens for diagnosis of human cystic echinococcosis
- Undertake longitudinal follow-up studies of human seropositivity in cystic echinococcosis endemic regions using defined recombinant antigens. What is the extent, variability, and reason for long-term serial antibody responses in ultrasound-negative people?
- Compare the efficacy, sensitivity, and specificity of new copro-PCR tests for canine echinococcosis, and establish strain-specific detection for *E granulosus* in dogs
- Research priorities for the EG95 vaccine for ovine echinococcosis include determining the three-dimensional conformational structure by computer modelling; optimising vaccine delivery with existing established vaccines—eg, sheep orf; and carrying out a demonstration programme for hydatid control using EG95 vaccine in sheep, praziquantel dosing of dogs, and health education in a South American region
- Investigate approaches for vaccination against established hydatid cysts both as a treatment option and for use in conjunction with oncosphere vaccines to reduce the time required to break the transmission cycle of the dog–sheep strain
- Research priorities for dog vaccines against *E granulosus* include characterising infection dynamics in natural dog populations; expressing adult excretory/secretory antigens in salmonella and vaccinia heterologous carriers; establishing optimum protocols for dog vaccination studies; prototype testing in different settings and endemic areas; developing improved delivery methods using appropriate and acceptable adjuvants; determining the longevity of protection; and determining whether there is an effect against *E multilocularis*
- Guidelines should be developed for consideration of definitions for control of cystic echinococcosis—ie, what levels of reduction in sheep, dog, and human infections constitute significant effects? Elimination of transmission of *E granulosus* in a controlled region should be defined

and young cattle and a decreasing annual incidence of human cases.[112] By contrast, only two of the continental programmes in Latin America (Region XII in Chile, and Rio Negro in Argentina) have been successful, and several others failed.[112,113] A programme in Uruguay had little effect on livestock or human cystic echinococcosis rates over the first 20 years of the campaign (1972–92).[114] Problems with this programme included too much reliance on rural owners to dose their own dogs, use of a purgative rather than a cestocidal drug, lack of local municipality staff and insufficient funds from the dog tax, baseline data for sheep (and dogs) was not collected to provide feedback to measure progress, and political upheaval.[112] Other hydatid control programmes have failed for different reasons: (1) the premature withdrawal of government funding (mid-Wales programme); (2) small and under-funded control authority (Turkana programme, Kenya), with virtually no educational, medical, or veterinary facilities, poor communication and road networks, and a dispersed population; (3) inadequate management of stray dogs (Sardinian programme); and (4) presence of political upheaval or security issues, or both (terrorism in Peru,[68] major social and political changes in the newly independent states of central Asia following the collapse of the former Soviet Union).[115]

Control of *E multilocularis*

By contrast with the predominant domestic animal transmission cycles that sustain *E granulosus* worldwide, the closely related species *E multilocularis* is transmitted only in the northern hemisphere and mainly within wildlife cycles. A number of fox species are highly susceptible to infection with the adult tapeworm, and a wide range of rodents (especially microtine voles) and small mammals can act as intermediate hosts. Human infection with the larval stage, alveolar echinococcosis, is consequently a rarer zoonosis than cystic echinococcosis. However, the greater pathogenicity, treatment difficulty, and higher mortality risk of alveolar echinococcosis has led to consideration of its control by intervention trials/programmes in endemic areas of Alaska (St Lawrence Island), Europe (southern Germany and northern Switzerland), northern Japan (Hokkaido), and southwest China.[116] In China, both human alveolar echinococcosis and cystic echinococcosis are coendemic in several western regions, and therefore combined control of both these *Echinococcus* species may be warranted.[117,118]

Control of the wildlife transmission cycles of *E multilocularis* would be very difficult in most regions, although targeting fox populations is an option (see below). In many alveolar echinococcosis endemic areas, however, it seems that the domestic dog probably has an important role in zoonotic risk.[119–122] There are three main options for control of *E multilocularis* and reducing or eliminating alveolar echinococcosis as a public-health problem: (1) eliminate the fox population, (2) treat the fox population with anthelmintic baits, and/or (3) treat the rural dog population with anthelmintic (praziquantel).

The first option, to eliminate the fox population, is almost always too drastic a measure, but could be highly effective to control a known outbreak/emergence, or in an area with a small fixed population as was the case on Reuben Island, Japan.[116] The second option, to apply praziquantel baits at regular intervals to treat fox

populations, has shown good efficacy and potential over small areas (100–3500 km²) for short durations (12–26 months) in Germany and Switzerland, where prevalence of *E multilocularis* in red foxes declined from more than 30% to less than 1–5%.[123–125] The third option, based on monthly supervised praziquantel dosing of dogs, was successful in substantially reducing the prevalence of *E multilocularis* in commensal vole populations in Alaska (53% to 1% over 10 years) and thus ultimately decreased the zoonotic risk via owned dogs.[126] Currently, a government-based monthly praziquantel dog dosing programme is underway in ten alveolar echinococcosis/cystic echinococcosis coendemic counties of Sichuan Province, China (Wang Qian, Sichuan Centers for Disease Control, Sichuan, personal communication). There is currently no vaccine for *E multilocularis* in canid hosts, and it is not known whether the prototype *E granulosus* dog vaccine described by Zhang and colleagues[105] would provide cross-protection.

Shortcomings of control interventions and additional measures to improve efficacy

Dog treatment campaigns face the problem of coverage, incomplete sensitivity to identify positive dogs, and incomplete treatment effectiveness. Associated costs and the need for equipment (eg, ELISA reader) are major limitations with the coproantigen ELISA test in endemic areas. The application of the test in areas of low endemicity can be hampered by a predictive positive value that would be expected to be low and where potential cross-reactions with other *Taenia* spp may occur.[127]

Although substantial advances in evaluation of sheep vaccines have been made, some issues remain to be improved. Combined vaccines that also protect against other sheep pathogens would enhance coverage and feasibility of sheep immunisation campaigns. More data on the longevity of protection are still required. In terms of dog vaccination, the initial promising data are preliminary and need to be repeated in different settings and endemic areas. There is also a need to improve the method of delivery to use an appropriate and acceptable adjuvant. Oral baiting is a highly appropriate approach and oral vaccination would certainly be a practical way to introduce the vaccine into carnivores. On the same line, a combined rabies/echinococcal oral vaccine would be a major advance for many endemic areas. It is, however, not known whether the existing *E granulosus* dog vaccine candidates would work orally. The absence of data on the longevity of protection raises another key issue when considering the dog lifespan. Wide-scale production would present additional potential logistic and feasibility problems. It also remains to be assessed whether *E multilocularis* homologous proteins to the *E granulosus* proteins used to protect against dog echinococcosis would provide protection in this species.

Search strategy and selection criteria

Most information for this Review comes from ongoing work from the authors or was identified through searches of their extensive files. Additional references were identified by searches of Medline between 1969 and 2006, and references from relevant articles. The search terms "hydatidosis", "hydatid disease", or "*Echinococcus*" were used. Papers published in English or Spanish were reviewed. The final reference list was generated based on relevance to the topics covered in the Review.

The panel summarises the research needs and recommendations for effective prevention of echinococcosis transmission.

Conclusion

Hydatid disease remains endemic in many regions around the world. Advances in knowledge and development/design of new control tools for hydatid disease including new diagnostics and antiparasite vaccines for the definitive and intermediate hosts provide an excellent prospect for improved control programmes. Incorporation of these new measures has the potential to increase the efficiency of current control programmes and could reduce the time required to achieve effective prevention of disease transmission from the previously estimated 20 years or more to as little as 5–10 years.

Conflicts of interest

We declare that we have no conflicts of interest.

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References

1 McManus DP, Zhang W, Li J, Bartley PB. Echinococcosis. *Lancet* 2003; **362:** 1295–304.

2 Ci-Peng J, McManus PD, Malcom J. Liver alveolar echinococcosis in China: clinical aspect with relative basic research. *World J Gastroenterol* 2005; **11:** 4611–17.

3 Raether W, Hänel H. Epidemiology, clinical manifestations and diagnosis of zoonotic cestode infections: an update. *Parasitol Res* 2003; **91:** 412–38.

4 Thompson RC, Lymbery AJ, Constantine CC. Variation in echinococcus: towards a taxonomic revision of the genus. *Adv Parasitol* 1995; **35:** 145–76.

5 Khuroo MS. Hydatid disease: current status and recent advances. *Ann Saudi Med* 2002; **22:** 56–64.

6 Craig PS, Rogan MT, Allan JC. Detection, screening and community epidemiology of taeniid cestode zoonoses: cystic echinococcosis, alveolar echinococcosis and neurocysticercosis. *Adv Parasitol* 1996; **38:** 169–250.

7 Budke CM. Global socioeconomic impact of cystic echinococcosis. *Emerg Infect Dis* 2006; **12:** 296–303.

8 Schantz PM, Gottstein B, Ammann R, Lanier A. Hydatid and the Arctic. *Parasitol Today* 1991; **7:** 35–36.

9 Eckert J, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol* 2004; **17:** 107–35.

10 Somocurcio JR, Sanchez EL, Naquira C, et al. First report of a human case of polycystic echinococcosis due to *Echinococcus vogeli* from neotropical area of Peru, South America. *Rev Inst Med Trop Sao Paulo* 2004; **46:** 41–42.

11 Xiao N, Qiu J, Nakao M, et al. *Echinococcus shiquicus* n. sp., a taeniid cestode from Tibetan fox and plateau pika in China. *Int J Parasitol* 2005; **35:** 693–701.

12 Polat P, Kantarci M, Alper F, Suma S, Koruyucu MB, Okur A. Hydatid disease from head to toe. *Radiographics* 2003; **23:** 475–94.

13 Kjossev KT, Losanoff JE. Classification of hydatid liver cysts. *J Gastroenterol Hepatol* 2005; **20:** 352–59.

14 WHO Informal Working Group. International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. *Acta Trop* 2003; **85:** 253–61.

15 Macpherson CN, Milner R. Performance characteristics and quality control of community based ultrasound surveys for cystic and alveolar echinococcosis. *Acta Trop* 2003; **85:** 203–09.

16 Wen H, Craig P. Immunoglobulin G subclass responses in human cystic and alveolar echinococcosis. *Am J Trop Med Hyg* 1994; **51:** 741–48.

17 Dottorini BS, Sparvoli M, Bellucci C, Magnini M. *Echinococcus granulosus* diagnosis of hydatid disease in man. *Ann Trop Med Parasitol* 1985; **79:** 43–49.

18 Force L, Torres JM, Carrillo A, Busca J. Evaluation of eight serological tests in the diagnosis of human echinococcosis and follow-up. *Clin Infect Dis* 1992; **15:** 473–80.

19 Lorenzo C, Ferreira HB, Monteiro KM, et al. Comparative analysis of the diagnostic performance of six major *Echinococcus granulosus* antigens assessed in a double-blind, randomized multicenter study. *J Clin Microbiol* 2005; **43:** 2764–70.

20 Ito A, Ma L, Schantz PM, et al. Differential serodiagnosis for cystic and alveolar echinococcosis using fractions of *Echinococcus granulosus* cyst fluid (antigen B) and *E multilocularis* protoscolex (EM18). *Am J Trop Med Hyg* 1999; **60:** 188–92.

21 Siracusano A, Ioppolo S, Notargiacomo S, et al. Detection of antibodies against *Echinococcus granulosus* major antigens and their subunits by immunoblotting. *Trans R Soc Trop Med Hyg* 1991; **85:** 239–43.

22 Maddison SE, Slemenda SB, Schantz PM, Fried JA, Wilson M, Tsang VC. A specific diagnostic antigen of *Echinococcus granulosus* with an apparent molecular weight of 8 kDa. *Am J Trop Med Hyg* 1989; **40:** 377–83.

23 Shepherd JC, McManus DP. Specific and cross-reactive antigens of *Echinococcus granulosus* hydatid cyst fluid. *Mol Biochem Parasitol* 1987; **25:** 143–54.

24 Rigano R, Profumo E, Bruschi F, et al. Modulation of human immune response by *Echinococcus granulosus* antigen B and its possible role in evading host defenses. *Infect Immun* 2001; **69:** 288–96.

25 Verastegui M, Moro P, Guevara A, Rodriguez T, Miranda E, Gilman RH. Enzyme-linked immunoelectrotransfer blot test for diagnosis of human hydatid disease. *J Clin Microbiol* 1992; **30:** 1557–61.

26 Lightowlers MW. Immunology and molecular biology of echinococcus infections. *Int J Parasitol* 1990; **20:** 471–78.

27 Poretti D, Felleisen E, Grimm F, et al. Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies. *Am J Trop Med Hyg* 1999; **60:** 193–98.

28 Lawn SD, Bligh J, Craig PS, Chiodini PL. Human cystic echinococcosis: evaluation of post-treatment serologic follow-up by IgG subclass antibody detection. *Am J Trop Med Hyg* 2004; **70:** 329–35.

29 Leggatt GR, Yang W, McManus DP. Serological evaluation of the 12 kDa subunit of antigen B in *Echinococcus granulosus* cyst fluid by immunoblot analysis. *Trans R Soc Trop Med Hyg* 1992; **86:** 189–92.

30 Virginio VG, Hernandez A, Rott MB, et al. A set of recombinant antigens from *Echinococcus granulosus* with potential for use in the immunodiagnosis of human cystic hydatid disease. *Clin Exp Immunol* 2003; **132:** 309–15.

31 Wattal C, Malla N, Khan IA, Agarwal SC. Comparative evaluation of enzyme-linked immunosorbent assay for the diagnosis of pulmonary echinococcosis. *J Clin Microbiol* 1986; **24:** 41–46.

32 Li J, Zhang WB, McManus DP. Recombinant antigens for immunodiagnosis of cystic echinococcosis. *Biol Proced Online* 2004; **6:** 67–77.

33 Li J, Zhang WB, Wilson M, Ito A, McManus DP. A novel recombinant antigen for immunodiagnosis of human cystic echinococcosis. *J Infect Dis* 2003; **188:** 1951–60.

34 Paredes R. *Echinococcus granulosus:* association between the bovine humoral immune response, apoptosis and infertility in hydatid cysts. PhD thesis, Universidad de Chile, 2005 (in Spanish).

35 Blanton RE, Gideon BA, Kijobe J, King C. Antibody responses to in vitro translation products following albendazole therapy for *Echinococcus granulosus. Antimicrob Agents Chemother* 1991; **35:** 1674–76.

36 Lawn SD, Bligh J, Craig PS, Chiodini PL. Human cystic echinococcosis: evaluation of post-treatment serologic follow-up by IgG subclass antibody detection. *Am J Trop Med Hyg* 2004; **70:** 329–35.

37 Rigano R, Ioppolo S, Ortona E, et al. Long-term serological evaluation of patients with cystic echinococcosis treated with benzimidazole carbamates. *Clin Exp Immunol* 2002; **129:** 485–92.

38 Zhang W, McManus DP. Recent advances in the immunology and diagnosis of echinococcosis. *FEMS Immunol Med Microbiol* 2006; **47:** 24–41.

39 Thompson RC, McManus DP. Towards a taxonomic revision of the genus Echinococcus. *Trends Parasitol* 2002; **18:** 452–57.

40 Zhang W, Li J, McManus DP. Concepts in immunology and diagnosis of hydatid disease. *Clin Microbiol* 2003; **16:** 18–36.

41 Reiterova K, Miterpakova M, Turcekova L, Antolova D, Dubinsky P. Field evaluation of an intravital diagnostic test of *Echinococcus multilocularis* infection in red foxes. *Vet Parasitol* 2005; **128:** 65–71.

42 Stefanic S, Shaikenov BS, Deplazes P, Dinkel A, Torgerson PR, Mathis A. Polymerase chain reaction for detection of patent infections of *Echinococcus granulosus* ("sheep strain") in naturally infected dogs. *Parasitol Res* 2004; **92:** 347–51.

43 Abbasi I, Branzburg A, Campos-Ponce M, et al. Copro-diagnosis of *Echinococcus granulosus* infection in dogs by amplification of a newly identified repeated DNA sequence. *Am J Trop Med Hyg* 2003; **69:** 324–30.

44 Deplazes P, Dinkel A, Mathis A. Molecular tools for studies on the transmission biology of *Echinococcus multilocularis. Parasitology* 2003; **127:** S53–61.

45 Naidich A, McManus DP, Canova SG, et al. Patent and pre-patent detection of *Echinococcus granulosus* genotypes in the definitive host. *Mol Cell Probes* 2006; **20:** 5–10.

46 Moro PL, Gilman RH, Verastegui M, Bern C, Silva B, Bonilla JJ. Human hydatidosis in the central Andes of Peru: evolution of the disease over 3 years. *Clin Infect Dis* 1999; **29:** 807–12.

47 Davis A, Dixon H, Pawlowski ZS. Multicentre clinical trials of benzimidazole-carbamates in human cystic echinococcosis (phase 2). *Bull World Health Organ* 1989; **67:** 503–08.

48 Horton J. Albendazole for the treatment of echinococcosis. *Fundam Clin Pharmacol* 2003; **17:** 205–12.

49 Franchi C, Di Vico B, Teggi A. Long-term evaluation of patients with hydatidosis treated with benzimidazole carbamates. *Clin Infect Dis* 1999; **29:** 304–09.

50 Kern P. *Echinococcus granulosus* infection: clinical presentation, medical treatment and outcome. *Langenbecks Arch Surg* 2003; **388:** 413–20.

51 Dziri C, Haouet K, Fingerhut A. Treatment of hydatid cyst of the liver: where is the evidence? *World J Surg* 2004; **28:** 731–36.

52 Saimot AG. Medical treatment of liver hydatidosis. *World J Surg* 2001; **25:** 15–20.

53 Khuroo MS, Wani NA, Javid G, et al. Percutaneous drainage compared with surgery for hepatic hydatid cysts. *N Engl J Med* 1997; **337:** 881–87.

54 el-Mufti M, Kamag A, Ibrahim H, et al. Albendazole therapy of hydatid disease: 2-year follow-up of 40 cases. *Ann Trop Med Parasitol* 1993; **87:** 241–46.

55 Gil-Grande LA, Rodriguez-Caabeiro F, Prieto JG, et al. Randomised controlled trial of efficacy of albendazole in intra-abdominal hydatid disease. *Lancet* 1993; **342:** 1269–72.

56 Nahmias J, Goldsmith R, Soibelman M, el-On J. Three- to 7-year follow-up after albendazole treatment of 68 patients with cystic echinococcosis (hydatid disease). *Ann Trop Med Parasitol* 1994; **88:** 295–304.

57 Teggi A, Lastilla MG, De Rosa F. Therapy of human hydatid disease with mebendazole and albendazole. *Antimicrob Agents Chemother* 1993; **37:** 1679–84.

58 Todorov T, Vutova K, Mechkov G, Petkov D, Nedelkov G, Tonchev Z. Evaluation of response to chemotherapy of human cystic echinococcosis. *Br J Radiol* 1990; **63:** 523–31.

59 Horton RJ. Chemotherapy of echinococcus infection in man with albendazole. *Trans R Soc Trop Med Hyg* 1989; **83:** 97–102.

60 Wen H, Zou PF, Yang WG, et al. Albendazole chemotherapy for human cystic and alveolar echinococcosis in north-western China. *Trans R Soc Trop Med Hyg* 1994; **88:** 340–43.

61 Horton J. Albendazole: a broad spectrum anthelminthic for treatment of individuals and populations. *Curr Opin Infect Dis* 2002; **15:** 599–608.

62 El-On J. Benzimidazole treatment of cystic echinococcosis. *Acta Trop* 2003; **85:** 243–52.

63 Polat C, Dervisoglu A, Hokelek M, et al. Dual treatment of albendazole in hepatic hydatidosis: new therapeutic modality in 52 cases. *J Gastroenterol Hepatol* 2005; **20:** 421–25.

64 Ayles HM, Corbett EL, Taylor I, et al. A combined medical and surgical approach to hydatid disease: 12 years' experience at the Hospital for Tropical Diseases, London. *Ann R Coll Surg Engl* 2002; **84:** 100–05.

65 Vildosola H, Sanchez L, Espinoza R. Albendazol en el tratamiento de la hidatidosis hepatica e intrabdominal. *Rev Gastroenterol Peru* 1989; **9:** 17–23 (in Spanish).

66 Davis A, Pawlowski ZS, Dixon H. Multicentre clinical trials of benzimidazolecarbamates in human echinococcosis. *Bull World Health Organ* 1986; **64:** 383–88.

67 Cobo F, Yarnoz C, Sesma B, et al. Albendazole plus praziquantel versus albendazole alone as a pre-operative treatment in intra-abdominal hydatisosis caused by *Echinococcus granulosus*. *Trop Med Int Health* 1998; **3:** 462–66.

68 Moro PL, McDonald J, Gilman RH, et al. Epidemiology of *Echinococcus granulosus* infection in the central Peruvian Andes. *Bull World Health Organ* 1997; **75:** 553–61.

69 Andersen FL, Everett JR, Barbour AG, Schoenfeld FJ. Current studies on hydatid disease in Utah. *Proc Annu Meet U S Anim Health Assoc* 1994; 78: 37–84.

70 Campos-Bueno A, Lopez-Abente G, Andres-Cercadillo AM. Risk factors for *Echinococcus granulosus* infection: a case-control study. *Am J Trop Med Hyg* 2000; **62:** 329–34.

71 Seimenis A. Overview of the epidemiological situation on echinococcosis in the Mediterranean region. *Acta Trop* 2003; **85:** 191–95.

72 Moro PL, Garcia HH, Gonzales AE, Bonilla JJ, Verastegui M, Gilman RH. Screening for cystic echinococcosis in an endemic region of Peru using portable ultrasonography and the enzyme-linked immunoelectrotransfer blot (EITB) assay. *Parasitol Res* 2005; **96:** 242–46.

73 Altintas N. Past to present: echinococcosis in Turkey. *Acta Trop* 2003; **85:** 105–12.

74 Sotiraki S, Himonas C, Korkoliakou P. Hydatidosis-echinococcosis in Greece. *Acta Trop* 2003; **85:** 197–201.

75 Chabalgoity JA, Moreno M, Carol H, Dougan G, Hormaeche CE. A dog-adapted *Salmonella typhimurium* strain as a basis for a live oral *Echinococcus granulosus* vaccine. *Vaccine* 2000; **19:** 460–69.

76 Torgerson PR. Mathematical models for the control of cystic echinococcosis. *Parasitol Int* 2006; **55:** S253–58.

77 Cabrera PA, Haran G, Benavidez U, et al. Transmission dynamics of *Echinococcus granulosus, Taenia hydatigena* and *Taenia ovis* in sheep in Uruguay. *Int J Parasitol* 1995; **25:** 807–13.

78 Cohen H, Paolillo E, Bonifacino R, et al. Human cystic echinococcosis in a Uruguayan community: a sonographic, serologic, and epidemiologic study. *Am J Trop Med Hyg* 1998; **59:** 620–27.

79 Craig PS, Zeyhle E, Romig T. Hydatid disease: research and control in Turkana. II. The role of immunological techniques for the diagnosis of hydatid disease. *Trans R Soc Trop Med Hyg* 1986; **80:** 183–92.

80 Coltorti E, Fernandez E, Guarnera E, Lago J, Iriarte J. Field evaluation of an enzyme immunoassay for detection of asymptomatic patients in a hydatid control program. *Am J Trop Med Hyg* 1988; **38:** 603–07.

81 Cabrera PA, Parietti S, Haran G, et al. Rates of reinfection with *Echinococcus granulosus, Taenia hydatigena, Taenia ovis* and other cestodes in a rural dog population in Uruguay. *Int J Parasitol* 1996; **26:** 79–83.

82 Roberts MG, Lawson JR, Gemmell MA. Population dynamics in echinococcosis and cysticercosis: mathematical model of the life-cycle of *Echinococcus granulosus*. *Parasitology* 1986; **92:** 621–41.

83 Gemmell MA, Lawson JR, Roberts MG, Kerin BR, Mason CJ. Population dynamics in echinococcosis and cysticercosis: comparison of the response of *Echinococcus granulosus, Taenia hydatigena* and *T ovis* to control. *Parasitology* 1986; **93:** 357–69.

84 Kachani M, Macpherson CN, Lyagoubi M, et al. Public health education/importance and experience from the field. Educational impact of community-based ultrasound screening surveys. *Acta Trop* 2003; **85:** 263–69.

85 Cabrera PA, Lloyd S, Haran G, et al. Control of *Echinococcus granulosus* in Uruguay: evaluation of different treatment intervals for dogs. *Vet Parasitol* 2002; **103:** 333–40.

86 Lightowlers MW, Flisser CG, Gauci CG, Heath DD, Jensen O, Rolfe R. Vaccination against cysticercosis and hydatid disease. *Parasitol Today* 2000; **16:** 191–96.

87 Heath DD, Lawrence SB. Antigenic polypeptides of *Echinococcus granulosus* oncospheres and definition of protective molecules. *Parasite Inmunol* 1996; **18:** 347–57.

88 Osborn PJ, Heath DD. Immunisation of lambs against *Echinococcus granulosus* using antigens obtained by incubation of oncospheres in vitro. *Res Vet Sci* 1982; **33:** 132–33.

89 Zhang W, You H, Zhang Z, Turson G, Hasyet A, McManus DP. Further studies on an intermediate host murine model showing that a primary *Echinococcus granulosus* infection is protective against subsequent oncospheral challenge. *Parasitol Int* 2001; **50:** 279–83.

90 Chow C, Gauci CG, Cowman AF, Lightowlers MW. A gene family expressing a host-protective antigen of *Echinococcus granulosus*. *Mol Biochem Parasitol* 2001; **118:** 83–88.

91 Woollard DJ, Gauci CG, Heath DD, Lightowlers MW. Epitope specifities and antibody responses to the EG95 hydatid vaccine. *Parasite Immunol* 1998; **20:** 535–40.

92 Lightowlers MW, Lawrence SB, Gauci CG, et al. Vaccination against hydatidosis using a defined recombinant antigen. *Parasite Immunol* 1996; **18:** 457–62.

93 Lightowlers MW, Gauci CG, Chow C, et al. Molecular and genetic characterisation of the host-protective oncosphere antigens of taeniid cestode parasites. *Int J Parasitol* 2003; **33:** 1207–17.

94 Woollard DJ, Gauci CG, Lightowlers MW. Synthetic peptides induce antibody against a host-protective antigen of *Echinococcus granulosus*. *Vaccine* 2000; **18:** 785–94.

95 Woollard DJ, Gauci CG, Heath DD, Lightowlers MW. Protection against hydatid disease induced with the EG95 vaccine is associated with conformational epitopes. *Vaccine* 2000; **19:** 498–507.

96 Woollard DJ, Heath DD, Lightowlers MW. Assessment of protective immune responses against hydatid disease in sheep by immunization with synthetic peptide antigens. *Parasitology* 2000; **121:** 145–53.

97 Lightowlers MW, Gauci CG. Vaccines against cysticercosis and hydatidosis. *Vet Parasitol* 2001; **101:** 337–52.

98 Lightowlers MW, Jensen O, Fernandez E, et al. Vaccination trials in Australia and Argentina confirm the effectiveness of the EG95 hydatid vaccine in sheep. *Int J Parasitol* 1999; **29:** 531–34.

99 Dempster RP, Harrison GB. Maternal transfer of protection from *Echinococcus granulosus* infection in sheep. *Res Vet Sci* 1995; **58:** 197–202.

100 Heath DD, Jensen O, Lightowlers MW. Progress in control of hydatidosis using vaccination—a review of formulation and delivery of the vaccine and recommendations for practical use in control programmes. *Acta Trop* 2003; **85:** 133–43.

101 Dueger EL, Moro PL, Gilman RH. Oxfendazole treatment of sheep with naturally acquired hydatid disease. *Antimicrob Agents Chemother* 1999; **43:** 2263–67.

102 Blanton RE, Wachira TM, Zeyhle EE, Njoroge EM, Magambo JK, Schantz PM. Oxfendazole treatment for cystic hydatid disease in naturally infected animals. *Antimicrob Agents Chemother* 1998; **42:** 601–05.

103 Herd RP. Resistance of dogs to *Echinococcus granulosus*. *Int J Parasitol* 1977; **7:** 135–38.

104 Herd RP, Chappel RJ, Biddell D. Immunization of dogs against *Echinococcus granulosus* using worm secretory antigens. *Int J Parasitol* 1975; **5:** 395–99.

105 Zhang WB, Zhang ZZ, Shi BX, et al. Vaccination of dogs against *Echinococcus granulosus*, the cause of cystic hydatid disease in humans. *J Infect Dis* 2006; **194:** 966–74.

106 Lopera L, Moro PL, Chavez A, Montes G, Gonzales A, Gilman RH. Field evaluation of a coproantigen enzyme-linked immunosorbent assay for diagnosis of canine echinococcosis in a rural Andean village in Peru. *Vet Parasitol* 2003; **117:** 37–42.

107 Jenkins DJ, Fraser A, Bradshaw H, Craig PS. Detection of *Echinococcus granulosus* coproantigens in Australian canids with natural or experimental infection. *J Parasitol* 2000; **86:** 140–45.

108 Lahmar S, Lahmar S, Boufana B, Bradshaw H, Craig PS. Screening for *Echinococcus granulosus* in dogs: comparison between arecoline purgation, coproELISA and coproPCR with necropsy in pre-patent infections. *Vet Parasitol* 2007; **144:** 287–992.

109 Deplazes P, Thompson RC, Constantine CC, Penhale WJ. Primary infection of dogs with *Echinococcus granulosus*. Systemic and local (Peyer's patches) immune responses. *Vet Immunol Immunopathol* 1994; **40:** 171–84.

110 Moreno M, Benavidez U, Welle M, et al. Local and systemic immune responses to *Echinococcus granulosus* in experimentally infected dogs. *Vet Parasitol* 2004; **119:** 37–50.

111 Rosenkranz CD, Chiara D, Agorio C, et al. Towards new immunotherapies: targeting recombinant cytokines to the immune system using live attenuated salmonella. *Vaccine* 2003; **21:** 798–801.

112 Craig PS, Larrieu E. Control of cystic echinococcosis/hydatidosis: 1863–2002. *Adv Parasitol* 2006; **61:** 443–508.

113 Moro PL, Schantz PM. Echinococcosis: historical landmarks and progress in research and control. *Ann Trop Med Parasitol* 2006; **100:** 703–14.

114 Gemmell MA. Workshop summary: hydatid—new approaches. *Vet Parasitol* 1994; **54:** 295–96.

115 Jenkins DJ, Romig T, Thompson RC. Emergence/re-emergence of *Echinococcus* spp—a global update. *Int J Parasitol* 2005; **35:** 1205–19.

116 Ito A, Romig T, Takahashi K. Perspective on control options for *Echinococcus multilocularis* with particular reference to Japan. *Parasitology* 2003; **127:** S159–72.

117 Li T, Qiu J, Yang W, et al. Echinococcosis in Tibetan populations, Western Sichuan Province, China. *Emerg Infect Dis* 2005; **11:** 1866–73.

118 Budke CM, Qiu J, Wang Q, Torgerson PR. Economic effects of echinococcosis in a disease-endemic region of the Tibetan Plateau. *Am J Trop Med Hyg* 2005; **73:** 2–10.

119 Craig PS, Giraudoux P, Shi D, et al. An epidemiological and ecological study of human alveolar echinococcosis transmission in south Gansu, China. *Acta Trop* 2000; **77:** 167–77.

120 Kern P, Ammon A, Kron M, et al. Risk factors for alveolar echinococcosis in humans. *Emerg Infect Dis* 2004; **10:** 2088–93.

121 Wang Q, Qiu J, Yang W, et al. Socioeconomic and behavioural risk factors of human alveolar echinococcosis in Tibetan communities in Sichuan, People's Republic of China. *Am J Trop Med Hyg* 2006; **74:** 856–62.

122 Yang Y, Sun T, Li Z, et al. Community surveys and risk factor analysis of human alveolar and cystic echinococcosis in Ningxia Hui Autonomous Region, PR China. *Bull World Health Organ* 2006; **84:** 1–8.

123 Schelling U, Frank W, Will R, Romig T, Lucius R. Chemotherapy with praziquantel has the potential to reduce the prevalence of *Echinococcus multilocularis* in wild foxes (*Vulpes vulpes*). *Ann Trop Med Parasitol* 1997; **91:** 179–86.

124 Tackmann K, Loschner U, Mix H, et al. A field study to control *Echinococcus multilocularis* infections of the red fox (*Vulpes vulpes*) in an endemic focus. *Epidemiol Infect* 2001; **127:** 577–87.

125 Hegglin D, Ward PI, Deplazes P. Anthelmintic baiting of foxes against urban contamination with *Echinococcus multilocularis*. *Emerg Infect Dis* 2003; **9:** 1266–72.

126 Rausch RL, Wilson JF, Schantz PM. A programme to reduce the risk of infection with *Echinococcus multilocularis*: the use of praziquantel to control the cestode in a village in the hyperendemic region of Alaska. *Ann Trop Med Parasitol* 1990; **84:** 239–50.

127 Christofi G, Deplazes P, Christofi N, Tanner I, Economides P, Eckert J. Screening of dogs for *Echinococcus granulosus* coproantigen in a low endemic situation in Cyprus. *Vet Parasitol* 2002; **104:** 299–306.