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A publication of the University of Rome "La Sapienza"
Official Journal of the Italian Society of Parasitology

Proceedings of the FIRST NATIONAL CONGRESS OF HYDATIDOLOGY

(Sassari, Italy, October 7-8, 2004)

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Front cover

Arixi (Cagliari, Sardinia, Italy): a popular "educational" mural by Pinuccio Sciola (1978) alarming about bad customs that perpetuate the echinococcosis/hydatidosis. [Archivio Centro Studi Ferretti].

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PARASSITOLOGIA

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Proceedings of

the FIRST NATIONAL CONGRESS OF HYDATIDOLOGY

I CONVEGNO NAZIONALE DI IDATIDOLOGIA

held at the Faculty of Biological Sciences, University of Sassari, Italy, October 7-8, 2004,

and

organized by the Department of Animal Biology, Faculty of Veterinary Medicine, University of Sassari, and by the Istituto Zooprofilattico Sperimentale della Sardegna "G. Pegreffi", Sassari,

in collaboration with

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Federazione Mediterranea Sanità e Produzione Ruminanti (FE ME S P RUM),
Società Italiana di Ecopatologia della Fauna (SIEF).
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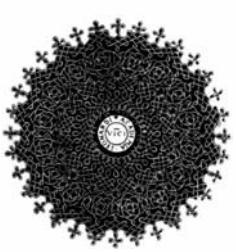

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The individual authors take responsability for linguistic quality of the articles and presentations.

Parassitologia 46: 351-352, 2004

Opening addresses

Sono lieto di inaugurare questo congresso, non solo per il suo interesse scientifico intrinseco ed il numero dei partecipanti, segni evidenti della qualità del lavoro dei colleghi di Sassari che l'hanno organizzato, ma perché segna una rinnovata collaborazione nel campo dell'idatidologia.

L'echinococcosi è per il clinico una malattia complessa, le cui manifestazioni vanno dalla totale assenza di sintomi alla compromissione drammatica della funzionalità dei distretti colpiti. Enormi progressi sono stati resi possibili negli ultimi 25 anni dall'adozione delle tecniche di immagine, dai miglioramenti delle tecniche chirurgiche, dall'impiego dei farmaci benzimidazolici e dall'introduzione delle tecniche di trattamento percutaneo, oltre che dallo sviluppo dei test sierologici e da avanzamenti nella comprensione della biologia del parassita. Molto, moltissimo, rimane però da fare.

Oggi ci troviamo di fronte a pazienti in cui la decisione terapeutica non è sempre facile, perché abbiamo a disposizione tre opzioni terapeutiche, ma non ci sono dati utilizzabili nel quadro di una moderna medicina basata sulle evidenze. La malattia è relativamente rara anche nelle zone di media endemia ed è difficile persino per chi si occupa giornalmente di questi pazienti accumulare una quantità di casi sufficiente ad avere un significato statistico. Pazienti provenienti da aree endemiche, particolarmente Nord-Africa ed Europa dell'Est, sono sempre più frequenti ed è necessario assicurare cure non basate sull'improvvisazione. Purtroppo le competenze in ambito medico sono frammentate fra chirurghi, infettivologi, radiologi, gastroenterologi e parassitologi e non è sempre facile accordarsi, con conseguente disorientamento del paziente.

Mancano dati epidemiologici, sia per la difficoltà di diagnosticare tutti i casi in cui l'infestazione è clinicamente silente (il metodo ideale sarebbe lo screening ecografico di massa della popolazione), sia perché il Decreto del Ministero della Sanità del 15.12.1991 ha posto l'echinococcosi nella classe V, che comprende malattie per le quali è previsto solo un riepilogo annuale da parte della ASL al Ministero, senza che le informazioni arrivino all'ISTAT.

In questa situazione, solo la collaborazione con i colleghi parassitologi veterinari, di gran lunga più organizzati ed efficienti rispetto ai pochi medici che si occupano sistematicamente di questa patologia nell'uomo, può permetterci di avanzare.

Questo congresso è dunque un'occasione preziosa per lavorare insieme, per scambiare idee e dati, e soprattutto per rilanciare questa collaborazione nella Società Italiana di Idatidologia. Gli Autori italiani hanno una lunga tradizione in questo ambito e sono apprezzati a livello internazionale. Collaborazioni con Università e centri stranieri, sia europei che extraeuropei, sono in corso. Alcuni di noi lavorano da anni nell'Informal Working Group on Echinococcosis dell'Organizzazione Mondiale della Sanità. Utilizziamo questo patrimonio e organizziamo nuove iniziative, per esempio un registro italiano dell'echinococcosi cistica, che ci aiutino a risolvere almeno alcune delle difficoltà.

Ho parlato dal punto di visto medico, ma altrettante questioni sul controllo della malattia, ambito veterinario per eccellenza, verranno affrontate oggi e domani.

Ringrazio tutti voi per essere qui ed a tutti faccio i miei auguri di buon lavoro, per questi giorni, ma soprattutto per il prossimo futuro.

Carlo Filice
Presidente
della Società Italiana di Idatidologia

As president of the Italian Society of Parasitology (SoIPa), when the organizers of the National Conference of Hydatidology, held 7-8 October 2004 in Sassari (Sardinia) asked me to support their Conference, I was extremely pleased to be part of this meeting. Echinococcus granulosus *is a worldwide, severe zoonotic infection. Furthermore, the spread of infection has very high economic costs on human health systems and on animal husbandry. In spite of the efforts in prevention and control, cystic echinococcosis is still present in many parts of Italy as clearly shown by the scientists throughout the Conference presentations.*

In this type situation, it is of interest that recently stressed by Dr Cristina Soro, a Sardinian veterinarian working in an area most affected by the infection, in a letter addressed to Dr Anto-

nio Scala from University of Sassari. The persistence of the infection is not only due to some "persistent ancient beliefs and mass media often spreading contradictory information" as stated by Mantovani (see page 353), or to poor socio-cultural background of people. The real problems include "assistance to breeders in the control of stray dogs, cost of dog treatment, cost and methods of sheep carcass disposal in the field, complicated by illegal slaughtering practices" as stated by Soro. It has to be pointed out that cystic echinococcosis is a zoonosis whose successful control demands continuous resources and activities in the long term, as many authors have stressed during the Conference.

Obviously, this Conference will not be able to solve the problem "echinococcosis", but I hope it will be an excellent opportunity to renew the debate and to awaken the local and national health authorities and politicians on this "old but still present" parasitic zoonosis.

Claudio Genchi
President,
Italian Society of Parasitology

Parassitologia 46: 353-355, 2004

Opening lecture
Notes on cystic echinococcosis in the Mediterranean

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Abstract. Cystic Echinococcosis (CE), caused by *Echinococcus granulosus*, is present from the beginning of history and in the Mediterranean it is linked to the dog-sheep cycle. The Mediterranean area possesses many features favouring CE. Positive and negative influences derived from the action of the European Community and from recent developments. The control measures of CE have political, economic, public health and environmental implications. Dog population and dog-transmitted zoonoses control, improvement of slaughtering procedures and the destruction of infected viscera, health education, interprofessional cooperation are able individually to constitute a contraposition to CE and combined to compose a control program. Epidemiological surveillance and control of CE in the Mediterranean are coordinated by the WHO Mediterranean Zoonoses Control Centre of Athens.

Key words: cystic echinococcosis, zoonoses, Mediterranean, cystic echinococcosis control.

When the history began, cystic echinococcosis by *Echinococcus granulosus* (CE) was here, as it was rabies (Lasagna *et al.*, *1995*). We suppose that hydatid cysts might be a part of the alterations used by haruspices (the diviners who interpreted the will of the Goods from inspection of the entrails of sacrificed animals) in their predictions.

In the Mediterranean CE has been linked to the dog-sheep cycle. It has been supposed that this parasite was originally present in canids as final hosts and in herbivores as intermediate hosts. It may be reasonably supposed that the parasite may have taken advantage of the formation of herds and of the slaughtering of animals in close vicinity of human settlements in which dogs have been present from the beginning of civilization. Man has remained an occasional, unimportant intermediate host even if it may be presumed that in some occasion (depending on civilizations, wars, *etc.*) dogs may have had access to human bodies (Mantovani *et al.*, 2004).

Development and maintenance of CE in the Mediterranean

The features which may favour the development and maintenance of CE in the Mediterranean are the following (Mantovani, 1997b):

(1) many rural families have small lots of land where they keep animals of different species;
(2) dogs are numerous and many live free in contact with people and find freely food (Mantovani, 1997);
(3) wolves are increasing in number and supported by important campaigns;
(4) home slaughter is largely practised and dogs are fed on offal;
(5) sheep or other animal carcasses are abandoned in the fields and may be preyed by dogs and/or wolves. In some places these carcasses are purposely left in the fields or put in feeding areas accessible to dogs to facilitate the survival of vultures or wolves;
(6) abattoirs exist which are insufficiently equipped and are accessible to dogs;
(7) some houses lack running water;
(8) vegetables accessible to dog defecation are used by families and sometimes served in restaurants;
(9) professional training of pastoralists, agricultural and animal industry workers, and health education of the public are often neglected;
(10) ancient beliefs persist and mass media often spread contradictory information.

Many of these conditions have been recently ameliorated and are ameliorating as a consequence of the improvement of living conditions and of preventive actions.

The wolf (Guberti *et al.*, 2004) has a confirmed role, and is a part of the dog-sheep cycle; a true wild cycle has not evolved in Italy, and possibly in other areas of the Mediterranean.

Urban infections may derive from communities having the habit to slaughter animals at home; in same cases animals are slaughtered in rural areas without veterinary inspection, imported in urban areas and their viscera may be fed to dogs. Special attention should be given to biological farming and agro-tourism.

The importance of dog populations surveillance and control, as a tool for the prevention of CE and

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other zoonoses, both in rural and urban or semi-urban territories, is progressively emerging (Ciarallo and Pozzi, 2004).

CE control programmes in the Mediterranean

The European Community does not consider CE a priority. Indirect control measures have been activated by the rationalisation of animal slaughtering and viscera destruction. On the other side, the closure of many small abattoirs distributed in the territory has increased illegal home slaughtering and dispersal of viscera.

Some expert believe that CE will be controlled as a consequence of the changes of human habits, agricultural practices and dog management and control, as has happened *e.g.* in the Po Valley; this seems possible only in areas of very intensive farming.

CE has been classified as a zoonosis "typical Mediterranean" (Mantovani and Prosperi,. 1995). The WHO Mediterranean Zoonoses Control Centre of Athens has organized and is coordinating the epidemiological surveillance and in many cases the control of CE in Mediterranean countries (Battelli, 1997b; Battelli *et al.*, 2002; Mantovani, 1988; Seimenis, 2003; Seimenis and Battelli, 2003; Seimenis *et al.*, 1997, 2001). With the exception of Cyprus and Malta, the infection has remained endemic and important in all countries and the control measures have received scarce political support.

Main features which characterise the development of CE control programmes in the Mediterranean are (Battelli *et al.*, 2002; Mantovani, 1997b; Mantovani, 2001):

(1) the programmes are lasting for decades, *i.e.* longer than the usual period of a political mandate;
(2) they are expensive and costs are evaluated more easily than benefits; resources are not always available for the specific control of CE;
(3) all actions used in CE control are useful also to control other zoonoses (*e.g.* leishmaniosis and rabies) and to face other public health problems (food safety, dog populations control, *etc.*);
(4) CE *per se* receives scarce attention by mass media and by the population at large; among the components of a CE control program strong attention is received by the problems of dog populations control and by dog-connected problems involving urban areas.

Moreover, it should be considered that human hydatidosis is sporadic even in endemic areas, develops slowly (years) and is rarely fatal; its diagnosis and therapy are very advanced compared with other diseases of poorly developed areas.

The scarce attention and success obtained by CE control programs in the Mediterranean Region have suggested to consider the motivations and the different components of a program, and to analyse their practicability and usefulness for CE control, both applied separately, and fused to form a combined CE control program. We will try to investigate this thesis.

Major veterinary public health issues influenced by a CE control programme may be the following:

(1) control of human infections (CE and other zoonoses);
(2) dog population control;
(3) control of dog transmitted zoonoses;
(4) improvement of slaughtering procedures and destruction of infected organs;
(5) improvement of food safety;
(6) health education (Parodi *et al.*, 2001);
(7) interprofessional cooperation.

Implications of CE control measures

Implications of CE control measures are different in nature and may be considered from the political, economic, public health, and environmental standpoints (Battelli *et al.*, 2004 *l.c.*; Mantovani, 2001 *l.c.*).

The main political implications are:

(1) involvement of more generations of public administrators and health operators;
(2) establishment of a network of permanent activities;
(3) collaboration among public health, agriculture, environment, consumers and others;
(4) permanent legislation and funding from different administrations (health, agriculture, environment, *etc.*);
(5) organisation of a policy of animal welfare.

The main economic implications are:

(1) socio-economic cost of the human and animal infections (Battelli 1997; Battelli and Martini, 1992);
(2) costs of control activities;
(3) losses in animals attacked by dogs;
(4) possible finalisation of resources allotted for other programs also for CE control;
(5) subdivision of costs among different programs and activities;
(6) possible financing by different projects (slaughterhouse rationalization, viscera destruction, dog populations control, health education, *etc.*).

The main public health implications are:

(1) control of human infection;
(2) improvement of food safety;
(3) control of dog-connected infections and other problems.

The main environmental implications are:

(1) dog populations management;
(2) wolf and wildlife management;
(3) appropriate garbage disposal;
(4) carcasses disposal;
(5) slaughterhouse management;
(6) contamination following dog deworming.

Conclusion

CE is an historical infection of the Mediterranean Region, present mainly in rural and less developed areas. It is originating valuable research and attracting scientific attention, centred on aetiology, epidemiology, therapy of the human disease and control measures. Its importance involves human medicine, veterinary public health, economy, food safety, environment and education.

CE has received and still receives scarce interest by the public administration and by mass media, both at the national and European level. Control programs in the Mediterranean had scarce success with limited exceptions. It has to be considered that the components of a CE control program (dog populations and dog-transmitted zoonoses control, improvement of slaughtering and viscera destruction procedures, health education and interprofessional cooperation) when practiced as a routine procedure have each one originated an important impact; when properly combined these components constitute a CE control program.

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Papers

Parassitologia 46: 359-362, 2004

Socio-economic impact of cystic echinococcosis and of its control: some data and considerations

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Abstract. The socio-economic impact of cystic echinococcosis (CE), caused by *Echinococcus granulosus,* is reviewed with special reference to the following topics: consequences in man and livestock, costs and benefits of control programmes and economic procedures for evaluating control programmes. Examples of some important costs and benefits are given. Many consequences in man and livestock are difficult to evaluate from an economic point of view, because some basic data are difficult to obtain in many countries. However, the socio-economic evaluation of the consequences of CE and of the present and future control actions proves indispensable to best use available resources and possibly tailor control stategies.

Key words: *Echinococcus granulosus,* cystic echinococcosis, control, socio-economics.

Echinococcus granulosus infection or cystic echinococcosis (CE) is persisting in many parts of the world, especially in the Mediterranean Region, Latin America and Africa south of Sahara, and is emerging or re-emerging in some countries, such as Bulgaria, Kazakistan and China, but our knowledge of this point is incomplete (Eckert, 2001). Control programmes have been successful in interrupting parasite transmission in a few countries, mainly in island situations. It should be pointed out that CE is a zoonosis whose successful control demands continuous resources and activities in the long term. In the present paper, a brief review is made on the the socio-economic impact of CE and of its control. The data presented, unless otherwise stated, have been taken from Battelli (2001; 2004), Battelli and Martini (1992), Battelli *et al.* (2002), VV. AA. (2003).

Socio-economic consequences of CE

In *humans*, CE may have various consequences, including the following: cost for diagnosis; medical and surgical fees and costs of hospitalisation, nursing and drugs; loss of working days and/or "production"; cost of travel to seek treatment for both patient and family members; mortality; suffering and social consequences of disability; abandonment of farming or agricultural activities by affected or at-risk persons. It should be noted that some of the above consequences are difficult to evaluate from an economic point of view and others can be mainly or exclusively evaluated in social terms. For instance, the value of human life is still a controversial subject and has been calculated in a number of ways (e.g. as potential loss of income) (Torgerson, 2003). The case fatality rate for CE is generally reported to be 1-2%. Among the costs associated with identification and treatment of CE, those related to the duration of hospitalisation and convalescence represent the most important components. According to experiences in the Mediterranea Region and Latin America, it has been calculated that the duration of hospitalisation varies from about 2 weeks to more than one month in case of surgery, and is about 8 days for diagnosis and therapy, alone. Where efficient services and modern techniques and interventions have been applied for diagnosis, admission, surgery and treatment, the hospitalisation period has decreased by about 50% within a few years. Such an implementation leads also to a better control of the convalescent period (and to a decrease in the working days lost) which would normally last 3 to 4 weeks.

In Italy, at the main hospital of Bologna, the 1995 mean specific cost of a surgical case was about 14,000 US$, and that of a clinical case about 2,500 US$. The mean number of days spent in hospital was 28 and 8 for surgical and clinical cases, respectively. Almost all the cases were hepatic infections. For surgical cases, the cost considered were the following: days spent in hospitals (net cost of stay) (73.4% of total costs); laboratory examinations (6.8%); imaging examinations (4.3%); drugs (0.5%); pharmaceutical material (0.1%); anaesthesia (1.5%); surgical facilities (3.8%); blood and blood products (2.1%); histological examinations (6.2%); consultations (0.4%); surgical dressing (0.4%); personnel of operating theatre (0.4%). Still in Italy, in 1999, a study was performed to evaluate the costs of diagnosis and chemotherapy with albendazole of liver CE in out-patients. Considering a 10-year period of post-diagnosis follow-up and that a relapse occurs in nearly 25% of the patients, the present value of the mean costs was evaluated equal

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to about 2,000 US$ (nearly 70% paid by the patient) at a 5% discount rate. It should be noted that these costs do not include the working hours lost, the costs of travel and correlated expenses for both patients and family members.

In the Rio Negro Province, Argentina, the 1997 costs of surgical cases in two hospitals varied approximately from 4,600 to 6,000 US$, and the mean costs per infected patient amounted to approximately 4,500 US$. The latter costs were about 31% lower than in 1980, mainly due to the introduction of chemotherapy with albendazole and of the PAIR technique (Puncture, Aspirate, Inject a scolecidal agent and Re-aspirate the cyst) (mean costs per patient approximately 1,350 and 2,000 US$, respectively). With regard to the improvement of PAIR procedure, the experiences from many countries indicate that this technique, mainly for the treatment of liver cysts, is effective with comparable outcomes in terms of rates of success, complication and mortality. It also shortens the time of treatment and final recovery and it is a valuable alternative to surgery also in terms of cost-containment and the mean hospitalisation time.

In *livestock*, the following consequences of CE must be considered: reduced yield and quality of meat, milk and wool; reduced birth rate; delayed performance and growth; condemnation of organs; costs for destruction of infected viscera and dead animals. There are also other possible indirect detrimental consequences, such as ban on export of animals and their products if these are required to be free of CE. In livestock, the importance of the above-mentioned losses will depend, to a large extent, on the characteristics of the animals or of the farming or livestock industry. Quantification, standardised evaluation of such losses and exclusion of biasing factors in animal production are very difficult, therefore the available data should be interpreted with caution.

Losses in sheep with CE have been reported to approximate 7-10% of milk yield, 5-20% of meat or total carcass weight, and 10-40% of wool production. It has been estimated that birth weight of lambs from infected sheep may be 20-30% less than that of lambs from healthy sheep. In Sardinia, with a population of 3 million dairy sheep, a loss in milk production was estimated to about 13.7 million US$ in 1982. This evaluation was based on a presumed decreased milk production of 7% in infected sheep and on 80% prevalence of CE in the sheep population. This sum approximately represented about 80% of the total yearly losses in livestock productivity caused by CE. In Italy, in 1980, an evaluation was proposed of the reduction of the commercial value per sheep infected with CE equal to 10%, this percentage including the value of the condemned viscera. The quantification of losses caused by infected viscera is influenced by both the legislative rules of each country and the number of animals slaughtered under veterinary supervision. It should also be stressed that the costs of an efficient destruction of condemned offal may be high, particularly as a starting investment to provide proper facilities (e. g. incinerators). In Estremadura, Spain, in 1991 the costs of condemned viscera were estimated to be approximately 2% of the total yearly costs of CE, both in livestock and in man. In southern South America, it was estimated that the viscera of 2 million cattle and 3.5 million sheep are condemned every year, and that the cost of such condemnation (1999) amounts to 6.3 million US$ in Argentina and 2.5 million US$ in Chile (Thakur, 2002).

Costs and benefits of CE control programmes

The awareness of the socio-economic impact of the disease has stimulated the implementation of control programmes in certain areas or countries. Of particular interest in this connection is a reliable cost estimation as a basis for selecting an adequate control strategy. When CE control is financed with public funds, the true costs that should be evaluated are the opportunity costs.

The main *costs* for control programmes are summarised as follows: education; dog control and treatment; detection and destruction of infected viscera; diagnosis and therapy in humans; surveillance and monitoring of human and animal disease (recently, ultrasonography has been used in some endemic communities as a screening method); administration and evaluation of the programme. If the control includes vaccination of intermediate hosts, the costs must be considered of vaccine and stock vaccination. It should be noted that some of the expenses sustained for CE control may simultaneously be beneficial to control programmes against other diseases (e.g. rabies, tapeworm infections).

According to information from the Rio Negro Province, the costs of a dog dosing programme in 1997 were 37 US$ per animal, including the costs for dog testing with arecoline, drug distribution to dog owners and for praziquantel. Compared with 1980, a reduction of costs by 16 US$ per animal was achieved. In Spain, in the years from 1986 to 1996, the Ministry of Health and Consumers Affairs provided economical support to CE prevention and control programmes; the total amount was nearly 707 million pesetas for six autonomous Communities. The sums were allocated and used for the following interventions: dog treatments with praziquantel (62%), building of kennels (17%), septic tanks and wells (10%), health education (5.7%), experts' travel expenses (3.6%), transport vehicles (1%), and incinerators (1%).

The *benefits* of control programmes may be financial and non-financial (the latter category is difficult to evaluate). The most relevant are the following: increase in farm animal production; increase in working days per year and per person; decreased costs for hospitalisation, diagnosis and treatments;

improvement of veterinary and public health services, hygiene and primary health care; improvement of the physical, psychological and social status of the population; reduction of other health or zooeconomic problems such as rabies, food-borne infections, cestode larval infections in farm animals, etc.

Until now, few examples are available of economic analyses of CE control programmes. With regard to the Mediterranean Region, a prospective analysis was performed of a 10-year project involving Sardinia. Assuming a reduction of the disease prevalence in sheep from 80 down to 10%, the net present value of the gained milk production was evaluated at 18.3 million U$/1982. Reducing the human cases per year from 235 down to 15, 669 years of human life gained were reckoned. The total cost of the programme was evaluated equal to 8.8 million U$/1982 and the internal rate of return equal to 53.6%. In the Rio Negro Province, some financial advantages from the CE control programme were evaluated. Compared with 1980, in 1997 a reduction was achieved of 67.5% in human incidence, of 48.4% in the hospitalisation period, of 31.3% in the costs per patient, and of 77.7% in overall costs of medical care per year. In Chile, the weight of lambs and adult sheep in the years 1969-78 (without control programme) and in the years 1979-88 (with a 10-year control programme) was compared, in the absence of climatic variations or pressure of grazing. As an effect of the programme, an increase was found of 2.8% and of 5.6% in the weight of lambs and of adult sheep, respectively. In Uruguay, in 1997, compared with 1991 (first year of the control programme), a reduction was observed of 66.7% of condemned livers, corresponding to a benefit of nearly 3.2 million US$ (Orlando, 2002). In the Community of La Rioja, Spain, a programme of prevention and control of CE was initiated in 1987. In 2000, this programme led to a reduction of 97.2% in the prevalence in dogs, of 74.4% in the prevalence in sheep, and of 78.9% in the incidence in humans. These reductions were estimated to yield an increasing cumulative cost/benefit balance that was already positive on year 8 of the programme, and that reached 1.96 in year 2000.

With regard to PAIR procedure, it offers not only economic benefits but also opportunities for making treatments available to communities in endemic areas that have limited access to modern hospital facilities. However, a long term follow-up is needed to evaluate the effectiveness of this technique.

Economic evaluation of CE control programmes

For many years, some methodologies have been applied to the evaluation of CE control programmes. Among the economic procedures*, mention should be made of* cost-effectiveness *analysis and* cost-benefit *analysis. These procedures may be applied before a programme is implemented (prospective analysis) when a project is to determine the optimal policy, or after or during implementation to review the economic consequences or to modify the interventions (retrospective or "mixed" analysis).*

Regarding the evaluation of costs and benefits, their identification and quantification may sometimes prove complex and/or "twisted" when one intends to estimate all costs and benefits, especially the secondary ones or when benefits are essentially social or hard to calculate/predict. In this case, the outcome of a programme can be partially evaluated in social terms (social benefit-cost analysis). In addition, the efficiency of interventions may be expressed by such different measures as net present value of the benefits (preferable), benefit-cost ratio, and internal rate of return. It should be pointed out that in analyses of the prospective or retrospective types, e.g. a cost-benefit analysis, costs and benefits of each year of the programme must be converted into present values by procedures known as "discounting" and "compounding", respectively. Recently, the use of mathematical models has been introduced to compare different intervention strategies aiming at controlling CE. These models, however, are hardly applicable due either to the lack of accurate qualitative (epidemiologic and economic) data or to their insufficient number. In some cases, therefore, such models must be evaluated with due caution.

Final considerations

The evaluations of the socio-economic impact of CE and of its control programmes often prove difficult. Their validity strongly depends, besides on the specialised skills of those performing them, also on an efficient information system (not only sanitary) capable of providing reliable, real data and minimising exclusively personal evaluations. Sophisticated analyses not based upon qualitatively and quantitatively sufficient data may prove useless and give a false impression of precision. Despite extant restraints, the socio-economic evaluation of the consequences of CE and of the present and future control actions proves indispensable to best use available resources and possibly tailor control stategies. Evaluations taking into account only few but sufficiently accurate and time-verifiable parameters and data, and based on correct methodologies, may assist in reaching the objective.

Acknowledgements

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Natural history of cystic echinococcosis in humans

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Abstract. This study provides a contribution to understanding of the natural history of the hydatid cyst during its evolution in the human liver examining the high morphostructural variability of larval forms of *Echinococcus granulosus*. A detailed study of a large sample of intact cysts removed from patients surgically treated by means of total pericystectomy, has enabled to outline the different developmental stages of the parasite over time, up to its death and complete degeneration.

Key words: human hydatid cysts, morphology, parasite evolution, *Echinococcus granulosus*.

In spite of a reduction in parasite pressure, the current pattern of *Echinococcus granulosus* diffusion in humans in Sardinia registers almost 150 surgical operations per year, about 75% of which concern hepatic localizations (Ecca *et al.*, 1998; Conchedda *et al.*, 2002). Many of the observations were carried out at the "Clinica Chirugica" of Cagliari University directed by professor M. Cagetti, where possible by total pericystectomy of intact cysts. With this technique not only spheroidal (Fig. 1a), but also lobed and multilobed (Fig. 1b) cysts or forms with highly irregular shape (Figs 1c,d) can be excised intact with minor parenchyma loss.

Unlike to what happened in the past when cysts were normally opened and drained during surgery, total pericystectomy enables the cyst to be studied in its entirety and the structural organization of the parasite examined in minute detail, making it possible to define different typologies of cystic echinococcosis cysts.

Results and discussion

Extending an earlier study on the morphostructural aspects of hepatic hydatid cysts (Bortoletti *et al.*, 2002), observation of a total of 118 cases has provided further evidence of the variability of *E. granulosus* larval forms, enabling to continue the characterization of 10 different typologies. In short, the cyst types identified can be classified as: *classic* (6.8% of examined sample), *multivesicular* (26.3%), *hyperlaminated* (5.9%), *hyperlaminated caseous* (40,7%), the most common, *hyperlaminated gelatinous* (7.6%), *hyperlaminated granular* (9.3%), *caseous* (0.9%), *acephalocyst* (0.9%), *serous* (0.9%) and *septated* (0.9%).

The resulting pattern appears extremely variable, also considering the high prevalence of "transitional forms", i.e. of cysts showing morphostructural features intermediate between two different typologies (Bortoletti *et al.*, 2000). Therefore, if we consider the single cases as representing different steps of the development process of *E. granulosus* larval forms in humans, it is possible by ordering the observed pictures, to follow the course of events that characterize the evolution, degeneration and ultimately the death of the parasite. In this way it is possible to draw up an outline of a sort of "natural history of the hepatic cyst in humans".

Similarly to the experimental *Taenia taeniaeformis*/mouse model (Bortoletti and Ferretti, 1985), *E. granulosus* hexacanth embryo that reaches the liver undergoes an initial proliferative phase, forming a sort of "morula" a few hundreds microns in size. Within a few days, it vacuolizes differentiating into germinal membrane able to proliferate and expand in the surrounding parenchyma. This development is facilitated, among other factors, by the larval production of secreted material, rich in acid mucopolysaccarides that interposing between the parasite *sensu stricto* (germinal membrane) and host immune cells enables the larva to survive and slowly develop (Conchedda *et al.*, in press). Slackened or reduced production as well as an accelerated host response may fatally impair parasite development insomuch as the symbionts are thought to engage in a race in time.

If early stages succeed in developing, larva starts to grow very slowly, the germinal membrane gradually expanding. This produces an outer laminar layer towards the pericyst, and brood capsules and protoscoleces inside towards the cyst cavity, then cyst evolving into the *classic type* characterized by a cavity filled just with cyst fluid and hydatid sand (Fig. 2a).

During the years, possible protoscolex vesiculation produces endogenous daughter cysts (DC), the primary cyst evolving into the *multivesicular* form (Figs. 2b,c). As long as cyst cavity permits, the DC remain turgid and spheroidal in shape (Fig. 2b). As their number increases they begin to collapse due to mutual compression (Fig. 2c). Consequently, within the cyst cavity the DC walls appear tightly pressed together and packed into compact masses of sheets of laminar tissues (SLT), repeatedly back folded and always arranged in a circonvoluted and often intri-

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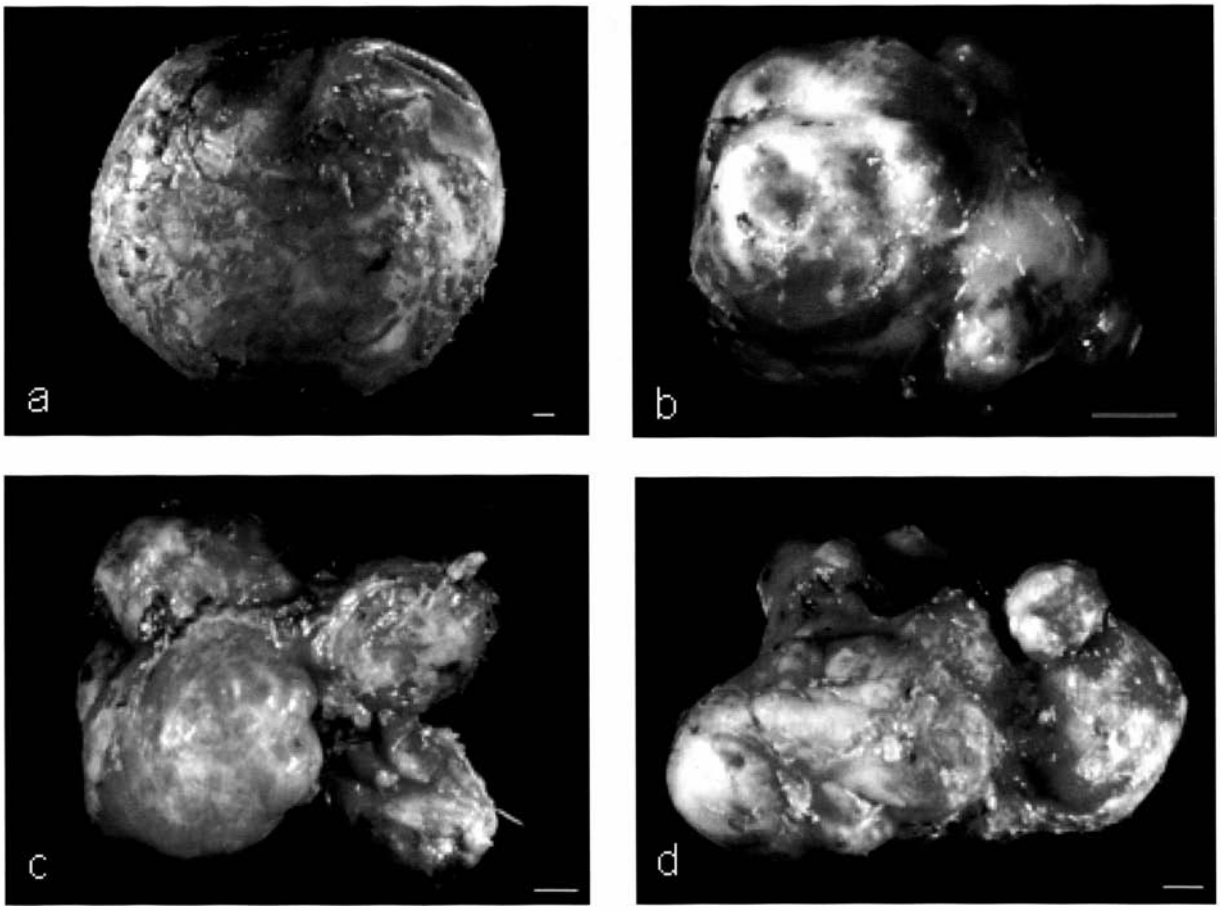

Fig. 1. Intact hepatic hydatid cysts excised by pericystectomy varying from spheroidal (a) to multilobed (b) or very irregular in shape (c, d). (Bar = 1 cm).

cate pattern (Figs 2c,d). Further production of daughter or grand-daughter cysts occupying the remaining spaces, completely fills the cavity with SLT, the cyst becoming *hyperlaminated* (Fig. 2e). In many multivesicular cysts, expansion and detachment of the endocyst of the primary cyst also contributes to this process, sometimes forming considerable masses of SLT.

This evolution also influences larva fertility. In fact, while practically all classic and multivescicular cysts are fertile, cyst fertility gradually decreases as more and more DC collapse forming SLT. Once a virtual space has been created in the cyst cavity, no DC germinal membrane part is anymore able to produce brood capsules and protoscoleces. Practically, what happens is a sort of "boomerang effect" insomuch as continuous or overproduction of DC becomes deleterious to the larval form itself. Over the years the larva paradoxically becomes sterile, moving towards the end of its life cycle. In these cases it is also possible to observe degenerative aspects, ranging from loss of endocyst in the mother cyst, now detached and collapsed into the cavity, to the presence of a largely calcified pericyst, lined within by a layer of yellowish material of a fibrous-caseous consistency. In theses cases in addition strongly degenerated SLT are present.

During the slow course of the above described events, cysts may further degenerate with accumulation of caseous, granular or gelatinous material. In particular, caseous degeneration, probably due to bacterial contamination or infiltration, is most common picture (more than 60% of examined cases), slowly leading to the formation of *hyperlaminated caseous* cysts (Figs $2f_1$,h,m,l). This process may begin early on, when the cyst is still multivesicular, even in hyperfertile cases with DC and grand-daughter cysts still turgid, or later during degeneration after SLT formation. In these cases, also owing to endocyst detachment and DC packing, the cavity appears to be near completely filled with SLT with little caseous matrix (Figs $2f_1$,h). In other cases caseous degeneration may conversely occur after formation of a small number of DC, the cyst evolving towards a hyperlaminated form characterized by a large quantity of caseous matrix with several convoluted layers of SLT (Fig. 2m). In those cysts where caseous degeneration commences prior to DC formation and the endocyst is detached and convoluted, the cavity is characterized by a prevalent

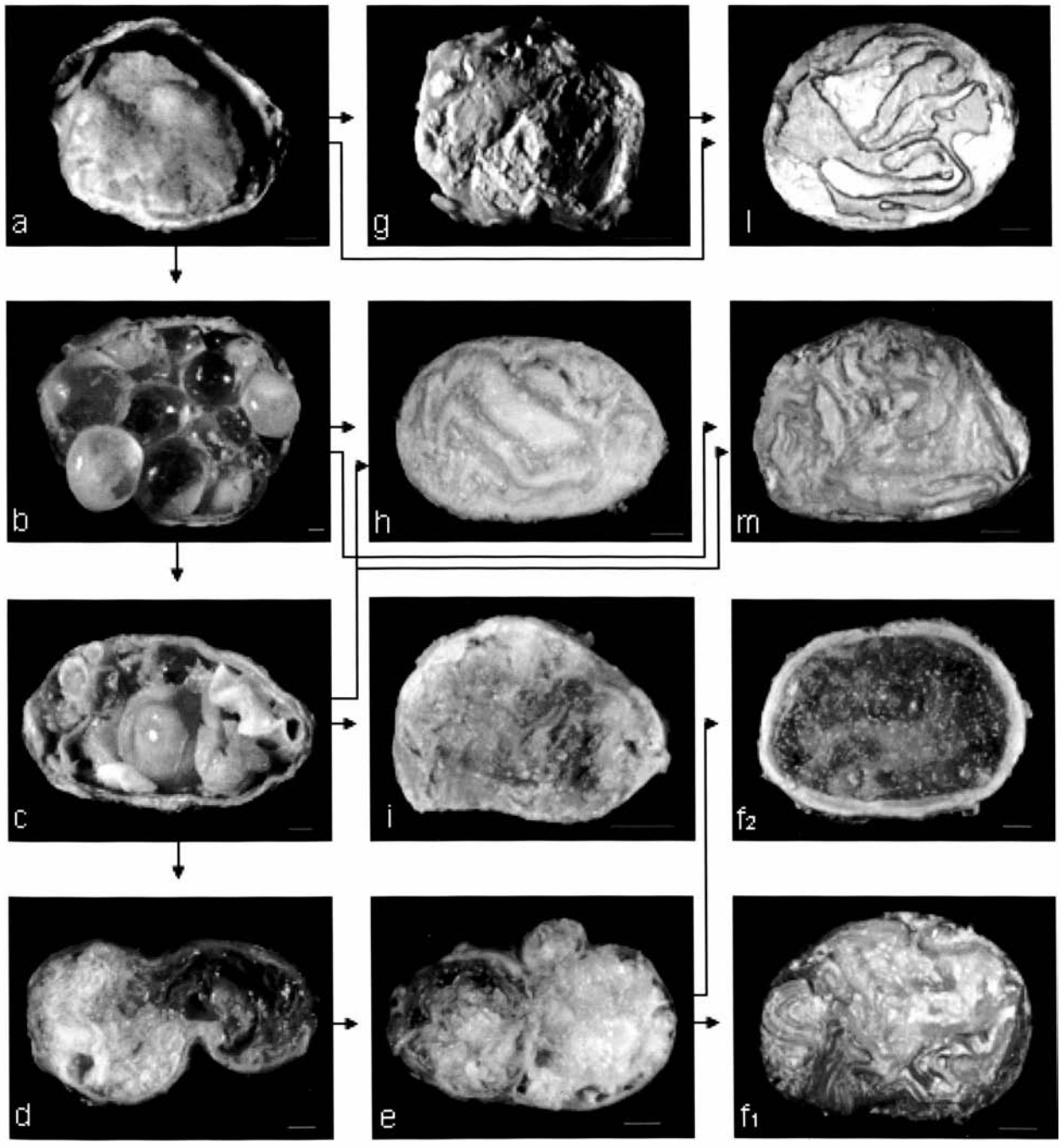

Fig. 2. Pattern of possible evolution of the *E. granulosus* larval form in human liver. Arrows indicate the course of transformation undergone by different cyst typologies over time. (a): *classic*; (b), (c): *multivesicular*; (d): *transitional multivesicular-hyperlaminated form*; (e): *hyperlaminated*; (f_1), (h), (m), (l): *hyperlaminated caseous*; (f_2): *hyperlaminated gelatinous*; (i): *hyperlaminated granular*; (g): *caseous*. (Bar=1 cm).

caseous matrix over little STL (Fig. 2l). Lastly, if degeneration occurs before proliferation and endocyst detachment, the cyst results *caseous* (Fig. 2g).

Hyperlaminated granular cysts are, on the contrary, largely filled with SLT and minor amounts of granular aggregate especially close to the pericyst (Fig 2i). This suggests that the degenerative process begins in old cysts formed several years before, after possible detachment and folding of the endocyst and possible DC production.

Lastly, the *hyperlaminated gelatinous* cysts show on their inside practically only SLT, that, because of tissue degeneration, assume a diaphanous appearance and gelatinous consistency characterizing this

typology (Fig. $2f_2$). Similarly to the hyperlaminated granular cysts, this degeneration likely begins in old hyperlaminated cysts.

Clearly, the different types of hyperlaminated cysts are related to dead forms of the parasite, that nevertheless can remain silent for years in the host.

The residual hooks or highly degenerated protoscoleces observed in the majority of hyperlaminated cysts, irrespective of type of degeneration, suggests that these specimens are formerly fertile cysts, highlighting the degenerative process described.

The other observed typologies of cysts, i.e. *septated*, *serous* and *acephalocysts*, as described in detail in Bortoletti *et al.* (2002), can all be appropriately located in the described parasite transformation process.

Conclusion

As a whole the data presented describe what happens or what may happen to the larval form of *E. granulosus* over time in the human liver during the different phases of development, evolution, degeneration and death. A knowledge of these events, together with imagery and information gathered from the study of immune response kinetics, resulting in identification of prognostic immunological markers, can assist in optimizing therapeutic choice (surgery, PAIR, chemotherapy etc.), including the "wait and see" approach when considered appropriate in relation to cyst typology, position within the organ and size, patient age and medical condition.

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Twenty years of percutaneous treatments for cystic echinococcosis: a preliminary assessment of their use and safety

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Abstract. Image-guided percutaneous treatments for echinococcal cysts were introduced in the mid-eighties. Today they represent a third therapeutic option, after surgery and benzimidazole derivatives. Two types of percutaneous treatments are available, based on the destruction of the germinal layer or the evacuation of the endocyst. To assess the extent of their use and their safety, a Medline search of the literature on this subject was performed. The number of cysts treated, their anatomical sites, the complications and, length of follow-up (when available), were all examined. The results show that percutaneous treatments for cystic echinococcosis are safe and efficacious in selected anatomical sites, provided basic safety issues are correctly addressed. However, before drawing final conclusions, a more detailed analysis of the literature is needed. Percutaneous treatments could be simplified and made more effective if a scolecidal agent could be found that melts the entire endocyst without causing harm to the biliary epithelium.

Key words: percutaneous treatments, PAIR, cystic echinococcosis, hydatidosis.

Percutaneous treatments for abdominal cystic echinococcosis (CE) are 20 years-old (Mueller *et al.*, 1985). Introduced in the mid-eighties, they were met with skepticism, but their increasing use over the years showed them to be an acceptable third therapeutic option for the treatment of cystic echinococcosis, after surgery and benzimidazole derivatives (mebendazole and albendazole).

The aim of these treatments is to destroy the germinal layer with a scolecidal agent or to evacuate the germinal and laminated layers, i.e. the entire endocyst (Table 1). The germinal layer is the key target of any treatment: hundreds brood capsules cover each square centimeter of its surface. Each brood capsule contains 4-5 protoscolices - each protoscolex can generate a daughter cyst.

The first percutaneous treatment to be employed was to Puncture, Aspirate, Inject a scolecidal agent and Re-aspirate the cyst (PAIR). This procedure was only effective with unilocular cysts, and therefore new percutaneous procedures were sought to deal with the other types of cysts.

Table 1. Types of percutaneous treatments by cystic structure.

Destruction of germinal layer	Evacuation of the cyst content
PAIR	PEVAC
Radio Frequency Thermal Ablation	DMFT
	Cutting devices
	Large-bore catheters

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Background: A summary of the current options

Group 1. Destruction of germinal layer

PAIR

The basic PAIR technique is widely known. Several series with minor variations of the essential steps have been published. However, there are only two randomized, prospective studies reported (Khuroo and Dar, 1993; Khuroo and Wani, 1997) and only one paper reports a follow-up reaching 11 years(Giorgio and Tarantino, 2001). Khuroo and Wani (1997) showed PAIR in association with benzimidazole derivatives to be as effective as open surgical drainage and to be less expensive with fewer complications.

Giorgio and Tarantino (2001) showed that PAIR of multivesiculated cysts never allowed complete healing (solidification) and in 30% of cases resulted in an intracystic recurrence that required up to four repeat procedures. Smego and Bhatti (2003) conducted a meta-analysis of 21 articles that involved 769 patients with 1072 hepatic cysts undergoing PAIR and compared the findings with 952 era-matched historical control subjects undergoing surgical intervention.

The articles all had acceptable details of the treatment, complications, outcomes and follow-up. The rate of clinical and parasitologic cure was greater in patients receiving PAIR plus chemotherapy.

Disease recurrence, major complications (anaphylaxis, biliary fistula, cyst infection, liver/intra-abdominal abscess, and sepsis), minor complications and death occurred more frequently among surgical control subjects. The mean durations of hospital stay were 2.4 days for patients treated with PAIR and 15.0 days for the surgical control group. The study

concluded that, compared with surgery, PAIR together with chemotherapy is associated with greater clinical and parasitologic efficacy, lower rates of mortality and morbidity, lower disease recurrence rates and shorter hospital stays.

Radio Frequency Thermal Ablation

Radiofrequency thermal ablation has been shown to be a safe method of destroying the germinal layer. Using the same kind of needle-electrode employed in the ablation of tumors, we treated 2 patients having solid cysts with daughter cysts without causing any complications (Brunetti and Filice, 2001). Because the contents of the cyst are destroyed by heat rather than a chemical agent, the procedure is simpler than the PAIR treatment since it avoids the need to inject and aspirate a scolecidal agent. However, we have not prevented the recurrence of cysts (Brunetti, unpublished) and therefore further investigations need to be carried out before it can be recommended as an effective percutaneous treatment.

Group 2. Evacuation of endocyst

These techniques are generally reserved for cysts whose contents are difficult to drain, or that tend to relapse after PAIR (multivesiculated cysts or cysts with prevalently solid content and daughter cysts). Cutting devices and large-bore catheters are employed to extract the germinal and the laminated layer from the cavity.

Cutting devices

A large-bore cutting aspiration device was used by Saremi and McNamara (1995) in 32 patients in order to fragment and evacuate daughter cysts and laminated membrane. The catheter drainage was continued for four weeks. A two-year follow up showed a high rate (90%) of success and a low incidence of major complications (3%). A Chinese group (Vuitton *et al.*, 2002), treated 699 multivesicular and abdominal cysts with a device called dilatable-multi-function trocar (DMFT). This is linked to an aspiration apparatus which extracts endocyst, daughter cysts and other cystic contents. Then the cavity is irrigated with 10-20% saline and, if necessary, curettage is done. The catheter remains in the cavity for 2-3 days. No deaths but four anaphylactic reactions were observed. The recurrence rate in a 3-year follow-up was 2.3% in situ and of 1% in other locations.

Large-bore catheters

Large-bore 14 Fr van Sonnenberg sump drainage catheters, under sonographic guidance, were used by Haddad and Sammak (2000) to treat 9 solid cysts with daughter cysts. They evacuated the cyst contents and irrigated the cavity with hypertonic saline. At 15 months, there were no major complications, no deaths, and no recurrences.

Schipper and Lameris (2002) treated unilocular and multivesiculated cysts, including complicated ones, with PEVAC (short for PERcutaneous EVACuation). Aspiration and evacuation of cyst content were performed with a 14 French catheter. Cystobiliary fistulae were treated with an endoprosthesis introduced endoscopically into the common bile duct. Twelve patients were treated. In a mean period of 17.9 month (range 4-30), seven cysts disappeared and five decreased in size. The main complications of this procedure were cystobiliary fistulae and infections. These complications prolonged hospitalization from 11.5 days (8-14) to 72.3 days (28-128). PEVAC is a safe and effective alternative percutaneous treatment for multivesiculated cysts and for those communicating with the biliary tree, but treatment morbidity is high and the catheter time can be very long.

Safety

Although there are no standard protocols for the treatments described, their safety must be assured by addressing at least the major risks of puncturing echinococcal cysts, namely anaphylactic shock, and secondary echinococcosis from spillage of cystic fluid during the procedure. For treatments involving the intracystic injection of a scolecidal agent, a third risk is chemical cholangitis due to contact of the scolecidal agent with the biliary tree.

To reduce those risks, three conditions must be met:

(1) Resuscitative measures must be available in case of anaphylactic shock.
(2) Peri-interventional prophylaxis with benzimidazole must be performed.
(3) Communications with the biliary tree must be excluded before injection of any scolecidal agent, as they all can cause chemical cholangitis.

Evaluation of the literature. Methods

In order to evaluate the extent of use of percutaneous treatments and their overall safety, a Medline search of the literature published in English on this subject was performed using the key words "Echinococcal cysts", "Hydatid cysts" "Cystic Echinococcosis", "Hydatidosis", "PAIR", "Percutaneous treatment", "Percutaneous drainage", "Ultrasound" as keywords. Papers published from 1983 through 2004 were included. The authors' files were used as well. If the original article was not available, the abstract was used if it reported the number and location of treated cysts and major complications. Major complications were defined as death, anaphylactic shock, secondary echinococcosis due to spillage of fluid and peritoneal seeding. All other complications were defined as minor. The stratification of these minor complications by stage of the disease and anatomical location of the cyst was not performed. Cysts punctured for diagnostic purposes were included in the calculations of the risk of ana-

phylactic shock but excluded from calculations of the risk of other complications.

Results

The results of our survey are summarized in Tables 2, 3, and 4. Data on percutaneous drainage of 4209 cysts in 3005 patients from 96 articles were obtained. Of these articles, 46 were not available and their abstracts were used instead. Of 4209 cysts, 166 were punctured for diagnosis and 4043 for treatment (Table 2). As expected, the majority of those cysts occurred in the abdomen, mostly in the liver. Treatment of cysts located in specific abdominal sites, like kidney, pancreas and spleen, was reported in some papers. Treatments of cysts with rare locations (i.e. thyroid, parotid, eye, spine and breast) were reported as well (Table 3).

It was encouraging that major complications were rare. Only two deaths (0.047%), related to anaphylactic shock, were reported. Anaphylactic shock that responded to resuscitative measures was reported in 14 cases. Overall anaphylactic shock rate was 0.38%. Minor complications rate was 6.62%. Recurrence rate was 1.27 % (Table 4).

Table 2. Tabulation of cysts and patients by diagnostic and therapeutic percutaneous procedures.

	Total number	Diagnostic aspiration	Therapeutic drainage
Cysts	4209	166	4043
Patients	3005	164	2841

Table 3. Anatomical location of cysts.

	Location of cysts No.	%
Liver	2278	54.1
Lung	19	0.45
Kidney	23	0.54
Spleen	21	0.50
Pancreas	4	0.09
Abdomen	1636	38.86
Thyroid	3	0.07
Parotid	1	0.023
Ocular	1	0.023
Spinal	7	0.16
Breast	4	0.09
Soft tissues, muscles	12	0.28
Miscellaneous (abdomen, thorax, bone, soft tissues, thyroid, breast, spine)	168	3.4
Not available	32	0.7

Discussion

Mortality and major complications rates are acceptably low, probably as a result of taking the basic precautions mentioned above. All groups reported the availability of resuscitation maneuvers and most groups adopted peri-interventional prophylaxis for secondary echinococcosis. Few groups reported taking precautions against the risk of chemical cholangitis when scolecidal agents were injected into the cyst cavity. No groups reported any cases of cholangitis. These results show that the fear of anaphylactic shock as a reason not to perform these treatments (Yaghan and Heis, 2004) is unwarranted.

We found no mention of secondary echinococcosis as a result of spillage of fluid. This may be the result of albendazole prophylaxis, or of the difficulty in diagnosing this side effect (abdominal CT should be regularly performed 3-5 years after the procedure), or both. We found no reports of monitoring patients for secondary echinococcosis following spillage of cystic fluid. We also found no report of cholangitis secondary to the damage of biliary epithelium from contact with scolecidal agents. It is not clear if this is a rare event or the result of precautions taken during the procedure to rule out cysto-biliary fistulas by looking for bile-stained fluid or doing cystography before injecting scolecidal agents.

The rate of minor complications is also low. It will be important in future literature evaluations and investigations to enumerate minor complications in order to be able to assess the safety and effectiveness of various percutaneous treatments.

Percutaneous treatments have been used outside the abdomen, usually when other methods have failed. Some sites are intrinsically difficult to treat: for example, percutaneous puncture of spinal and

Table 4. Complications and recurrences of percutaneous treatments, 1983-2004.

	No. punctured cysts	No. of events	% Complications
Deaths due to anaphylactic shock	4209	2	0.047
Major complications *	4209	16	0.38
Minor complications **	4043	268	6.62
Recurrence ***	3830	49	1.27

* Major complications (anaphylactic shock) were calculated on all cysts punctured.
** Minor complications were calculated only on cysts punctured for therapeutic reasons (4043).
*** Rate of recurrence is calculated only on cysts punctured for therapeutic purpose (4043). No information about recurrence was available in 19 studies for 213 cysts.

paraspinal cysts have failed, but it is well known that any treatment in such areas, short of early radical surgery, is bound to fail. For some locations such as the lung, percutaneous treatments should only be used as a last resort.

The recurrences was low (1.27%), but this figure must be interpreted carefully. No information was available on 213 cysts in 19 studies. Further, the definition of therapeutic success or cure is too complex in this disease to be covered in this short presentation. The duration of follow-up is still an unresolved question. Unfortunately, of 65 papers only 4 had a follow-up longer than 4 years. Because cyst morphology, when monitored after treatment, has often been shown to change very slowly, the minimum follow-up period should be 5 years. We have seen one case of local recurrence in a successfully treated cyst 10 years after PAIR (Brunetti, unpublished).

The importance of long term follow-up is shown in the paper by Giorgio and Tarantino (2001) who reported repeated failures of PAIR in multivesiculated cysts. These findings should prompt clinicians to use PAIR exclusively for unilocular cysts, with or without detached endocyst. In multivesiculated cysts other percutaneous treatments (cutting devices, large bore catheters) should be used. However, these treatments are cumbersome when compared to PAIR. Recently, an experimental study on the effect of different scolecidal agents on the endocyst showed complete melting of the endocyst in a few minutes with a 2.5% solution of sodium hypochlorite and in 1 hour by a 0.1% sodium hypochlorite solution (Karaoglanoglu and Akinci, 2004). This may represent an important advancement if further investigations showing that this agent in these concentrations is not harmful to the biliary epithelium.

Finally, our survey highlights the importance of a uniform sonographic classification for assessing studies of echinococcosis treatments. The WHO Informal Working Group on Echinococcosis (WHO-IWGE) recently proposed such a classification of echinococcal cysts based on their ultrasound appearance (2003). We urge all groups working in this field to adopt this classification scheme in order to test its usefulness in defining the extent and condition of the cyst and its modifications after treatment.

Conclusions

This brief summary of the literature shows that percutaneous treatments of echinococcal cysts are performed frequently and represent a safe, therapeutic alternative to the traditional surgical removal of endocyst. It is clear that anaphylactic shock is a rarity, eliminating this complication as a concern for percutaneous treatment of echinococcal cysts. However, there needs to be a more systematic study of the frequency and types of other complications by the method of percutaneous treatment used and by the stage of the disease. The safest and most effective technique (PAIR) has been shown to be effective only for unilocular cysts with or without detached endocysts. For other types of cysts (multivesiculated cysts and solid cysts with daughter cysts), there are no accepted methods though new approaches are being explored. Finally, as a means of evaluating future studies of percutaneous treatments, it is important to classify cysts by the sonographic characteristics recently proposed by the WHO Informal Working Group on Echinococcosis (WHO-IWGE).

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Epidemiology of hydatidosis in the province of Sassari, Italy

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Abstract. Cystic echinococcosis is endemic in certain parts of the world, including Sardinia, Italy. It was performed a study in the province of Sassari in order to evaluate the incidence of the infection in man and the effects of control programs since 1964 to 2002. Data obtained by surgical records, hospital discharge forms, radiological and pathological files were collected using a case report form. During the years 1964-2002, 2702 new cases were identified (average annual incidence: 17 per 100,000) and 1981 (73.3%) were submitted to surgical treatment. In 57.3% municipalities no cases were observed during the years 1998-2002. Males are more affected (56.2%), mostly farmers-shepherdess (68.6 per 100,000) and pensioners (59.6 per 100,000). Control measures led to a significant decline in the incidence rate of hydatidosis during the period 1964-2002, dropping by 27.6 per 100,000. The mean age of surgical patients increased during the years of surveillance, such as the surgical liver/lung ratio as a consequence of a *cohort effect*. The durability of control programs is the corner stone for obtaining a significant decrease of this infection.

Key words: hydatidosis, Sardinia, surveillance, incidence rates.

Hydatidosis is a widespread zoonosis affecting numerous animals, including man that may accidentally becomes intermediate host as a consequence of eating raw vegetables contaminated by the feces of the dogs or intimate contact with infected dogs. Cystic echinococcosis is endemic in certain parts of the world (Sayek *et al.*, 2004). The highest Italian frequency of the disease is found in Sardinia, island of the Mediterranean sea (24,090 km^2), where many people are involved in sheep raising (Gabriele *et al.*, 2004).

In Italy, the first control program was set up since 1955 in order to decrease the high parasite prevalence in definitive host. Five years later, a regional campaign for Sardinia based on anthelmintic treatment of dogs, war to stray, health education campaign, surveillance municipal slaughterhouses, started in the province of Nuoro, followed by Sassari and Cagliari. In 1978, the second Sardinian control program started, followed after 10 years by a new strategy, pointing out the need of therapy of infected dogs, war to stray (subcutaneous micro-processor), municipal slaughterhouses, health education. In 1993 lack of financial support blocked evident advantages (Arru *et al.*, 1999).

In order to study the epidemiology of hydatidosis in Northern Sardinia and the effects of preventive programs, a surveillance system has been performed since 1964 (Bo *et al.*, 1978; Mura *et al.*, 1981; Maida *et al.*, 1988, 1994, Castiglia *et al.*, 2001).

Materials and methods

Data on hydatidosis in Northern Sardinia during the period 1998-2002 were collected and reviewed as in our earlier studies(Bo *et al*, 1978; Mura *et al.*, 1981; Maida *et al.*, 1988, 1994; Castiglia *et al.*, 2001). Source of data were hospital discharge forms, surgical registers, radiological and pathological records. Case report form for each person was used in order to enter demographic (age, sex, job, residence) and clinical data (year of admission and/or of operation, relapse, signs and symptoms, number and localization of cysts, classification of hydatid cysts, treatment adopted). Information obtained after epidemiological surveillance updated a pre-existing database, containing information since 1964. Descriptive analysis was performed using STATA software.

Average annual incidence rates were calculated on the basis of resident population. The incidence linear trend by year of diagnosis was tested according to linear regression model (α=0.01).

Results

In the present survey (1998-2002), 94 new cases (4.1 per 100,000 inhabitants) and 10 relapses were found (males: 54.8%; surgical treatment: 74%).

During the years 1964-2002, 2702 new cases and 190 relapses were identified in the province of Sassari. Surgical treatment was performed in 1981 new cases (73.3%) and in 92 relapses (48.4%). Cases, including symptomatic and asymptomatic patients, were identified using mainly surgical records and hospital discharge forms, while a lot of asymptomatic persons were identified using radiological or pathological files only.

The mean annual incidence rate changed between 1964 and 2002, from 31.1 per 100,000 to 3.5 per 100,000, with a significant decreasing incidence linear trend (b=–0.72, p<0.001) as a consequence of the 3 control campaigns started in 1962, 1978 and 1987 (Fig. 1).

Since 1964 it has been evident the effect of con-

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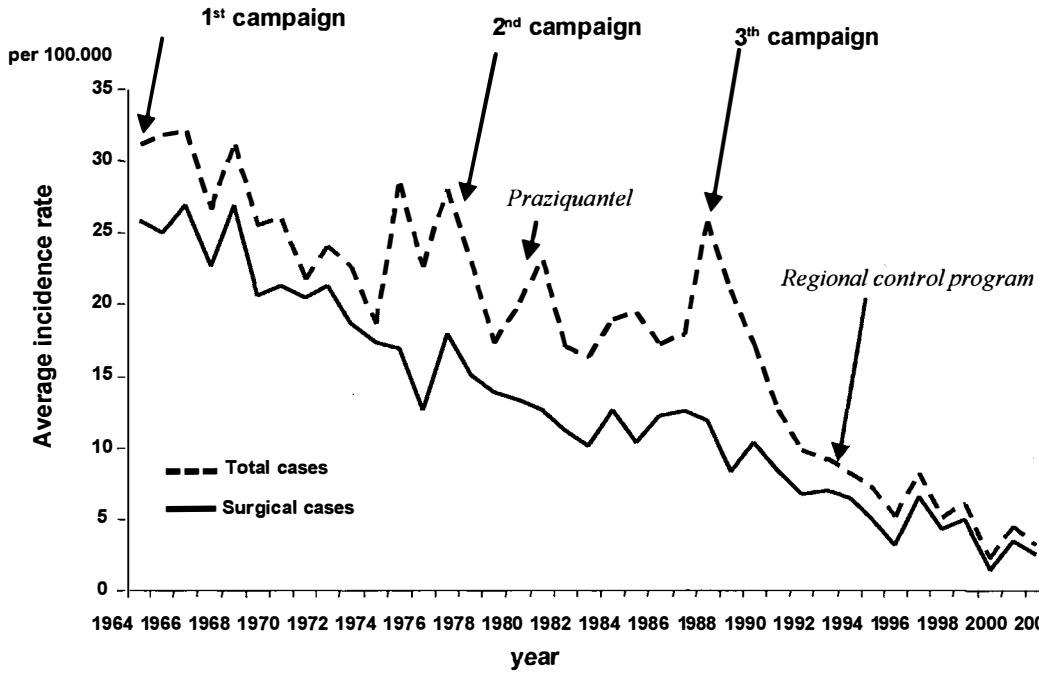

Fig. 1. Trends of hydatydosis incidence per 100,000 inhabitants in province of Sassari during the period 1964-2002.

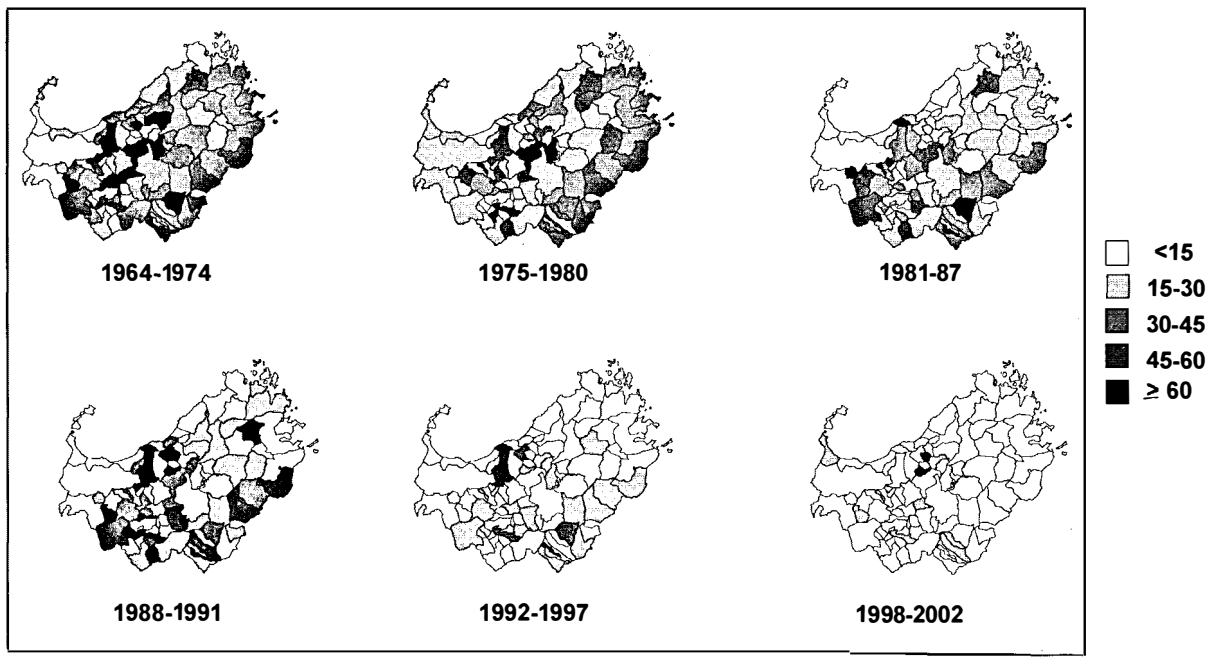

Fig. 2. Incidence rates per 100,000 in Northern Sardinia during six consecutive periods considering municipal areas.

trol measures (Fig. 2): at the present time mean annual incidence rate is below 15 per 100,000 in 30.3% of the municipalities (74.1% of the resident population), above 25 per 100,000 in 3.4% (0.7% of the resident population) and no cases in 57.3% (22.6% of the resident population).

Males are more frequently affected (56.2%), though it was observed a reversed male/female ratio in young-adult age (20-29 years old).

During the surveillance period, it was observed that *E. granulosus* affected mostly people aged above 50 year-old, in particular retired people and housewives. The mean age of surgical patients increased during the years of surveillance, such as the liver/lung surgical ratio increasing from 1.4 to 12.9.

Main professional categories affected by cystic echinococcosis were farmers and shepherdess (68.6

per 100,000), followed by pensioners (59.6 per 100,000). It is remarkable a relative persistent number of cases among students in the last epidemiological assessments.

Discussion

The incidence rate drastically declines during the years. This decreasing trend is significant considering the availability of actual diagnostic examinations (high specificity and sensitivity) associated to the effect of preventive measures elaborated from 1960s. Therefore, we think that the last incidence rates are biased: an increasing number of old patients in the recent investigations may be classified as "prevalent cases", identified through the high sensibility of diagnostic procedures (ultrasonography). This aspect, associated to the introduction of new therapies, such as percutaneous aspiration, explains the increasing surgical liver/lung ratio evidencing an important *cohort effect*; consequently, the parasite burden might be lower than the surveillance system evidenced. Nevertheless, after 4 decades from the first hydatidosis eradication plan is still endemic in Sardinia. Despite imperfect preventive campaigns, results appear comparable to those reported from La Rioja, Spain (Jeménez *et al.*, 2002).

In order to obtain better results, it is necessary to concentrate resources on uncontrolled animals and on an intensive and permanent health information among young people, looking at the relative persistent number of cases among students.

Financial support influence durability of a preventive program and permanent availability of economic resources represents the basis for a long term success.

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Immunobiology of cystic echinococcosis

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Abstract. The present report focuses on the ability of cystic echinoccocosis metacestode to survive for a long time, despite host immunity, by developing avoidance strategies. The tactics believed to come into play, ranging from intrinsic parasitic factors to host-related mechanisms, are briefly discussed and the importance of studies on experimental models is highlighted particularly in relation to furthering the theoretical understanding of the dynamic equilibrium between host and parasite, and to the feasibility of practical approaches in controlling the disease by artificial manipulation of the immunoregulatory mechanisms.

Key words: cystic echinococcosis, immune response, avoidance strategies, experimental secondary hydatidosis, human infection.

The host/parasite (h/p) relationship is interactive and exists in balance and tension. On the one hand, the host aims to eliminate the parasite minimizing immunopathological reactions that may result from while the parasite attempts to survive in the host as long as possible. In order to do this, to avoid falling prey to the elicited immune response, the parasites have selectively evolved a wide range of strategies for confusing, deflecting or evading the host immune response. In the simplest terms, while an effective immune response may clearly lead to the parasite death, at the same time an inappropriate response may also be detrimental to the parasite, which continuing its unrestrained growth, may eventually lead to the death of the host, with the inevitable repercussions on its own survival. Therefore a regulated immune response appears paradoxically useful in many cases for parasite survival and growth.

Because of h/p interplay dynamic, the outcome of infection depends on the balance achieved by the combination of the different variables involved with the host immunity and the parasite avoidance strategies (virulence of parasite species or strains, susceptibility/resistance of the host, both genetically controlled, parasite burden, infection frequency, etc.).

Cystic Echinococcosis (CE) and its experimental models provide a fertile ground for studies in this sphere. The natural history of echinococcosis (Bortoletti *et al.*, 2005) has shown extremely variable pathogenesis ranging from spontaneous cure (abortive forms) to chronicity, suggestin that it can be regarded as a "polar disease" with very diverse features depending on the kind and intensity of the host immune response.

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Avoidance strategies

A variety of avoidance stategies are believed to be involved in CE (antigenic polymorphism, parasite sequestration, antigen mimicry, low immunogenicity, inefficient or blocking antibody isotypes production, inappropriate effector T cells, production of immunosuppressive molecules, polyclonal activation, shift of Th1/Th2 polarization, etc.), ranging from intrinsic parasitic factors to host-related mechanisms.

Concomitant immunity and "race-in-time"

Larval infection in CE is characterized by the slow growth of cysts in the internal organs of suitable host and frequently throughout its entire life span. Once the cyst is established, it seem unrestrained by host immunity though an effective immune resistance against egg re-infection occurs. This pattern indicates a state of "concomitant immunity", due to the co-existence of immunity to oncospheral challenge and tolerance to implanted larvae.

The theoretical background for the subsequent development of the recombinant oncospheral antigen vaccine EG95 (Lightowlers *et al.*, 1996; 1999; 2004; Heath *et al.*, 2003) was from early observations on resistance to egg challenge in sheep (Sweatman et *al.*, 1963; Yarulin, 1968; Aminzhanov, 1976), seminal studies with *E. granulosus* and *Taenia ovis* during the 1960s by Gemmell, his field observations on prevalence of the infection in sheep according to host age in endemic or hyperendemic areas (Gemmell, 1962; 1964; 1966; 1967; Gemmel and Soulsby, 1968; Gemmell *et al.*, 1987), together with significant data gathered from research with the *Taenia taeniaeformis* model in laboratory animals (Campbell, 1936; 1938; Rickard and Bell, 1971; Kwa and Liew, 1977; Ayuya and Williams, 1979; Rajasekariah *et al.*, 1980a; 1980b; 1982; Conchedda and Ferretti, 1983; 1984). Antibody and complement-mediated lysis of oncospheres appears to be the major host-protective mechanism involved, although other means of defence cannot be excluded. Seroprevalence of antibodies able to kill oncos-

pheres *in vitro* (Rogan *et al.*, 1992) is higher in endemic than in non-endemic areas, also in apparently cyst-free subjects (Craig, 1993).

Antibody role is further emphasized by passive transfer of protection with serum in experimental hydatidosis in mice (Dempster *et al.*, 1992), as in the *T. taeniaeformis*/mouse system. In this model, IgG2a from susceptible hosts confer protection to recipient animals if transferred before or early after infection, the parasites rapidly becoming invulnerable through mimicry (Bortoletti and Conchedda, 1983). In the "race in time", we suggested to take place between rate of antibody production by the host and loss of susceptibility to Ab-mediated killing by the parasite (Conchedda and Ferretti, 1984), larvae are precociously destroyed in rapid-responder mice. Slow-responder strains on the other hand fail to produce enough antibody in time before larvae become insusceptible and parasites are rapidly surrounded by a halo of acid mucopolysaccarides preventing contact between host cells and parasite tegument (Bortoletti and Ferretti, 1985). No halo is observed (probably suppressed or lost) around cysts destined to be killed (Bortoletti *et al.*, 1985), which are in fact surrounded by a large quantity of inflammatory cells, mainly neutrophils, eosinophils and macrophages.

Sequestration, regulated Ag exposition and laminar layer role, Ag masking

Although the hydatid cyst probably represents the largest antigenic structure able to survive in the host tissues, with a potential ability to elicit a major inflammatory response, after resolution of an initially more intense reaction, mild inflammation around the established cyst occurs causing minimal disruption of host tissue. The laminar layer (LL) formation is thought to be the pivotal point in the avoidance process. Its structure seems to be a relatively impermeable barrier with resulting *sequestration* of parasite antigens. Actually (Coltorti and Varela-Diaz, 1974; Conchedda *et al.*, 1988; D'Amelio *et al.*, 1989), LL is permeable both to host macromolecules and to parasite Ag, as indicated by the recovering of host IgG in the cyst fluid and of circulating parasite Ag or immune complexes into the host. In the light of this, a more advanced form of antigen sequestration, i.e. a *regulated antigen exposition*, is believed to take place. Several data support it: e.g. infected sheep, though exhibiting a low Ab production, develop an anamnestic response to cyst fluid injection (Lightowlers *et al.*, 1986). Preliminary results of our on-going research into the immunological aspects related to CE clinical features (Conchedda *et al.*, 2004) in the different morpho-structural cyst typologies illustrated by Bortoletti (Bortoletti *et al.*, 2005, and Bortoletti *et al.*, 2002), show that almost invariably Ab titres increase immediately after excision (within 2 months) when cysts are opened and drained during surgery, compared with pericystectomy of intact cysts.

As is well known from other parasites, one evasion mechanism is antigen masking through accretion of host proteins. This strategy is operative in the *Taenia taeniaeformis* model, as shown by our experiments on parasite surgical transplantation from mice to rats (Bortoletti *et al.*, 1983). Transferred strobilocerci are rapidly destroyed in the recipient host (rats) immunized against the donor host (mice), conversely surviving in non-immunized controls. Evidence of molecular mimicry has been provided by several *Taenia* species, the parasites escaping immune response through host-like molecules, such as substances with blood group specificity (*E. granulosus*), or cytovillin or fibronectin-like molecules (*E. multilocularis* and *Taenia solium* respectively) (Dixon and Jenkins, 1995). Specifically in CE, Fc-binding molecules have been observed on the protoscolex surface, binding mainly to γ1 and γ3 isotypes (Baz *et al.*, 1998), though interference with IgG1 and IgG3 mediated effector functions has not been explored nor has parasite or host-origin been determined.

Complement role in evasion strategies

Potential triggers for both classic and alternative pathways of complement (C) are present in the hydatid cyst (immune complexes and neutral carbohydrates respectively) while the MBL pathway does not seem to be involved.

Infective stages (oncosphere for primary cysts and protoscoleces for secondary cysts) are lysed by C *in vitro* and dead protoscoleces or their soluble extracts are also able to activate the alternative pathway of C (Kassis and Tanner, 1976; Irigoin *et al.*, 1996).

In vivo a great deal of intraperitoneally inoculated protoscoleces are precociously destroyed, while the surviving parasites appear to become invulnerable to C-mediated killing once vesiculation and differentiation into cysts has commenced (Kassis and Tanner, 1976). The consumption of C components by disrupted protoscoleces, lowering them around the living parasites remaining, has been indicated as an evasion mechanism during early infection.

At the metacestode level, the cyst fluid, a potentially strong C activator by high molecular weight carbohydrates, is on the other hand "sequestered" within the cyst and released at an insufficient rate to cause considerable activation, unless traumatic cyst rupture has occurred. In addition, active parasite C-activation controlling mechanisms occur, such as sequestration of the host-derived negative regulator factor H by a non-identified parasite factor (probably sulphated glycosaminoglycans like in *T. taeniaeformis*), and inhibition of alternative pathway C3 convertase (able to act synergistically with the first) by a parasite-derived, heat-stable non proteinaceous inhibitor.

Immunomodulation

Data for human natural infection and for secondary experimental hydatidosis show glycoconjugates to

be involved in immunomodulation in CE. Actually carbohydrate epitopes of protoscoleces are major immunogens (Miguez *et al.*, 1996), but they induce production of low avidity Ab (Ferragut and Nieto, 1996), particularly IgG3 and IgM in mice (Severi *et al.*, 1997), and IgG2 in hydatid patients (Sterla *et al.*, 1999), acting as "blocking" Ab to effector systems mediated by other isotypes (IgG1 and IgG4).

In the experimental model, modulation of the cellular response is also thought to be mediated by a carbohydrate rich fraction ($E4^+$) from protoscoleces (Dematteis *et al.*, 2001). This component, besides apparently being involved in immunosuppression phenomena, through IL-10 production, polarizes the immune response towards a Th2 response.

The influence of metacestodes on immune response involves both cytotoxicity and immunoregulation. They are not mutually exclusive options, and immunomodulatory mechanisms, such as non specific mitogenesis, may paradoxically act in combination with immunosuppression and inhibition of lymphoproliferative response to mitogens. In mice infected with protoscoleces a rapid initial proliferation in paracortical areas of the draining lymph node is followed by a marked depletion of the T cells, B cell proliferation suggesting polyclonal expansion (Riley *et al.*, 1985; 1986; Riley and Dixon, 1987). Study of subpopulation alterations indicated a decrease in Lyt1+:Lyt2+ ratio, i.e. a depletion in CD4+ cells from 14 days p.i., after an initial expansion, and a parallel increase of CD8+ from day 8 p.i.

In vitro studies confirm that metacestode secretions contain non specific mitogenic factor(s) for T-cells together with broadly T-cell suppressive factors. Experimental data, indicating induction of mitosis accompanied by paradoxical decline in S-phase activity, suggest cell-cycle dysregulation to be a general feature in CE (MacIntyre *et al.*, 2000; 2001).

Immunoregulatory effects of hydatids appear to be expressed locally around the cyst, apart from draining lymphonodes. In the experimental alveolar hydatid lesions in mice, granulomatous cell composition and proportion of T-cell subpopulations correlate with regression or progression of lesions (Bresson-Hadni *et al.*, 1990). Early after infection CD4+ predominate in all strains, persisting throughout development up to 6 months p.i. in resistant mice, and being conversely progressively replaced with CD8+ between 1 and 4 months p.i. in the susceptible strains. The same findings are also reported in humans infections, CD8+ being predominant in the pericystic granuloma of alveolar echinococosis active lesions and CD4+ in the few degenerating cases (Vuitton *et al.*, 1989).

Recent immunohistochemical observations show a similar picture in the pericystic adventitia of CE cysts from bovines (Sakamoto and Cabrera, 2003): infiltrating lymphocytes are composed mostly of CD4+ in regressive hydatid cysts, and of CD8+ in progressive ones. In addition eosinophils and their granules, up-regulated by IL-5 secreted by Ag-stimulated lymphocytes, accumulate adhering to the LL and infiltrate lesions producing vermiculate erosions and contributing to the formation of regressive lesions.

There is increasing evidence that anti-chemotactic parasite factors are able to immunomodulate reaction or factors influencing Th1/Th2 bias. It has recently been suggested that the 12-kDa subunit of AgB, known to inhibit neutrophil recruitment (Shepherd *et al.*, 1991), in addition may elicit a protective Th2 response (Riganò *et al.*, 2001) that benefits the parasite.

Th1/Th2 polarization

One of most significant advance in understanding immune response is the Th1/Th2 paradigm by Mosmann and Coffman showing how the immune system directs responses to different pathogens. Outcome of infection in terms of resistance/susceptibility is in fact related to pattern of T cell subpopulations and of cytokines produced. Parasitic infections frequently result in highly polarized response characterized by dominant Th1 or Th2 cytokine profile. Generally it has been reported that intracellular parasites induce a Th1-polarized response, whereas extracellular helminths preferentially trigger Th2-dominated responses. Actually the Th1/Th2 cell commitment is quite complex, as shown by data from hydatidosis in humans, and polarization is decided by a series of different factors. As is well known and highlighted in Siracusano report and in communications by Riganò and Ortona during the present Congress, elevated levels of IgG1, IgG4 and IgE isotypes are reported in CE patients and most surveys agree with a Th2 bias in chronic infections. Nevertheless, studies on immune response during chemotherapy indicate a more dominant Th1 cytokine profile in patients better responding to treatment (Riganò *et al.*, 1995; 1999), suggesting that, unlike Th2, the Th1 polarization in later phases of development may concur with chemotherapy in cyst killing. The experimental secondary hydatidosis model may be of great help in investigating bias during the developing stages compared to later phases, when immune evasion strategies are already operative. Our research (Conchedda *et al.*, 1998; 2001 and unpublished) on cytokine production during experimental secondary infection shows an early Th1/Th0 profile, with high IFN-γ production during the first weeks, when the majority of inoculated protoscoleces are killed, unless injected within diffusion chambers protecting them from cell attack. Adherent PEC, i.e. IFN-γ-activated macrophages, are the central cells of this killing, probably by NO release, whereas Th2 (IL-4 and IL-10 production) becomes dominant later, probably because Ags of these more advanced phases (particularly by action of LL) down-regulate inflammation producing an inhibitory effect on macrophage activation. Polarization shifts during infection, from an effective protective reaction (Th1), to a response

more functional to parasite survival (Th2) (Conchedda *et al.*, 1998; 2001 and unpublished). Despite this, the cysts would still be potentially susceptible to Th1-driven macrophage attack (Steers *et al.*, 2001), but when crude extract of LL is added to *in vitro* cultures or injected *in vivo* in secondary infection, a dose-dependent reduction of NO release is observable due to the phagocytosis of LL fragments by the macrophages.

Elevated NO levels have been reported by now in some hydatid patients (Touil-Boukaffa *et al.*, 1998), if and how it correlates with the fate of cysts and their different morpho-structural involution requires further investigation. Our preliminary results on immune pattern according to cyst degeneration show that seropositivity increases from unilocular cysts to multivesicular with daughter cysts, it persists in transitional cysts but decreases in highly degenerated cysts (Conchedda *et al.*, 2004). Additional comparative analysis with cytokine patterns in these different well-detailed morpho-structural and clinical pictures will likely provide a more comprehensive understanding of immunological cyst killing and, in conjunction with research in experimental studies, may assist in identifying potential triggers for a protective Th1 bias.

Conclusive remarks

In conclusion, investigations on the CE host/parasite relationship and in particular on the ability of the metacestode to survive by avoidance strategies, combining results from natural and experimental infections, appear a very profitable area of study. Research, on account of rapidly accumulating knowledge on the issues dealt with, should contribute to furthering the theoretical understanding and suggest new practical approaches for controlling this disease by means of artificial manipulation of the immunoregulatory mechanisms.

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Veterinary public health activities at FAO: echinococcosis/hydatid disease

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Abstract. Cystic hydatidosis is a zoonotic disease that remain as a significant cause of human morbidity and mortality in many parts of the world. The disease has veterinary public health implications. FAO is involved with some activities in the control of echinococcosis/hydatid disease: within the Animal Production and Health Division the Veterinary Public Health (VHP) Programme is constituted by members of the different Services (Animal Health, Animal Production, and Livestock Policy) within the Division. FAO regular programme has also established a global network of professionals directly involved in VPH. Furthermore FAO's Technical Cooperation Projects (TCP) is a tool to assist member countries in responding to urgent and unforeseen demands.

Cystic hydatidosis is a zoonotic disease that remain as a significant cause of human morbidity and mortality in many parts of the world. The disease has veterinary public health implications. Moreover, hydatid disease affect ruminants, mainly sheep which leads to further economic losses. The economic impact of echinococcosis is divided in three categories: a) cost due to the disease in humans; b) cost due to the disease in livestock and c) cost of the control programmes to mitigate or eradicate the disease.

In many lesser developed and restructuring countries, parasitic zoonoses such as echinococcosis/ hydatidosis, cause serious human suffering and considerable losses in agricultural and human productivity, thus posing a significant hindrance to their development. Although effective and reliable tools for the diagnosis, prevention and control of parasitic zoonoses are now available, these parasites remain an important problem in many countries. This is primarily due to the lack of awareness of their presence or their impact. In addition, often the needed intersectorial cooperation, resource management and political commitment for their control are (also) absent.

In humans, the disease is initially without any symptoms until gradually the cyst increased in size, causing local pressure effects. In animals, the disease does not produce any clinical signs and is usually only discovered during meat inspection at the slaughterhouse, where the viscera (mainly liver and lung) are condemned.

It is well known that the main factor for the persistance of the disease is the feeding of infested parts (hydatic cysts) of sheep to dogs. Breaking the cycle is one of the main control measures. This however, largely requires awareness creation and public education.

The main constrains to control the disease could be further summarized as follows: high level of infection in endemic areas; lack of resources; difficulties in early diagnosis; low public awareness; stray dogs without control; unsupervised or illegal slaughter.

As a consequence the disease can cause: high morbidity rates; high economic losses. In the scientific literature, a multivariate analysis determined the following main risk factors: agricultural workers; livestock ownership; herding occupation; living in a rural area, being illiterate; having contact with dogs; being nomadic; overgrazing conditions; age and gender.

Anthelmintic treatments using praziquantel to prevent transmission by definitive hosts (dogs) is one of the most used strategies in control programmes. However, although great efforts were undertaken in many countries and regions, the success in the eradication of hydatid disease it is not always a feasible task.

Vaccines that can prevent infection in the intermediate host, provide an additional tool to assist with control of the disease. A vaccine based on a cloned recombinant antigen derived from *E. granulosus* eggs was developed and showed high level of protection in sheep. Recombinant DNA techniques provide the opportunity to produce antigens in quantities suitable for use as practical vaccines that in experimental trials induced high level of protection (95-100%) against either experimental or naturally-acquired infections.

Preliminary encouraging results prompted vaccination trials in New Zealand, Australia and Argentina. The vaccine reduced considerably the number of viable cysts in sheep challenged with *E. granulosus* eggs. Although there are questions about its usefulness, this vaccine could be an additional measure to programmes based on dog control and could potentially decrease the duration of control required to achieve very low levels of transmission or eradication. In addition, it has the potential to prevent hydatidosis in vaccinated humans.

The development of coproantigen and serodiagno-

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sis techniques in animals and humans has great potential in the diagnosis of hydatidosis in the laboratory and in the field in particular during surveillance and control programmes.

FAO's specific activities

Within the Animal Production and Health Division the Veterinary Public Health (VHP) Programme is constituted by members of the different Services (Animal Health, Animal Production, and Livestock Policy) within the Division. In addition, it links up with other units within the organisation on issues related to VPH. The VPH Programme has developed its Website (http://www.fao.org/ag/vph.html) on which information on ongoing activities, references and full text publications and manuals can be readily accessed. In addition, a number of fact sheets on zoonotic and food-borne diseases are provided as well as a database containing the addresses and contacts of veterinary faculties world-wide.

FAO regular programme has also established a global network of professionals directly involved in VPH, and is currently establishing four regional networks located in Asia, Africa, Eastern and Central Europe, and Latin America. The networks provide a basic framework to spread information related to the diagnosis, prevention and control of major zoonotic diseases including echinococcosis. In addition electronic conferences, discussion fora as well as newsletters contribute to information dissemination and to the general discussion on VPH related issues. A Directory with contacts of individuals and institutions involved in VPH issues and zoonotic diseases was also elaborated.

As communities play a crucial role in the prevention and control of zoonotic diseases in general, and echinococcosis in particular, an expert consultation on community-based Veterinary Public Health delivery systems was organised by FAO in October 2003. Furthermore FAO's Technical Cooperation Projects (TCP) is a tool to assist member countries in responding to urgent and unforeseen demands.

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Human cystic echinococcosis in Sardinia during the 20th century

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Abstract. At the beginning of the 20th century in Sardinia human Cystic Echinococcosis was considered a highly endemic disease by all the health operators. In the early twenties the number of reported cases was 429, but incidence appeared rising due to increase in availability of data and to improvements in diagnosis. The fall of personal and public hygienic conditions together with the drift from urban to rural areas during the second world war, had in fact facilitated the possibilities of infection, causing a strong growth of surgical cases during the post war years, so that incidence rate remains high and almost unvaried until the end of the eighties. Comparative analysis of young and probably old cysts indicates that this high rate of surgical cases was attributable to treated hepatic cysts in elderly. On the contrary the continuous reduction in the number of young cysts clearly shows a slow but constant decrease of the infection rate.

Key words: human cystic echinococcosis, Sardinia, epidemiology, history.

Due to widespread sheep breeding, Sardinia has always been a high-risk area for Cystic Echinococcosis (CE). However the first description of an human CE case as a *Taenia echinococcus* cyst dates from just 1874 (Pintor Pasella), as an autoptic finding in a young woman dead in childbeard. The exact term "echinococcus cyst" appeared for the first time in 1889 in the registers of Cagliari General Hospital and in 1893 in those of Sassari Hospital. Only between the late eighteen hundred and the early nineteen hundred a few papers were published describing some cases from personal surgical experience. Although human CE was thought to be an actual plague in Sardinia, and even if rare localizations were discussed, nevertheless no attempt was made to quantify regional or local diffusion rates. Finally, in 1920 Prof. Roberto Binaghi assigned to a medical student a graduation thesis on "Echinococcosis in Sardinia". In this study, later published (Cabras, 1930), all CE diagnosed cases (almost all surgically treated) were collected from public and private Hospitals, in a first attempt to give a complete epidemiological picture of the disease in a specific area. The 324 reported cases might appear very few as referred to a highly alarming disease, but probably this low number was attributable to the sketchiness of available data. In fact, encompassing the cases reported by Pinna (1906), the total number of cases increased to 351 while some years later Putzu (1925), including cases of his personal survey and data from other authors, reached a total of 429 cases. On the other hand, the trend described by Cabras (1930; Fig. 1) shows a relevant and continuous increase from 1889 to August 1920. It has to be ascribed to an increase in availability of data (keeping hospital registers, formal notifications) and to improvements in radiological, serological and clinical diagnosis, rather than to a real infection rising. In addition the age group distribution (Fig. 2) shows a clustering in the adult classes respect to younger and older groups, due to the difficulties encountered at that time in paediatric and elderly surgery comparing to more recent periods (Gabriele *et al.*, 2004) insomuch as mortality hospital rate was 9.8% (Putzu,

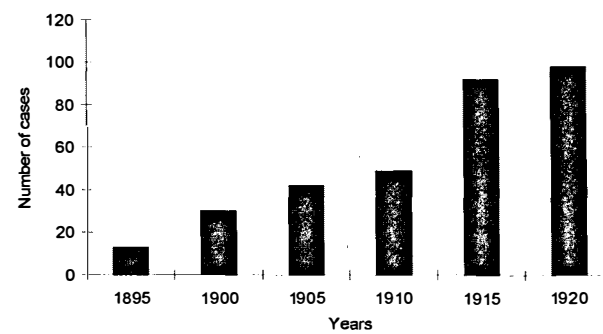

Fig. 1. Number of human cases of CE registered in Sardinia from 1889 to August 1920.

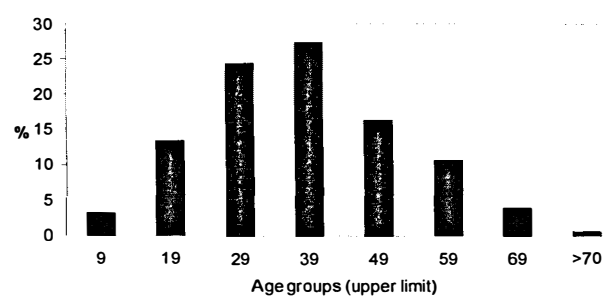

Fig. 2. Human CE cases registered in Sardinia until the early twenties. Distribution of % frequencies according to age group.

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Table 1. Human CE cases registered in Sardinia until the early twenties. Distribution of percent frequencies according to cyst localization.

Liver	Lung	Other organs
52,5%	16.2%	31.3%

1925). The very high incidence rate respect to more recent data (Conchedda *et al.*, 1985; Gabriele *et al.*, 1990) reported for housewives, as for farmers and shepherds, was certainly related to the greater participation of women in agro-pastoral activities and conversely to their little involvement in other jobs. As shown in Table 1, the distribution of cases by cyst localization appears anomalous. In fact, according to hygienic and health conditions in Sardinia in the early nineteen hundred, the rate of pulmonary cysts should have been much higher than cysts in liver or in other localizations (Giromini and Granati, 1954; Gabriele *et al.*, 2004). This can be explained considering the backwardness of thoracic surgery insomuch as 50% of untreated pulmonary cysts resolved in hydatid vomica (Pinna, 1906).

No data regarding whole Sardinia was published from the early twenties to 1940, but the general trend was probably unchanged, as suggested by cases from Cagliari hospitals ranging from 152 in 1920 to 272 in 1929 (Putzu Doneddu, 1930).

In 1954, Giromini and Granati published a study on all CE human cases surgically treated in Italy from 1941 to 1952. Although the lack of population census from 1936 to 1951 does not permit to calculate the incidence per 100,000 inhabitants, real data shows a sharp increase in surgical cases in Sardinia in the second half of the forties (Fig. 3). The fall of personal and public hygienic conditions together with the drift from urban to rural areas during the second world war, had in fact facilitated the chances of infection. Due to the slow larval growth and the disease latency, the effect of this increase of the infective pressure, began to appear in terms of surgical treatments only with a delay of several years. When Census restarted in 1951, systematic collection of surgical cases by some research groups allowed to draw a picture of human CE diffusion in Sardinia covering almost entirely the second half of the twentieth century. As shown in Fig. 4, the rise observed during the forties markedly continued during the following decade, whereas at the beginning of the last survey the incidence appeared reverted to the rates registered around the fifties. The trend appears nearly unchanged up to the early nineties, with a sharp increase between 1984 and 1988.

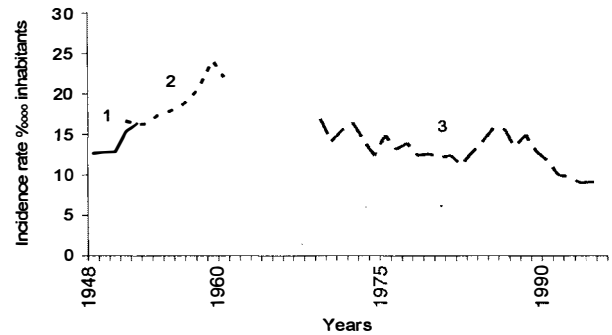

Fig. 4. Annual mean incidence rate ‰ inhabitants of CE in Sardinia from 1948 to 1995.

Considering the improvements in public health and personal hygiene and because of changes in the structure and management of agropastoral activities, a virtually unchanged trend appears in contrast with the persistence of an unvaried infection rate. Actually, different variables contributed to the high number of surgically treated cases (Gabriele *et al.*, 2004). Advances in instrumental investigations and increasing availability of ultrasound scan together with improvements in surgical as well in anaesthesiological skills resulted both in detection of cysts otherwise silent (Gabriele *et al.*, 1989) and in an increase in number of previously inoperable cases, particularly in elderly.

Study of young cysts, i.e. pulmonary cysts and cysts detected in young people has in fact demonstrated that rise of infection rate was actually due to a real increase in infections only for cases operated

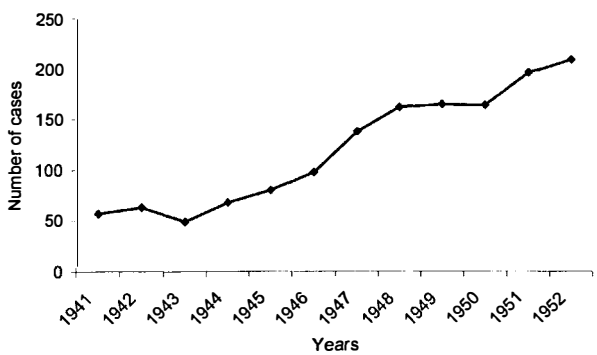

Fig. 3. Number of human CE cases surgically treated in Sardinia from 1941 to 1952.

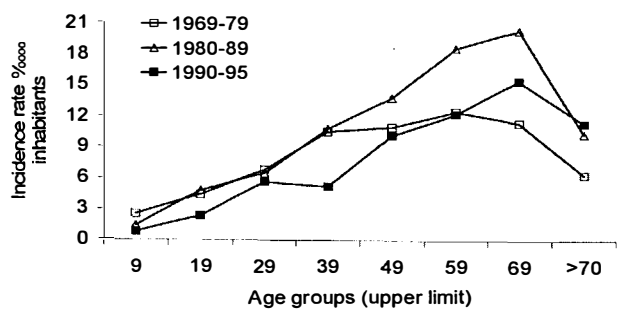

Fig. 5. Human CE in Sardinia. Annual mean incidence rate ‰ inhabitants of hepatic cases in three subsequent periods, according to age groups.

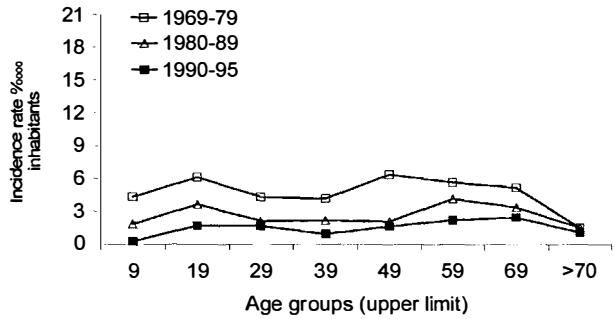

Fig. 6. Human CE in Sardinia. Annual mean incidence rate ‰ inhabitants of pulmonary cases in three subsequent periods according to age groups.

until the early sixties. Vice versa, the persisting high rates in the following decades is only attributable to hepatic cysts and generally to cysts recovered in elderly (Gabriele *et al.*, 2004).

Regarding the most recent survey (1969-95), the analysis by age group and cyst localization carried out throughout 3 subsequent periods confirms that hepatic cysts in elderly only made an incidence rate artfully high and were responsible for the unexpected increase observed during the eighties, while rates of pulmonary, therefore young, cysts invariably decreased in all age groups (Figs. 5 and 6).

Conclusive remarks

The very few available data referring to the early decades of the twentieth century, do not allow to provide an exhaustive picture of human CE, but suggest the heaviness of the disease insomuch as it was considered one of the endemic plagues of Sardinia such as malaria, tuberculosis and trachoma (Putzu, 1936). Social and economic upheaval caused by second world war considerably worsened the epidemiological situation extending its effects to the following decades insomuch as the deep socio-structural changes occurred also in Sardinia (Conchedda *et al.*, 1997) are only recently resulting in a slow but constant decrease in treated cases.

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Cystic echinococcosis in Italy from the 1950s to present

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Abstract. In Italy the epidemiological pattern of cistic echinococcosis (CE) is incomplete and the information for most regions is out of date, contradictory, and almost exclusively limited to the intermediate hosts. The disease is found most frequently in particular social and economic conditions: widespread use of extensive or semi-extensive sheep farming, illegal slaughtering, and high numbers of sheepdogs and other types of dogs. The highest incidence in sheep is found in Sardinia (70.6-92.8%), Sicily (6.5-36.5%), Basilicata (5-28%), Abruzzo (22%) and Tuscany (47%). It North Italy, it is never higher than 0.5% in slaughtered sheep. No data are available on the biomolecular characterization of the strains of *E. granulosus* in Italy, apart from Sardinia and recently Lazio. G1 (Sheep strain), G7 (Pig strain) G2 (Tasmanian sheep strain) have been identified in Sardinia and G1 and G3 (Buffalo strain) have been recently isolated in Lazio. In Italy, CE has was also found in buffaloes (2.63-9.8%) and horses (<1%). However, further epidemiological surveys and genotyping study are necessary. The small quantity of up to date information on the diffusion of *E. granulosus* in dogs (Abruzzo 4%, Sardinia 6-10% and Sicily 19.3%) highlights the need for modern, fast, sensitive and low risk diagnostic methods which would provide a true picture of the pattern of the infection in this host.

Key words: *Echinococcus granulosus*, livestock, dogs, epidemiology, Italy.

Cistic echinococcosis (CE) is an important problem for public health and the economy in the many parts of the world where the infection is endemic or hyperendemic. It is of particular importance in the Mediterranean Region (MR), where it is one of the principal parasitic infection in livestock. It is the most important parasite zoonosis in the area and it is of great social importance. In MR, CE is directly linked to ovine strains (G1) although others of 10 *Echinococcus granulosus* genotypes (G1-G10) identified in the world have been found in the area. The equine strain (G4) has been found in Spain, Italy, Lebanon and Syria, the camel strain (G6) in North Africa and the Middle East and the swine strain (G7) in Spain, the Slovak Republic and Poland (Thompson and McManus, 2002).

CE is a particular problem in the areas where grazing, and in particular pastoralism, is widespread. The close relationship between dog, sheep and man, makes more likely that the life cycle of the parasite can be completed. At the moment, the sheep strain (G1) seems to be the only one which is found in all the MR and it is the main responsible for the disease in humans. Its high prevalence is closely connected to the following factors, which are linked to the social and economic conditions of the population: continued widespread use of traditional techniques when raising small ruminants (extensive or semi-extensive grazing), illegal slaughtering of the animals, and the presence of high number sheepdogs which gravitate to the sheep raising areas.

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Although there are numerous studies on the geographical distribution and the prevalence and incidence of CE in animals, these are fragmented both in geographical area and time, and of little use in defining a sufficiently precise pattern of the epidemiological situation. Only partial and sporadic studies have been made on CE in the countries on the Southern and South Eastern areas of the Mediterranean basin. Also data from EU Mediterranean countries is not complete, being limited to some geographical areas. Although the data has to be treated with caution, it indicates that in certain countries the disease is alarmingly widespread and that the infection has to be considered endemic or highly endemic in most MR countries, with the exception of Malta and the Republic of Cyprus where the infection has almost completely disappeared (Seimenis and Battelli, 2003).

The epidemiological pattern of CE in animals is also incomplete in Italy. The data for numerous regions is often contradictory, as it is often limited to information on the prevalence of the disease in slaughtered animals and is also out of date. Data on the diffusion of the parasitosis in dogs is always limited to a number of areas. There are no systematic epidemiological investigations which document how many, and what strains of *E. granulosus* are actually present in the country. Recent studies in Sardinia have found G1 (Sheep strain) in sheep, cattle and pigs and G7 (Pig strain) in pigs (Varcasia *et al.*, 2004a). Further research has found G2 (Tasmanian sheep strain) in cattle in Sardinia and G1 and G3 (Buffalo strain) in pigs in Lazio (Busi *et al.*, 2004). CE has been found in horses with G4 (Horse strain), although its presence has not been confirmed by molecular investigation.

The first investigation of CE in slaughtered animals, which covered all areas of Italy, dates back to 1952 (Pellegrini and Cilli, 1955). This confirmed, as had previously been observed, that the parasitosis was more widespread in the islands and South Italy. It was found in 4.08% of cattle in North Italy, 11.45% in Central Italy, 13.27% in Southern Italy and 55.09% in Sardinia. The figures for its presence in sheep were as follows: 15.93% in North Italy; 14.94% in South Italy; 21.74% in Central Italy with a peak of 41.67% in Tuscany; 68.72% in Sardinia; 21.44% in Sicily. It was found in 1.82% of goats in North Italy (6.22% in Piedmont), 8.14% in Central Italy (31.37% in Tuscany), 10.33% in South Italy, 7.55% in Sicily and 12.75% in Sardinia. Swine CE varied from highs of 19.81% in Sardinia, 14.66% in Basilicata, and 13.24% in Umbria, to lows of 3.21% in Sicily and 2.01% in Emilia.

Using annual data collected by the Ministry of Health in 1972-1977, Schiavo *et al.*, (1979) found the following prevalence: sheep 11.6%, goats 5.6%, cattle 1.54%, pigs 1.11%, horses 0.45%. Another investigation which provided "a reasonably approximate pattern, even though underestimated" of the diffusion of CE in Italy was that by Romboli *et al.* (1980), which used official information and data. From the national data for 1968-1978, they found that CE was present in 8.1-15.2% of cattle, 8.1-15.3% of sheep, 2.7-8.9-% of goats 0.7-1.2-% of and 0.4-0.9% of horses. Regional data for 1972-1978 in the different animal species confirmed that CE was more prevalent in all species in Central and South Italy and the islands than it was in North Italy.

Finally the more recent investigation by Lorenzini and Ruggieri (1987) confirmed that the parasitosis was more widespread in South Italy and the islands and in particular to those areas where animal husbandry was less developed and the animals, mostly sheep, were raised using traditional methods. The study was limited by the method of collecting data (questionnaires sent to the principal abattoirs in each province, and the fact that the data for sheep and goats was aggregated).

The results of national investigations on cattle and buffalo slaughtered by Inalca S.p.A (Ospedaletto Lodigiano), found CE in less than 1% of cattle in North Italy and in 2.63% of buffaloes. In Central Italy, the prevalence in cattle was far higher (10%-12.53%), while in buffaloes it was 3.53%. The figures for South Italy were even higher: cows 22.04%, bulls 11.11%, calves 1.85% (CE was absent in calves in other areas), buffaloes 9.8% (Fattori *et al.*, 2000). This data confirms that the parasitosis is not widespread in North Italy but also that it is not diminishing in the rest of the country.

There is much less epidemiological data for North Italy than there is for the South and islands, and it is generally data collected from slaughterhouses. Data are limited for Valle d'Aosta, Piedmont and Emilia-Romagna. In Valle d'Aosta, CE prevalence ranged 0.08% to 0.18% in "slaughtered animals" in 1995-2003 (data supplied by Valle d'Aosta Health Authorities). The "2002 Report of the veterinary services of the Piedmont Region Health Services" found CE in 0.011% of slaughtered cattle. It was found in 0.51% of slaughtered sheep and 0.085% of sheep in general. However deeper examination of the reports shows that the prevalence was calculated for, respectively, the number of "sheep and goats" slaughtered and the total number of sheep and goats registered in the animal husbandry survey. Faggioli *et al.* (2001) found that the presence of CE in legally slaughtered animals in Emilia Romagna between 1996 and 1999 was as follows: pigs 0.95/million, cattle 0.39%, sheep 0.30%, goats 0.39%, horses 0.34%.

There is no information on the diffusion of canine echinococcosis in the northern Italy; the CA-ELISA immunoenzymatic method (Echinotest, Bommelli, Bern, Switzerland) found positive for the infection 9 of 19 (alpine) farm dogs and 27% of 60 in the Val di Susa (Rossi, pers. comm., 2004).

There is more data available for Central and South Italy, but it is still not enough to create a sufficiently clear pattern of the spread, and in particular of the infection trend over time. Let us take the Abruzzo region as an example. The evolution of the parasitosis in livestock, and in particular in sheep and goats, does not appear to be linear because in some periods the region was one of the most affected ones and in others the infection prevalence decreased. In 1981, Manilla (1986) found EC in 50.8% of adult sheep in Teramo. CE was present in an average of 10.6% of "sheep and goats" in the period 1972-1984 (Gargiulo *et al.*, 1987). In a study confined to adult animals in the period 1985-1989, Schiavo *et al.* (1992) found a reduction in the number of positive cattle (3.5%-2.3%) and horses (3.8%-1%) and a great stabilization of the situation in pigs (0.3%-0.6%). In goats there was a fall from 16.3% to 4.5%, but with a peak of 22.5% in 1988. On average, the situation for sheep was stable during the 5 year period. More recent data for the province of Teramo (1985-1994) shows that CE was present in an average of 32.14% of "sheep and goats" and in between 4 and 6% of cattle, horses and pigs (Tieri and Gatti, 1995). Recent surveys ("Research Programs Relevant National Interest" PRIN 2003), found CE in 22% of 2-5 year old sheep in the province of Teramo, with total fertility of 4.6%. The only data available for dogs in Abruzzo showed 4% of *E. granulosus* positive dogs, of which 2.66% were stray dogs and 50% sheepdogs (Di Ventura *et al.*, 1995).

The last epidemiological data showed that CE was found in 47% of sheep slaughtered in the province of Arezzo (Bio and Fagiolo, 2004).

These high prevalences in the intermediate hosts confirms that in Central Italy the previous available data were probably underestimated and further investigations are necessary to better understand the diffusion of the infection.

Past research in Apulia found a reduction in *E. granulosus* in dogs from 12.9% in 1955-1958, to 10.51% in 1962-1963 and 5.73% in 1971-1974 (Puccini *et al.*, 1975). There were more epidemiological investigations into CE in intermediate hosts. Between 1975 and 1982, CE was found in an average of 4.88% of sheep and 3.9% of goats (Puccini and Tassi, 1983). In the period 1989 to 1993, Schiavo and Pansini (1996) found that the presence of CE varied between 3.2% and 0.47% in sheep and 5.88% and 0.35% in goats slaughtered by Health Service 1 of Taranto. Puccini (pers. comm.) found CE present in the following amounts in 4 slaughterhouses in the province of Lecce in the period 1978-1987: cattle 7%, sheep 14%, and goats 12%. In the slaughterhouse of Foggia in 2003, CE was present in the following amounts: cattle 5.74%, adult sheep 5%, horses 0.02% (Puccini, pers. com.).

In 1996-2002 in Basilicata, Quaranta (2003) found CE in 2-3% of cattle, 5-28% of sheep, 4-25% of goats, 0.05-0.5% of pigs and 0.04-0.1% of horses. Surveys in Campania in cattle, sheep, goats, swine and horses slaughtered in 26 abattoirs in the provinces of Avellino and Salerno found an average presence of less than 5%. In certain districts, the presence in sheep and cattle was between 16% and 21% (Cringoli *et al.*, 1998). Capurso *et al.* (1968) found relatively high diffusion in dogs in the city of Naples and that it was present in 1% of dogs in some districts of the province.

Finally, the finding of CE in buffaloes, not only in this region but also in other areas of Italy, highlights the need for further research aimed to establish the diffusion, the epidemiological situation which favours the presence of the parasite in this species and to clarify which specific genotypes are responsible for it.

There has been more research on the parasitosis in Sicily. The first research was carried out by Bertocchi (1951) who found CE in 6-10% of cattle slaughtered in Palermo, Messina and Catania and 2.08% of dogs in the province of Palermo. Panebianco and Sciutteri (1955), found CE in 4.6% of dogs in Messina and slightly less (3.4%) was found in Palermo (Gallo and De Girolamo, 1960). By contrast, Virga and Giannetto (1998) found that the prevalence in sheepdogs treated with arecoline hydrobromide was significantly higher in the provinces of Agrigento (23.2%) and Palermo (16.2%). Samples taken between 1981 and 1985 at the Palermo slaughterhouse found the highest prevalence in sheep was 29.41%, in cattle 2.87%, in horses 2.69% and in pigs 0.32% (Demma *et al.*, 1987). In 1988-1989, Virga (1991) examined the registrations carried out by the veterinary inspectors to comply with the O.M. 21 April 1964, and found the following highest prevalences: cattle 2.29%, sheep/goats 10.90%, pigs 0.82%, and horses 3.1%. Magliarditti and Niutta (1995) found an even serious situation for local adult animals, with CE present in 11.13% of cattle, 43.22% of sheep, 2.85% of goats and 4.71% of pigs. Poglayen *et al.* (2001) found 49% positive results in the Sicilian Black Pig of the Nebrodi Park (ME) and more recent investigations (1988-2000) found CE present in 15.6% of them and fertility of 1.9% (Scala *et al.*, 2001). Later Poglayen *et al.* (2003), found CE in 15% of legally slaughtered sheep in 8 provinces, with a maximum of 36.5% in Palermo and a minimum of 6.5% in Agrigento. They also found an average fertility of 31%. This varied from 90% in Agrigento to 13% in the provinces of Enna, Messina and Ragusa. Finally *Taenia hydatigena* was found in 10.5% of the dogs in the municipal dog pound of the province of Trapani (Nobile *et al.*, 1993) and *E. granulosus* in 19.3% and *T. hydatigena* in 17.5% of sheepdogs in the provinces of Agrigento and Palermo (Giannetto *et al.*, 1997). This confirms how easy it is for the dogs to have access to the viscera of slaughtered animals or of those which had a natural death. While there was not enough data to establish a complete pattern of the situation on the island, it was clear that there were risks for man and domestic animals, above all in rural areas where less advanced social conditions and animal husbandry encourages the spread of CE.

The presence of CE in horses in various regions of South and Central Italy and in Sicily means that greater epidemiological awareness is necessary not only for buffaloes, as was said above, but also for this species, and bio-molecular research must be carried out to establish if G4 (horse strain) is present in Italy, given that biological tests and morphometric investigations by Macchioni and Gallo (1967) found that it was present in Sicily.

Unlike in other areas of Italy, CE in Sardinia has always been investigated organically as can be seen from the large quantity of literature on the subject, although due to limitations of space it is impossible to cite these exhaustively. The island has always been an ideal model for the study of the parasitosis. Firstly there are a large number of sheep and goats (about 3,000,000 sheep and 250,000 goats), with an advanced branch of production which has encouraged advances in the whole sector. Secondly the high number of dogs (150,000) (Cannas *et al.*, 1990), the sheep farming methods used (extensive, semi-extensive), illegal slaughtering and the social economic and cultural conditions of the shepherds have resulted in the level of CE in sheep remaining at 87%, in goats at 24%, in cattle at 30% and in pigs at 20%. The fertility rate varies from 40% in sheep to 31% in goats, 25% in cattle and 25% in sheep (Arru *et al.*, 1990; Conchedda *et al.*, 1997). In dogs it is found on average in 11% of stray dogs and 25.42% of sheepdogs with an overall average regional presence of 16.2% (Arru *et al.*, 1990). In addition, despite the fact that three eradication campaigns have been conducted in Sardinia (in 1960, 1978 and 1987) (Arru *et al.*, 1999), recent data showed that the prevalence of CE was still high, being present in 75.6% of sheep in the province of

Sassari, with fertile cysts being found in 6.9% of the animals examined and in 9.1% of those infected (Scala *et al.*, 2000a). Research in the province of Cagliari (Scala *et al.*, 2000b) in 1999 found similar results, with CE present in 72.2% of sheep. The situation found in 2000 in Goceano, a district of Central Sardinia, between the provinces of Sassari and Nuoro is still more worrying, with CE present in 92.8% of sheep and fertile cysts in 27.1% (Soro *et al.* 2002). The preliminary results of a second study being carried out at present on animals legally slaughtered in the provinces of Sassari and Nuoro show the levels of infection (82.6%) and fertility (17.3%) to be significantly higher than those of the first study which were, respectively, 70.6% and 7.3%. This confirms that the parasitosis is closely linked to certain areas where traditional methods of sheep farming are used. After more than a decade of silence on the diffusion of *E. granulosus* in definitive hosts, research on this was carried out in 2003 as part of the research project PRIN 2003. Faecal sample were taken from 300 dogs and microscopic examination of these found 8.25% *Taenia* spp. positive. CA-ELISAs performed with the commercial kit (Echinotest, Bommeli CH) found 3% positive while two ELISA which employed monoclonal antibodies (Mabs: EmA9 and EgC3) found 6% and 10% positive respectively (Varcasia *et al.*, 2004b).

It is thus evident that at present, with the exception of the previously cited cases, there is a lack of epidemiological and diagnostic research and molecular characterization studies in Italy, both in dogs and in intermediate hosts. For example, data of cyst fertility have only been collected in the Italian islands and principally in Sardinia. There are few studies on the prevalence of the parasitosis in wild animals (particularly useful when the same strain of *E. granulosus* is present in both domestic and wild animals). Research carried out by Arru *et al.* (1986) found CE was present in 1.18% of foxes in Sardinia while Guberti *et al.* (1983) found CE in 16.9% of wolves. The positive results of immune-enzymatic CA-ELISA tests on faecal samples from 6 wolf packs (*Canis lupus*) in Piedmont (Val di Susa) and of fertile cysts in sheep carcasses which had been preyed on by wolves in the same area (Rossi, pers. com.) confirm that more studies are needed in this field.

In conclusion, it is clear that there is very little information on the diffusion of canine echinococcosis and also that there is a grave lack of knowledge on the epidemiological situation for CE in intermediate hosts, with the exception of the few examples. This is particularly true in certain areas of Central and South Italy where sheep farming is widespread. Standard data from the Public Health Services is of itself insufficient to determine the existence or absence of situations of possible risk in particular zones, or at the local level, and is statistically of such questionable value that it makes accurate evaluation of the evolution of the parasitosis over time more difficult.

Given that CE is still widespread in certain areas where the relationship between dogs, humans and sheep is very close, it is of great importance for public health to create a rapid and sensitive method for diagnosing the parasitosis in dogs, as they are the sole transmitter of the parasitosis to humans and animals.

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Health education and formation: essential tools into the Echinococcosis/Hydatidosis prevention's programs

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Abstract. Health Education represents essential elements in the control and eradication campaigns of Echinococcosis/Hydatidosis. The basic elements and the applicatory principles and methodologies are examined. Moreover, the most appropriate intervention methodologies are defined through reference target. There is also a description of the experiences and the methodologies adopted in different Countries of the Mediterranean basin, where the disease is endemic.

Key words: health education, Echinococcosis/Hydatidosis, formation, communication.

Communication in the education activities to health

How to transfer a message that is not only included, but it has to bring a changement into the behaviour? This is the goal that each operator suggest to himself, he is appointed to realize activities of health education (HE).

Health education is an essential tool of the veterinary public health and it is critical for zoonosis prevention and control, including Echinococcosis/Hydatidosis (E/H). It has been defined as "an educational process which is turned to make the population responsable for itself and other people health's protection, both as individual and as groups".

It is a subject between education and health, also it is based on multidisciplinary activities, where medical sciences, formation and communication skills are requested. In this field, the communication concept fully goes over the restrictive value. This value actually identifies itself in the communication all the activities and resources which are in some way connected to the mass means of communication (radio, television, publishing, computer science, telematics) among the different social ambits.

Communication, as part of the educative process, assumes a largest value and it suggests, to be effective, the knowledge of the context and the needs of the target population. It really becames effective when it modifies the view of reading the reality in the user,it modifying the meanings given until that moment. The first step to pursue this goal is that operators themselves, changing the way to relate to the user, considering him not just a target to attain the informations, but a co-actor with whom to share the language, the objectives, the resources and tools.

The main elements, which have to be taken into consideration to follow this model can be summerized in this way:

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- to get in touch with the user to know his needs, characteristics, his belonging context and the possible environmental conditions;
- to know the factors which could affect the preventive behaviours of users;
- to acquire effective communication techniques from operators, so that the target population could actually became the real consignee of the all educational run.

As regard this last aspect, it is very important to underline that most frequent obstacle to the effective communication in health field is the socio-cultural distance between educator and user. It can represent an overwhelming difference which can thwart the HE best programs. In most of case, the sanitary educator ownes a cultural formation which is considerably different from that owned by their listeners. What can seems clear to the speaker, sometimes it is not to the listener. Who suggests the message has to identify himself with the user's cultural world without never giving up the scientific precision of the informations, which have always to be truthful and complete.

This objective can be realized only following the habit of two interlocutors and so it requires a constant relationship based on trust with the socio-sanitary personnel on the territory. It does not have to be carried out in an occasional way and by means of changeble operators. Furthermore the user is not always in the cultural conditions to receiving educational messages (user limited receptivity) health problems, work, very difficult life contexts.

The methodology to be adopted to obtain an effective communication in the sanitary field, should meet the following rules:

- the content: it has to be well presented and it has to be supported by scientific informations;
- the message: it has to be clear and understandable and it has to obtain the recognition and interest by the consignee to join him.

In the past one assigned a special importance to the transferred message text and to the transfer modalities, while recently the attention has been

focused on the message effective utility to people who receive it. Infact, we have to bear in mind that the real message is not the delivered one, nor the transferred one, but it is the one included by the consignees. It is not very important to transfer many elements, but those have to be enough to stimulate a changement in the behaviour.

The communication procedure has to reach all the interested groups, it has to identify the consignees and has to adapt the contents to the specific features of the single groups.

It is important to underline that the new information, presented in an original way are easier to remember, mainly if its will be recurrently repeated. The sight is the sense that more contributes to learning (about the 87%), followed by the hearing (7%) and by the other senses.

The source of communication: it has to be credible to support the mentioned thesis.

The trial evaluation: it always has to be foreseen and put into practice.

The result evaluation: it always has to be foreseen and put into practice to verify the real impact of the educational action on population.

According to what it has been described, it is possible to state that an effective communication in the HE campaigns does not have to point out only simple cognitive-behavioural information/prescription, but it has to assume a formative valence. This means to stimulate in the target people the reason of changement, through a critic reading of reality through behaving on the deepest convictions and values. It also has to stimulate different levels of consciousness, especially it has to make them the protagonists of some improving processes that they want to prime.

Sanitary education: objectives and activities

HE general objectives are: disease prevention and state of health support. These are direct to provide the tools to let people being able to:
- define their problems and necessities;
- realize what they can do to solve these problems through their resources which are associated to an external support;
- decide which actions are more appropriate to promote the life style and welfare of the entire comunity.

Accordingly, the HE includes all the activities correlate to information, education and formation (general and professional formation), which must not be considered mutually exclusive. They usually tend to overlap and to have no connection between them:

The information is the knowledge transferred from the "expert" to the target group. Usually, this activity is used to call the problem attention before the beginning of the control problems. The community will take part to it activily.

The HE *sensu stricto* includes all those target groups which are not professional connected to the specific problem (e.g. general audience and school's students). The final objective is a conscious and firm change of behaviours which can have negative effects on health.

The professional formation is directed to the people who should apply appropriate behaviours for the prevention of the specific problem during their activities (e.g. farmers, butchers).

Participation and methodologies

To achieve the control and the prevention of a disease it is necessary the participation of all the community not only as a support to the sanitary services, but also to define the main sanitary problems and to find human resources:
- voluntary participation in prevention and control's programs;
- declaration of community interest and definition of priority;
- interest in the definition of sanitary politics.

It is important to obtain the participation of several interested groups, such as:
- medical and veterinary services;
- sanitary committees and basic sanitary personnel;
- religious communities;
- child and adult schools;
- police or military unities.

The HE is an important component of every E/I control program and it should not be directed only to the application of specific measures, but also to a largest acquisition of personal responsabilities and of the whole community. It should be included in the programs after defining the main objectives: target groups, available resources and possible limited elements, the evaluation sistem, etc. The schematic methodology used in HE consists of four points:
- knowledge (problems and solutions' analisis);
- programming (definition of appropriate solutions);
- operation (activity and adoption of suitable behaviour);
- evaluation (impact evaluation and intervention results).

Sanitary education impact in the Echinococcosis/Hydatidosis control

HE is a basic component in the E/H control. Its meaning has already been underlined in the "OMS overseeing guide line, echinococcosis/hydatidosis prevention and control", published in 1984, and recently (2001), in the "People and animal echinococcosis WHO/OIE manual: a public sanitary problem from a global level".

The HE is important because an effective control is based on an active cooperation of different groups of the population, as sanitary and veterinary personnel, dogs owners, breeders, shepherds, butchers, slaughterhouse personnel and personnel which is responsible for the carcass and infected parts' destruction. The HE should also include occasional workers who do not know very much about local sanitary

problems. An E/H control program from a national or local level needs of decisions and politics obligations. The real epidemiological and socio-economic impact of the disease should be clearly illustrated to the community before starting a control program. There are different options to control the E/H. The E/H plays a dominant role in the horizontal approach to the control. This approach can be used in different circustances:

- if the E/H control is based on changing procedure in the breeding and in the slaughterhouse and/or in the social situation when it interferes with the biological cycle of *Echinococcosis granulosus* in the endemic areas;
- if a E/H control program, when the activities are based on individual components (e.g. canine population control and feeding sources, supervision and destruction of infected viscera, etc.) and there is the need to coordinate the different components.

Health educational role in the different stages of the control program

In the phase of planning and during the program initial stages, the HE can be useful to assure the public support to convince public administrators about the importance of the problem.

During the attack phase, it is very important that HE keeps on with leaning the different measures of control which had been adopted. For example, as regard to the prevention of feeding dogs with raw viscera, HE should change people habit and behaviour removing infected viscera, feeding dogs in a correct way. This objective is very important, but it requires a huge obligation. When the reduction of the canine population and of the mass anthelmintic treatments is applied, HE should be advised to guarantee the cooperation of dogs owners. This could be possible through our cooperation for a periodical parasitological exam of dogs and also for the pharmacological treatment of infected animals.

Cultural and religious traditions are very important to support control programs, such as customs tied to poverty and protein scarcity in the diet.

During the consolidation and maintenance phases of the program, it could be necessary to introduce specific laws to eliminate the residual infection and to restrict the activity of those people who usually do not respect control measures. In this case, educational programs should guarantee the application of the law.

Examples of sanitary education in different control programs

Italy

Veterinary services belong to Sanitary Administration. HE is a part of the institutional tasks since 1978, when it has been included in the National Sanitary Service. Since then, a remarkable experience on the topic has piled up. It also includes the pilot scheme for E/H control in Abruzzo of the first eighties. An other E/H important control program started in Sardinia in collaboration with the Sardinian Experimental Institute for Zooprophylaxis. This program was based on the experience aquired from other countries and adapted to the island particular conditions. It included the following phases:

(a) sanitary education;
(b) canine population control;
(c) slaughter overseeing.

HE has been considered as a supporting activity to other activities, and it was followed by an effective participation of the population to modify those incorrect behaviours which are tied to the relation man-animal and to the domiciliary slaughters. Mass media were used to spread messages which were easy to be understood. Radio, television, newspapers and brochures were used to pass messages to the population and to specific groups, such as breeders, butchers, hunters, etc.

Operators were educated through the constant veterinary presence and advices on the place of work and during proper meetings.

In order to stimulate a higher breeder responsibility, notable efforts were indispensable as regard the correct elimination of infected viscera of animals slaughtered at home, the notification of stray dogs, the canine population control and other adopted measures.

Sanitary education programs were expecially directed to schools to spread the information from school to family and were strengthened by clear concepts. The task was to prevent incorrect children behaviours (to feed dogs with infectous viscera). Teaching aids were adapted to schoolchildren of different ages. To the youngest ones it was prepared an easy to be remembered poster where relationships children-environment with suggestions on the main hygienic measures were drawn. Another poster was produced for primary and secondary schools where the biological life cycle of *E. granulosus*, the way of infection transmission and the control and prevention measures were illustrated. Moreover, team games were used to furnish the opportunity of learning through simulation ("a game to understand").

Spain

In the region of Castilla and León, the E/H campaign was based on a continuos interprofessional collaboration concerning the sanitary personnel and other professionalisms. A large collaboration was assured through public administrators involvement, parents of children at risk, people directly stroked by losses due to E/H, people who have been operated of E/H or people who were waiting for a surgical operation. HE was directed to different groups of the population, such as butchers and slaughterhouse operators, shepherds and breeders, sanitary personnel, autorities, teachers and general public. The activities were planned by territorial personnel.

The program evaluation was based on the evaluation of the level of knowledge acquired by each group through the dispensing of questionnaires both to the grocer and shepherds-breeders and to general public and school attendance.

Cyprus

An HE innovative method was used in Ciprus by going house to house and talking about topics regarding E/H control with families, and expecially with mothers. Information were given on the infection gravity, on the control program and on the precaution adopted to prevent the infection. Others methods were also used, such as domiciliary visits for breeders and educational activities in schools. Information opportunities were also offered during agricultural fairs, school shows and other public events.

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Cystic echinococcosis in Sardinia: the current role of sheep

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Abstract. Cystic echinococcosis is one of the most widespread parasitosis in the Mediterranean region. Unfortunately this is also true for Sardinia for various reasons, among which is the close relationship between sheep and dogs in the farms. This work first epidemiologically analyses hydatidosis in sheep in Sardinia and then examines the most important causes of the persistence of these metacestodosis in sheep. The work looks at the factors which are responsible for the enormous quantity of illegal slaughtering and the widespread habit of abandoning the sheep carcasses in the grazing areas, as well as possible initiatives which may eliminate these practices. Such initiatives must include reducing the financial costs and bureaucracy involved when sheep are slaughtered in abattoirs, and also increasing the value of sheep meat. In conclusion new checks and controls are hoped for at a political level which will increase the financial support for the farmers and encourage the use of recombinant vaccines which have already been tested in Sardinia. These are extremely useful for effectively curbing cystic echinococcosis.

Key words: cystic echinococcosis, sheep, Sardinia.

Cystic echinococcosis (CE) by the metacestode of *Echinococcus granulosus* is an important public health problem in many areas of the world. It is particularly important in the Mediterranean basin where it is one of the main forms of parasitosis in farm animals. It has a marked social impact because it is also frequently found in the human population (Eckert *et al.*, 2001). Sardinia, unfortunately, fits well into this pattern as the parasitosis has always been extremely common in this region, and even today it is still a serious health problem for humans and farm animals. Sardinia is a particular case, firstly because some 2/3 of all Italian sheep (which are the preferred intermediate host for this cestode) are raised here, secondly because there is a high level of epidemiological awareness, and finally because it is also the only Italian region in which there are in operation plans to control this disease. In addition there is also a very particular relationship between dogs, sheep and humans on the island. This relationship has always dominated sheep farming in the island, and it is linked to the slow rate of evolution of social, economic, cultural and biological conditions. These conditions are perfect for allowing *Echinococcus granulosus* to complete its life cycle (Bortoletti *et al.*, 1990). There are more than 3,000,000 Sarda breed sheep in Sardinia; they are also found in almost all the rest of Italy and in other Mediterranean countries, in particular in Tunisia (http://www.ara.sardegna.it). This is because the breed can adapt to extensive grazing in hilly and mountainous areas and also to intensive rearing in plains and irrigated areas. The Sarda sheep are good dairy sheep and supply some 100 or 170-180 litres annually during a 180 day lactation period, depending on whether they are raised extensively or intensively (http://www.ara.sardegna.it).The first epidemiological studies for parasitosis were conducted in the island in the 1950s, and these found that the situation was catastrophic. 99.4% of sheep were infected (Tanda, 1960), with 100% of the parasites fertile. The results for dogs infested with the tapeworm were also very high, varying from 8% (Papandrea, 1951) to 45.2% (Medda and Javedaia, 1960, Deiana and Arru, 1960; Arru *et al.*, 1990).

In a more recent study, Arru *et al.* (1990) confirmed that in 1990 the data for the definitive hosts (dogs), as well as those for the intermediate hosts (sheep), were not significantly different from those they had found ten years earlier (Arru *et al.*,1980), despite the campaigns to eradicate and/or control the parasitosis in the 1980s. These campaigns had not achieved the results which were hoped for, despite the extensive work carried out by the various professional groups involved.

Hydatidosis is also one of the most serious epidemiological risks for humans in the Mediterranean basin, with an average annual incidence of some 9.8‰. There are marked differences between the various provinces and also clear annual fluctuations (between 1969 and 1990 incidence varied between 15‰ and 8‰ respectively) (Ecca *et al.*, 1998).

In this context a detailed analysis of certain factors related to hydatidosis in sheep would be very valuable, given that they are the intermediate host of choice for this metacestodosis. This research defines the present epidemiological role of the parasitosis in Sardinia more precisely. New data on developments of the parasitosis give the feeling that with time something is changing. Analysing the situation in sheep is probably the most relevant aspect of this in Sardinia, where plans to control the parasite in dogs

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are more problematic, given that, for various reasons, so many of them cannot be systematically checked in any way (many unchecked stray dogs, no efficient statistical data base for dogs, and in particular sheepdogs, etc.).

Present epidemiological situation

Recent data on sheep in the island shows that the situation is still serious, but that it varies depending on the locality.

In the latest published data for north Sardinia hydatidosis was found in 76% of the sheep checked (Scala *et al.*, 2000). This is not substantially different from the previous survey (Gabriele *et al.*, 1992; Gabriele *et al.*, 1998). However if other epidemiological factors are taken into consideration, such as the percentage of host with fertile hydatids, mixed infestations (liver+lungs), and massive infestation (more than 10 hydatids/sheep examined), then it can be seen that the intensity of the parasitosis is declining in Sardinia (Scala *et al.*, 2000) (Table 1).

The reduction of these parameters does not, however, seem to be connected to a reduction in environmental contamination by the eggs produced by the dogs. The prevalence of infestation in sheep less than two years old (the sentinel animal group) is not significantly different from that of older animals (Scala *et al.*, 2000). Thus it seems that in this case the reduction of fertility may be due to the sheep themselves responding more efficiently to the tapeworm.

In those districts of north Sardinia where sheep rearing has become very efficient in recent years the reduction in parasitic pressure may be due to variety of causes, and in particular to the lower fertility of the hydatids. According to Bortoletti *et al.* (1990): "The epidemiological significance of sterile cysts is, at present, unknown and needs further study but some factors (probably synergistically concurrent) can be considered: a) reduction of transhumance (CENSIS: 1st report on the social situation of Sardinia); b) anthelmintic treatment of dogs; c) unfavourable environmental conditions for the survival and maturation of the eggs; d) genetic selection of animals; e) improvements in animal husbandry standards". However all the above mentioned factors do not take into consideration our information on the routine treatment of animals with benzimidazoles drugs, often more than once a year. It is well known in veterinary and human medicine that benzimidazoles devitalises the hydatids. Indeed 47% of Sardinian sheep are treated with these drugs (Scala *et al.*, 1999). This is a significant proportion of the population and it could be responsible for a certain reduction in the fertility of the metacestode. However this reduction of fertility is not evident in all of Sardinia, as has been emphasised by the recent data from the survey by Soro et al. (2002) in Goceano (a region of central Sardinia). Here they found an extremely serious situation, with the disease present in 92.8% of sheep and 27.1% of the animals with fertile cysts.

Thus it is clear that in Goceano, a particularly socially and culturally isolated district where public health instruction is more difficult, the parasite continues to spread in an uncontrolled way and that it has serious negative repercussions on the society, human health, and the economy.

There are also epidemiological data available for Sardinia on the percentage of fertile cysts in the lungs. This percentage is significantly higher than that of fertile cysts in the liver (Scala *et al.*, 2000). This means that the lungs are a more dangerous source of infection than the lungs in Sardinia, despite usually being underestimated.

A similar situation has been reported in Libya, where the liver was again the organ most commonly infected followed by the lungs and kidneys, although there were more fertile cysts in the lungs than the liver (Gusbi *et al.*, 1987).

Principal causes of the persistence of CE in sheep

Among the principal causes of the persistence of hydatidosis, and thus more generally echinococcosis, in sheep in Sardinia, is the low commercial value of sheep carcasses. As a result many sheep are slaughtered at home and not in slaughterhouses and the carcasses of sheep which have died in pasture land are left where they have fallen.

There is a series of reasons for the low commercial value of the carcasses. At present the bureaucratic authorities responsible for the logistical and economic aspects of slaughtering, share the outmoded belief that hydatid cysts are a normal part of the anatomy of sheep. This is also widely believed by many Sardinian shepherds, who also believe that the sheep is his own property to do with as he pleases. Consequently illegal slaughtering continues on 93.5% of Sardinian farms (Scala *et al.*, 1996). This

Table 1. Prevalence of sheep with fertile hydatids, mixed infestations (liver + lungs), and massive infestation (more than 10 hydatids/sheep examined) in the province of Sassari.

District	Total prevalence of CE	Mixed infestation (lungs+liver)	% sheep with fertile hydatids	% sheep with massive infestation	Authors
Sassari (1988)	85.1%	62.4%	36.8%	26.6%	Gabriele *et al.* (1992)
Sassari (1996-97)	76.7%	62.6%	16.9%	30.1%	Gabriele *et al.* (1998)
Sassari (1999)	75.6%	52.2%	6.9%	14.7%	Scala *et al.* (2000)

phenomenon is increasing due to the continually higher cost of slaughtering in abattoirs. Today slaughtering is so highly regulated and involves such difficult obligations, responsibilities and taxes that only the most profitable types of farm can afford them to comply with them. In is increasingly clear that the EU regulations have resulted in a drastic reduction in the number of small slaughterhouses which were previously found in the island, and the concentration of slaughtering in a few large, more modern and efficient structures. This is causing an aberrant evolution of our national slaughtering system in favour of industrial intensive farming and the abandonment of the previously widespread small unit productive system. Today the abattoir is a defective health institute whose role is solely to safeguard the quality of the meat which leaves it and its own environmental friendliness, while the remains of animals of low commercial value are either eaten without any health checks or are abandoned in the countryside (Cosseddu, 1998).

This situation is causing both a worsening of the health situation and also economic underdevelopment, and feeding the vicious circle of disease-poverty-disease which is so much feared by the WHO. The EU must bear this in mind and attempt to find a solution (Palmas and Ecca, 1995).

Thus, despite the fact that CE is still widespread in Sardinia, the shepherds and politicians do not attach particular importance to it and instead concentrate on other diseases which are of greater interest to the media (e.g. African Swine Fever, Scrapie and, recently, Blue Tongue in sheep). While these are indubitably serious, they are not more important than CE, especially when one bears in mind that it has, without question, important repercussions on animal health. This lack of interest is also linked to the fact that a control program for CE would certainly have to last more than five years, or, in other words, for longer than the life of a Sardinian regional legislature. As a result it is difficult to interest politicians in a program which would take so long to bear fruit, and which would not have an immediate impact on their public image.

Useful initiatives to control illegal slaughtering

In the areas where extensive methods of sheep farming are practiced, financial support should be given to encouraging the following indispensable initiatives: a) encouraging the slaughtering in slaughterhouses of animals of low commercial value such as sheep; b) developing the organisations involved in collecting the animals and taking them to the slaughterhouses; c) supporting organisations involved in finding ways of increasing the commercial value of the meat; d) establishing the norms used for slaughtering of local production (Cosseddu, 1998); e) providing adequate systems for disposing of the carcasses of animals which have died in the fields. This last point in particular could be solved by giving each farm a suitable container with the correct dimensions and structural characteristics. This would certainly reduce the number of carcasses abandoned in the fields, which then become a source of food for sheepdogs and/or the stray dogs that infest the island. In this respect, in the 90's, the previous Public Health authorities of Ales (Oristano) in took a useful initiative by inviting the shepherds to take the liver and lungs of dead sheep to the veterinary authorities. These were then kept in freezers and later incinerated. In this way the dead animals were removed from the stock list without any other bureaucratic formalities. This imitative was extremely successful but for various reasons, mainly bureaucratic, it was abandoned.

Certainly heightened awareness of the problem would be a positive step, as would be setting up a series of voluntary certification policies designed to give maximum value to animals raised extensively in "natural" environments. Certain commercial companies which already prepare sheep meat sausages and ham should also be encouraged. These are not adequately supported by the local authorities, unlike initiatives in other sectors. The local authorities are encouraging initiatives which follow the misguided approach used for wild ruminants in Valtellina (Lombardy), whose so-called "hams", and are being marketed, principally to elite and foreign tourists from North-East Europe, at high prices (some € 40/kg). The demand form the market is so great that at present the suppliers cannot satisfy it. The situation for sheep sausage is different. This is not always to the taste of the local Sardinian population, but is greatly appreciated by and in demand from the growing Muslim immigrant population. It is clear that if this activity was adequately financed it would provide a useful source of income for shepherds and this would reduce illegal slaughtering. Another possibility is exporting sheep at the end of their productive life to the Arab world, as Australia already does on a large scale (cf. Australia and Arabia, The incredible sheep war, "Il Venerdì di Repubblica" 24/10/2003). The sheep are then slaughtered and the meat prepared in a way which conforms with the rules of their religion. At a local level the consumption of mutton from adult sheep should be encouraged. At present only lambs are eaten, and these are usually only one month old. The sale of mutton could be encouraged by selling the types of cuts that the consumers value (e.g. cutlets and steaks ready for cooking) and above all by making the public aware of the positive virtues of sheep meat for human health, such as the quantity of unsaturated fatty acids it contains. This is widely appreciated by consumers as being of importance in the struggle against cholesterol (Santercole *et al.*, 2003).

Another action could also be taken to reduce the parasitic pressure on sheep. Restrictions could be placed on the transportation and sale of sheep from

farms where the sheep were found on slaughtering to have a level of infestation which is higher than the average for the area. A program of this type to check the spread of CE was used successfully in Tasmania in the 1970s (Thompsons and Lymbery, 1995). Obviously such a program would have to be supported by financial assistance to the farmers to avoid a boomerang effect and an increase in home slaughter.

Conclusions

From what is written above it is clear that the situation in Sardinia is, unfortunately, still very serious and integrated control measures on various fronts are necessary. At the same time one must remember that all the previous attempts to eradicate or control this important zoonosis in Sardinia ran into various obstructions and obstacles. Most of these were, however, financial and political, as Eckert *et al.* (2000) reported was also the case in other geographical areas where CE was fought with programmed plans. They state: "..., furthermore, financial restrictions and political instability are major obstacles in control and prevention of echinococcosis .....".

However not all is lost! While it is clear that routine or classical control measures will only be partially successful, if these are combined with a series of important innovations in the field of animal husbandry, and also a new sense of social solidarity, then important results can be achieved. The recent establishment of The National Reference Centre for Echinococcosis-Hydatidosis at the Experimental Institute for Zooprophylaxis of Sardinia could certainly encourage these new initiatives. Electronic tagging of sheep, which should come into force from 2006, should be particularly encouraged. So should research into a vaccine which impedes the development of metacestode in sheep. These are the most important intermediary hosts for the parasite in the island, and preventing its development in sheep is the most important aspect of the battle against CE. The results on the development of such a vaccine are encouraging (Lightowlers and Gauci, 2001; Tola *et al.*, 2002) and should be studied with great attention, because if the vaccine, which has already been developed and is presently being tested, passes the field tests then it will be without any question the real weapon to defeat this parasitosis.

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Critical points in the immunodiagnosis of cystic echinococcosis in humans

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Abstract. This study discusses the immunodiagnosis of cystic echinococcosis (ce, caused by *Echinococcus granulosus*). The detection by immunoblotting of antibodies specific for the 8 kDa subunit of antigen B and in particular the IgG4 subclass expression, seems the most promising serodiagnostic tool. Despite the development of molecular methods, nowadays there is no standard, highly sensitive, and specific test available for antibody detection in CE. Furthermore, because serological tests can give only a limited support to clinical findings there is a clear need for new advances in immunodiagnosis of *E. granulosus* infection.

Key words: *Echinococcus granulosus*, immunodiagnosis, serological tests.

Human cystic echinococosis (CE), caused by the metacestode *Echinococcus granulosus*, is considered the zoonosis most geographically widespread among the parasitic diseases (McManus and Zhang, 2003). Because prompt intervention can reduce morbidity, early diagnosis of CE by clinical or immunological methods is important (Craig, 1997; Siracusano *et al.*, 2002). The diagnosis of CE is critical on 5 points: (1) suspect CE whenever or wherever it may occur (frequent in immigrants from endemic areas); (2) carry out carefully a differential diagnosis process (non parasitic space-occupying lesions are common); (3) use fine-needle aspiration biopsy (FNAB) to confirm doubtful cases of CE by finding protoscoleces, hooks, antigen in the biopsy specimens (FNAB also helps in differential diagnosis of non-parasitic lesions); (4) try to diagnose CE as early as possible (treatment of young cysts is more successful); (5) evaluate viability status of the parasite (inactive cysts may not need any intervention). The diagnosis of echinococcosis, especially of the atypical forms, requires sophisticated laboratory investigations identifying and characterizing the echinococcal lesions by imaging techniques such as ultrasonography, X-ray examination, computerized tomography or magnetic resonance imaging. These techniques may also have limited diagnostic potential because the atypical appearance of the cysts often makes them difficult to distinguish from abscesses or tumours. Visualized lesions may also be insufficient in providing information about the involved species or about the viability of the parasite. The definitive diagnosis therefore invariably rests upon immunological tests based on serum antibody or circulating antigen detection.

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Immunological techniques

The choice of a serodiagnostic technique depends primarily on its sensitivity and specificity. The first problem is that most conventional tests give a high percentage of false negative results (up to 25%). Secondly, in areas where *E. granulosus* and *E. multilocularis* coexist, the two may be hard to differentiate owing to the presence of false positive reactions. Currently, preferred immunodiagnostic techniques for sensitive measurement of specific antibodies include initial screening tests to identify crude somatic antigens (hydatid fluid or protoscolex extracts), double diffusion, indirect haemoagglutination, and enzyme-linked immunosorbent assay (ELISA). Despite their relatively high diagnostic sensitivity, these tests still fail to detect a certain percentage of patients with CE. The preferred tests for confirming the immunodiagnosis are ELISA using species-specific antigens, and immunoblotting (IB), a technique that identifies antigen subunits. Despite the development of these sensitive techniques the immunodiagnosis of CE remains a complex task, not yet feasible in worldwide centers. This task is made even more difficult by the lack of standardisation of the various techniques, a drawback responsible for the discrepant results reported by the various laboratories. Another diagnostic strategy for identifying active or current infections is to develop a technique for detecting circulating antigen in serum or urine (Craig, 1986; Ravinder *et al.*, 2000). Research efforts now focus also on molecular tools for the diagnosis of CE. The two most promising methods developed involve isolating native or recombinant parasite antigens to detect specific serum antibodies in suspected echinococcosis patients, and producing monoclonal antibodies to detect parasite antigens in clinical samples (Siles-Lucas and Gottstein, 2001).

Table 1. Immunological markers of *Echinococcus granulosus* cyst viability*.

Cyst viability	Clinical markers	Imaging markers	Immunological markers
Active	Frequently symptomatic; exerting pressure on adjacent tissue; complicated (ruptured, recurrent). Pharmacological or surgical treatment is opportune.	Growing fast. Type CE1 (simple cyst); type CE2 (with detachment).	Highly immunogenic with serological tests frequently positive. IgG subclasses: IgG4>IgG1; IgE elevated in some cases. Subset Th2 activated (IL-4, IL-5, IL-6, IL-10).
Transitional	For treatment: "wait and see".	Cyst is usually starting to degenerate. Type CE3 (unilocular cyst which may contain daughter cysts).	Similar to active cyst.
Inactive	Asymptomatic. No treatment.	No growing. Type CE4 and CE5 (solidification, calcification).	Weakly immunogenic with serological tests slowly decreasing. IgG subclasses: IgG1>IgG4; IgE decreasing quickly. Subset Th1 activated (IFN-γ, IL-2, TNF-α).

* Following the classification reported in Pawlowski ZS, Eckert J, Vuitton DA *et al*. In: WHO/OIE Manual on Echinococcosis in Humans and Animals: A Zoonosis of Global Concern, eds Eckert J, Gemmel MA, Meslin FX, Pawlowski ZS. Paris: World Organisation for Animal Health; 2001: 2066.

Antigens

The parasitic antigens present in hydatid fluid that have major immunodiagnostic value in detecting *E. granulosus* are Antigen (Ag) 5 and AgB. Native Ag5, a 400 kDa thermolabile lipoprotein produces two subunits at 55 and 65 kDa in sodium-dodecyl sulphate-polyacrilamide gel electrophoresis (SDS-PAGE) under non-reducing conditions and 2 subunits at 38/39 and 20 kDa under reducing conditions. The 38/39 kDa component with phosphorylcholine epitopes may be responsible for a large proportion of cross-reactions with sera from patients infected with nematodes, cestodes and trematodes. Native AgB, a polymeric lipoprotein with a molecular mass of 120 kDa, produces 3 main subunits at 8, 16 and 20 kDa in SDS-PAGE under reducing and non-reducing conditions as well as other mass-subunits, probably polymers of the 8 kDa subunit. The 8kDa subunit, that induces a good humoral and cellular response, has been proved most useful target un diagnostic studies (Ioppolo *et al.*, 1996). The main aim of current research is to investigate synthetic peptides derived from sequences of Ag5 and AgB (Gonzalez-Sapienza *et al.*, 2000). Although the use of recombinant antigens has become one of most prominent fields of research in immunodiagnosis, only recently recombinant antigens have begun to play a role in immunodiagnostic techniques (Ortona *et al.*, 2000).

Immunoglobulin isotypes

Recent studies designed to assess the IgG-subclass response in human CE demonstrated a predominance of IgG1 and IgG4 antibodies to cyst fluid antigens and a differential antigen recognition by these 2 subclasses, with Ag5 being recognised primarily by IgG1 and AgB being recognised by IgG4 (Ioppolo *et al.*, 1996; Wen and Craig, 1984). This differential antigen recognition may be an important feature in making the clinical diagnosis and also in studying parasite survival mechanisms. Studies designed to identify immunological markers of the clinical outcome have underlined the importance to add IgE and IgG subclasse detection in the immunodiagnosis and in the follow-up of patients with CE (Table 1).

Conclusion

In conclusion, to improve immunodiagnosis we can suggest to detect, by IB, antibodies specific for antigen B, in particular the IgG4 subclass expression. CE serology may be also improved by combining several defined antigens (including synthetic peptides). Currently, however there is no standard, highly sensitive, and specific test available for antibody detection in CE. Overall, these results indicate that, despite the development of promising molecular tools, serological tests can give only a limited support to clinical findings and there is a clear need for new advances in immunodiagnosis of *E. granulosus* infection.

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An up-to-date on clinical management of human cystic echinococcosis

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Abstract. More than one thousand of patients with more than two thousand of *Echinococcus granulosus* hydatid cysts were observed during the last 20 years. The therapeutic approach ranged from chemotherapy with benzimidazole carbamates (mebendazole and albendazole) to surgery, percutaneous non conventional treatment, and a "wait and see" approach, on the basis of the clinical picture, of the compliance and of the socio-economic background of each patient. The observed results, mainly those regarding chemotherapy, were briefly summarized and discussed. Most important point to underline is the need of data about a more prolonged follow-up on wider casuistries of patients both treated (with chemotherapy, with surgery, with percutaneous non conventional treatment) and not treated, to obtain a more suitable evaluation of the long-term outcome of the performed clinical approach. Moreover, a multidisciplinary approach is advisable to assure a better clinical management to patients with cystic echinococcosis.

Key words: cystic echinococcosis, hydatidosis, mebendazole, albendazole.

In the past, surgery was considered the first choice for treatment for most cases of human cystic echinococcosis (CE) because of the potential of completely cure the patient totally removing the parasite (WHO-IWGE,1997). Nowadays, besides surgery, clinical management of CE may relies on many therapeutic approaches ranging from chemotherapy with benzimidazole carbamates (mebendazole and albendazole) to percutaneous non conventional treatment, like PAIR (percutaneous Puncture with sonographic guidance, Aspiration of the content of the cyst, Injection of a protoscolicidal agent like 95% ethanol or hypertonic saline solution, Reaspiration), or Radio-Frequency Thermoablation, and a "wait and see" approach (Felice and Brunetti, 1997; Brunetti *et al.*, 2004; Pawlowski, 1997). Each of these approaches can be chosen on the basis of the clinical picture, of the morphologic characteristics of the cysts, and of the compliance and of the socio-economic background of the patient.

Materials and methods

One thousand and twenty four patients (age range: 4-87 years) with 205 *Echinococcus granulosus* hydatid cysts were observed during the last 24 years. The therapeutic approach ranged from chemotherapy with benzimidazole carbamates (mebendazole and albendazole), to surgery, percutaneous non conventional treatment, and a "wait and see" approach, on the basis of the clinical picture, of the compliance and of the socio-economic background of each patient, as previously reported (Teggi *et al.*, 1993).

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Each hydatid cyst was carefully examined concerning its morphology and volume; the classification based on cysts sonographic findings elaborated by World Health Organization International Working Group on Echinococcosis (WHO-IWGE) in 2001 was followed. It can be briefly summarized as:
- type I : anechoic (liquid) hydatid cyst;
- type II : hydatid cyst with daughter cysts;
- type III : hydatid cyst with membrane detachment or with partial solidification;
- type IV : hydatid cyst with total solidification of the matrix;
- type V : calcificated hydatid cyst.

Only the cysts that were clearly identified and belonged to patients who completed treatment and follow up for at least 12 months were considered .

Concerning chemotherapy, 402 patients with 842 cysts were treated with albendazole 10 mg/kg/day for 3 months, and 136 patients with 305 cysts were treated with mebendazole 50 mg/kg/day for 3-6 months performing clinical, haematological, immunological and imaging controls during treatment and during follow-up, as previously reported (Teggi *et al.*, 1993; Franchi *et al.*,1999). Therapeutic cycles may be repeated.

Criteria to evaluate the outcome of therapy were based mainly on imaging techniques, as described elsewhere (Teggi *et al.*, 1993). Follow-up ranged between 12 and 220 months.

In 96 asymptomatic patients with 123 liver cysts type IV or V a "wait and see" approach was followed. A follow-up for at least 60 months was available only for 60 of these patients with 77 cysts, so only them and their cysts were considered for results and discussion. Follow-up ranged from 60 to 204 months.

Three hundred seventy four patients with 725 cysts were observed only before surgery or soon after surgery. For most of them follow-up data was not available, so these patients were not considered here for results and discussion.

Sixteen patients with twenty liver cysts were treated with percutaneous non conventional treatments; because for most of them follow- up data were not available, these patients were not considered for results and discussion.

Results

As concerning medical therapy, actual data confirm the results reported and analysed in details elsewhere (Teggi *et al.*, 1993; Franchi *et al.*, 1999). I could summarize my experience as follows, taking into account that because mebendazole and albendazole have the same mechanism of action, data concerning cysts treated with both benzimidazoles were sometimes pooled:

- about 75% of treated hydatid cysts showed degenerative modifications after therapy;
- only 10% of the treated cysts disappeared;
- degenerative modifications of the cysts progressed also after the end of the treatment in about 22% of the cases;
- about 30% of the responsive cysts relapsed after stopping treatment;
- relapse consisted mainly in reappearance of liquid areas within the previous solidificated matrix;
- relapse was observed most frequently in type II liver cysts, within the first 2 years after stopping treatment;
- a further therapeutic cycle of benzimidazole carbamates was effective in more than 90% of relapsed cysts, maybe because their high metabolism;
- the observed side effects consisted mainly in pain in the localization of the cyst, and increase of transaminases; this last effect was always reversible; it was observed quite only in patients with liver cysts; transaminase values ranged mainly between 2 and 5 folds normal values, decreased spontaneously often without stopping treatment, so probably in these cases it may be considered a marker of treatment effectiveness.

Among the 60 asymptomatic patients with 72 liver cysts type IV or V in which a "wait and see" approach was followed, in 57 of them (and in 67 cysts) there were no modifications of the clinical picture and of the imaging technique findings. In 1 patient, 2 of his 2 cysts showed liquid areas within the matrix after 8 years of follow-up: it showed solidification after a cycle of albendazole treatment. In 1 patient the liver cyst showed sign of infection after about 70 months of follow-up (hypo-hyperechoic areas within the liver cyst, abdominal and lung pain, fever, neutrophilia, right pleural effusion and pneumonia as for cyst rupture in the right lung): the patient was submitted to surgery and the diagnosis of cyst rupture was confirmed. In an other patient with 2 liver cysts, 1 of them showed sign of infection after about 100 months of follow-up (hypo-hyper-echoic areas within the liver cyst, mild abdominal pain, fever): the surgical intervention confirmed the diagnosis.

Discussion

Concerning clinical management of CE, it is very important to individualize the treatment of choise for each patient and for each hydatid cyst. To reach this goal, first of all we have to identify each single hydatid cyst, carefully focusing their morphology and volume, and to follow the common classification based on cysts' imaging techniques' findings, as recently elaborated by WHO-IWGE.

This classification is particularly useful because it is an effort to correlate the ultrasonographic findings of the hydatid cysts with their clinical activity, which is, in my opinion, decreasing from type I-II (representing most active cysts) to type V cysts, representing most inactive parasitic lesion. So, medical, surgical and percutaneous non conventional approach to this parasitosis could be chosen for the first 3 types of hydatid cysts, whilst "wait and see" management seems to be more appropriated in the last 2 types of hydatid cysts.

In the therapeutic approach to cystic echinococcosis (CE), it is advisable to distinguish the cases in which there is a concordance between clinical and immunological data and imaging findings, and the cases in which these findings are discordant. In the first cases, and for uncomplicated cysts, it is possible to choice among medical treatment, radical surgery and percutaneous non conventional treatment on the basis of clinical picture and patient compliance, taking into account also a "wait and see" approach for asympomatic patients with liver cysts, especially if their imaging findings suggest larval inactivity, as for type IV and type V of the WHO-IWGE classification, taking into account the potential risks (of rupture, of infection, and so on) that, however, can overcome also after years of quiescence

When clinical and immunological data and imaging techniques findings are not concordant or in presence of complicated hydatid cysts, surgical approach must be performed.

Soon after the clinical picture, the morphologic characteristics of the cysts and their localization, the compliance, the consensus of the patient about the therapeutic approach of CE and the social and economic background are most important factors to be taken into account. Also previous patients experiences influence the choice of the treatment: most of patients previously surgically treated choice chemotherapy, whilst surgical approach is often choose by patients never submitted to surgery.

If hydatid cysts are uncomplicated, and there are no contraindication to benzimidazole treatment (as pregnancy, marked impairment of liver, renal or haemopoietic functionality), a cycle of albendazole treatment can be proposed as first choice approach.

Concerning chemotherapy of CE, many factors related both to the host and to the parasite, can influence the outcome of the treatment:

- drug: albendazole is more effective than mebendazole, mainly for liver cysts;
- dosing schedule: both albendazole and mebenda-

zole are more effective in cycles of continuous treatment, without intervals;
- age: "young" cysts and cysts of young people are more responsive to benzimidazole carbamates;
- cyst localization: therapy with benzimidazole carbamates is more effective against lung cysts than liver cyst; cysts of bone or of central nervous system are very difficult to treat;
- cyst morphology: type I cysts show more frequently membrane detachment after treatment, whilst type II cysts showed more frequently solidification of the matrix;
- -intrinsic sensitivity to drug of each single hydatid cyst: sometimes different therapeutic results were observed in cysts of the same patient, with the same morphology and localized in the same organ.

In conclusion, therapy with benzimidazole carbamates is quite safe and well tolerated; up to date most relevant problem to solve are the relapses.

Until now, the effectiveness of further therapeutic cycles on relapsed cysts is very high (higher than the mean effectiveness rate observed during the first cycle of treatment), and the side effects are less frequently observed during further cycles of therapy than during the first cycle of benzimidazole carbamates. Nevertheless,there are few data about the side effects and the toxicity of prolonged treatment with benzimidazole carbamates and after the fourth or the fifth relapse both the patient and the physician are reluctant to perform further cycles of therapy. On the other hand, we have to underline that relapses take place also after surgery (especially after not radical surgery) and percutaneous non conventional treatments. Moreover, surgery has been associated with high rates of intra– and postoperative morbidity and mortality, which increase mainly with repeated interventions (Little, 1976).

Type II cysts, probably the most active type of hydatid cyst, are the more problematic to treat, both by chemotherapy and by percutaneous non conventional approach and also by surgery, because of the high rates of recurrence.

Collection of more data on wider casuistries about the relapses rate and about the side effects and complications observed after surgery, medical treatment and percutaneous non conventional treatments, and collection of more data about history of untreated hydatid disease should be necessary to compare the long-term outcome of the different therapeutic approaches and to reach a better management to patients with pluri-relapsed hydatid cysts. Furthermore, a prolonged and careful follow-up is advisable for all patients with CE, both for untreated patients and for patients treated by surgery, chemotherapy and percutaneous non conventional treatments, because this parasitosis is slowly-growing, often quiescent, but sometimes it can lead to unexpected surprises and complications.

Finally, a multidisciplinary approach to this disease with the full cooperation among experts in biology, immunology, epidemiology, veterinary, pharmacology, radiology, surgery and physicians is necessary to reach the best clinical management to the patients affected by CE.

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The diagnosis of *Echinococcus granulosus* in dogs

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Abstract. The problem of diagnosing *Echinococcus granulosus* in dogs has still only been partially resolved, even after the advent of biotechnology. The eggs of taeniid Cestoda are extremely similar, and thus identification by microscopic examination of the faeces is risky and non-specific. For this reason, Echinococcus *granulosus* was traditionally diagnosed in dogs *ante mortem* after an arecoline hydrobromate purge. The faeces were examined macro and microscopically to establish if the adult tapeworm or its proglottids were present. Although this method is 100% specific, it is bio-hazardous and time-consuming, requires trained personnel, and its sensitivity varies. In the 1990s copro-antigens were discovered and characterised. These are released by the adult worm in the faeces. This made it possible to use enzyme-linked immune-adsorbent assay (ELISA) for *in vitam* diagnosis of *Echinococcus granulosus*. In recent years several PCR protocols have been published on the identification of *Echinococcus granulosus* DNA from eggs or from adult parasites and new ways of diagnosing this cestode have been developed.

Key words: diagnosis, dogs, echinococcosis, copro-antigens, PCR.

The Gold Standard technique of *Echinococcus* infection in the definitive host is the recovery of adult parasites in the intestine after necropsy. The best known method is described by Eckert *et al.* (2001) as the "sedimentation and counting technique" (STC). This is based on examining the faeces in the small intestine of the dogs with a microscope and counting the number of adult parasites. Although this method is 100% sensitive and specific it is also time consuming and bio-hazardous and can only be used on dead animals.

This paper reviews other alternative methods for diagnosing echinococcosis in dogs. These may be used for routine *in vitam* diagnosis, epidemiological studies and disease control plans.

Before starting, it is important to remember that these experiments involve a potential risk of infection for humans, so safety precautions should be taken when handling this material. Eggs can be deactivated by freezing at –80°C for at least 4-7 days or by heating to ≥ 60°C for 5 min (Eckert *et al.*, 2001).

Classical methods

Arecoline purging

Ante mortem diagnosis of canine echinococcosis has traditionally been performed by purging with arecoline hydrobromate (Eckert *et al.*, 2001). Arecoline is a parasympathomimetic drug that, when given to dogs in tablet or liquid form at doses between 1.75-3.5 mg/kg body weight, purges the entire intestinal contents, increases intestinal peristaltic movements, and paralyses the tapeworms. These can then be collected and identified.

This technique has been used in many control programs all over the world in recent decades. It has got 100% of specificity, however it has certain limitations. For example, its sensitivity is limited since not all dogs respond to the purge, and not all infected dogs eliminate *Echinococcus granulosus*, it is also bio-hazardous and time-consuming and must be administered by trained personnel (Eckert *et al.*, 2001). In situations where endemic rate of *Echinococcus granulosus* in the dog population is low, the predictive value of the test diminishes as the percentage of infected dogs decreases (Schantz, 1973).

This is an unpleasant technique but is the only quantitative technique that can be used on living dogs and it continues to play an important role in epidemiological studies. Most epidemiological data, and the models developed from them, come from the results of this method (Torgerson *et al.*, 2003).

Macroscopic and microscopic examination

Adult parasites or proglottids can be recovered and identified by macroscopic exam of faeces, but unless purging is used the chances of finding the adult Cestoda are extremely inconsistent. In addition copro-microscopic examination (sedimentation and flotation technique) to detect *Echinococcus granulosus* eggs is, unfortunately, not a useful method of diagnosing for this parasite.

Echinococcus granulosus eggs are morphologically indistinguishable to those of other taeniid Cestoda, emission of eggs is variable and inconstant (and naturally not present in the prepatent period). Copro-microscopic examination may be used successfully if it is combined with other more specific techniques, such as PCR examination of DNA from the isolated eggs.

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Detection of serum antibodies

Specific antibodies against oncosphere and protoscolex antigens can be readily detected in the serum of infected dogs (Heath *et al.*, 1985; Jenkins and Rickard, 1985). This is not yet a practical method, as it does not differentiate between current and previous infections and there are some problems related to cross-reactivity or specificity of the test (Gasser *et al.*, 1994).

The detection of serum antibodies using parasite antigens in ELISA has been considered unsuitable for reliable diagnosis of intestinal *Echinococcus* spp. infections because there is a poor co-relationship between the presence of specific antibodies and the worms (Eckert *et al.*, 2001). The development of diagnostic methods based on the detection of faecal copro-antigens has superseded this diagnostic method. Indeed a comparison of copro-diagnosis and serology detection found that identifying copro-antigens was 2.5 times more sensitive than antibody detection (Walters and Craig, 1992; Craig *et al.*, 1995).

New techniques

Copro-antigens detection

An alternative to arecoline testing, based on a faecal antigen-detection antibody sandwich enzyme-linked immunosorbent assay (ELISA), has been developed recently. This has shown particular promise, as copro-antigens can be detected shortly after infection (10-14 days) and their level declines rapidly following expulsion of the worms (within 3-4 days) (Malgor *et al.*, 1997).

The test is based on a parasite-specific layer of captured IgG antibodies which retains antigens from faecal supernatants. Copro-antigen detection ELISA tests have been developed that uses polyclonal antibodies to *Echinococcus granulosus* excretory/secretory (ES) antigens. *Post mortem* examination of naturally infected dogs showed 56% sensitivity and 96% specificity (Deplazes *et al.*, 1992).

Allan *et al.* (1992), using antiserum to somatic antigens in copro-antigen detection, found this was 88% sensitive in naturally infected dogs. False negative results in these studies have been attributed to low worm burden. Indeed, when the results were compared with those of *post mortem* examination, overall sensitivity was 63% but this increased to 92% in dogs with more than 100 worms (Deplazes *et al.*, 1992).

Copro-antigens can be detected prior to the release of the eggs by *Echinococcus* worms, and therefore are not related to egg antigens (Deplazes *et al.*, 1992; Sakashita *et al.*, 1995). Positive ELISA results were obtained during the prepatent period in dogs as early as 5 days post infection (Deplazes *et al.*, 1992; Sakashita *et al.*, 1995; Nonaka *et al.*, 1996). This has the advantage of being able to detect prepatent infections.

ELISA copro-tests can also detect heat-stable antigens. They have been used in a number of studies in the Middle East, Wales, Southern and Eastern Europe, and South America (Deplazes *et al.*, 1992; Sakashita *et al.*, 1995; Eckert *et al.*, 2001). The high sensitivity of monoclonal antibodies (MAb) to parasite specific antigens could increase the reliability of copro-antigen detection. Some sandwich ELISA systems have been evaluated for their ability to detect *E. granulosus* copro-antigens. These used a monoclonal antibody produced against somatic extract of *E. multilocularis* (Sakai *et al.*, 1995; Malgor *et al.*, 1997). Although the test was very sensitive (100%) in naturally and experimentally infected animals, there were also cases of cross-reactivity with *Taenia hydatigena* (Malgor *et al.*, 1997).

Recently the first MAbs for *E. granulosus* copro-antigen detection were produced: two IgM murine monoclonal antibodies (MAbs), EgC1 and EgC3, against the excretory/secretory (E/S) products of *E. granulosus* adult worms (Casaravilla *et al.*, 2004). A copro-antigen capture ELISA was developed using a rabbit polyclonal antibody against E/S products from adult tapeworms as catching antibodies, and each of the MAbs as detecting antibody. The assays detected 7 out of 8 (EgC1), and 8 out of 8 (EgC3) experimentally infected dogs (worm numbers ranging from 61 to 57,500), and none (n=8) of the negative control samples. Faecal samples from 2 dogs experimentally infected with *E. multilocularis* were not recognised by the EgC1 assay. This suggests that this is a potential species-specific diagnostic tool for discriminating *E. granulosus* and *E. multilocularis* infections (Casaravilla *et al.*, 2004). These advances made in applying MAbs in ELISA tests for copro-antigen detection suggest that this may be an interesting research line in order to develop new and more sensitive kits for diagnosing echinococcosis in dogs.

PCR protocols

It would be useful to develop more specific techniques in cases where the presence of the parasite in the dog population is relatively low (Christofi *et al.*, 2002), as well as for discriminating between dogs with *Echinococcus* and those with other taeniid infections. Several PCR tests were developed for detecting *E. granulosus*-specific DNA (Cabrera *et al.*, 2002; Abbasi *et al.*, 2003; Dinkel *et al.*, 2004; Štefanic *et al.*, 2004).

Two different protocols could be used for isolating the DNA: one extracts DNA from the total amount of faeces, and the second isolates and concentrates first taeniid eggs by combining sequential sieving with flotation solutions (Mathis *et al.*, 1996). This second protocol seems to be most useful because: faeces could contain substances that inhibit DNA amplification, only a limited amount of material can be processed in DNA extraction, and laborious purification of the DNA is often indispensable (Štefanic *et al.*, 2004).

DNA is obtained by alkaline treatment (to lyse the

eggs), neutralisation, proteinase K digestion and DNA purification using organo-solvent extractions and/or DNA adsorbing matrices or, more recently, by the use of commercially available kits designed for DNA isolation from faeces (Abbasi *et al.*, 2003; Štefanic *et al.*, 2004). The available protocols mentioned above for detecting *E. granulosus* are all designed to detect G1 *E. granulosus* (Sheep strain). The protocol evaluated by Dinkel *et al.* (2004) describe primers that detect strains G5 (Cattle strain), G6 (Camel strain) and G7 (Pig strain), with the additional possibility of strain typing by means of a second PCR.

Only the protocols of Abbasi *et al.* (2003) and Štefanic *et al.* (2004) were tested on faecal or environmental material, while Cabrera's *et al.* (2002) had a sensitivity limit of at least 100 eggs per gram of faeces when applied to infected dogs (Abbasi *et al.*, 2003).

The PCR test used by Abbasi *et al.* (2003) found 100% sensitivity and specificity using DNA samples extracted from 0.3 ml of faeces from 34 infected and 18 non-infected dogs, and found positive results even when the sample contained only two *E. granulosus* eggs.

Conclusions

Although PCR is a very sensitive technique for detecting parasite-specific DNA from a very small number of eggs, it is not quantitative and is not suitable for large scale screening of samples. So, at the moment the method of choice for *in vitam* diagnosis of *E. granulosus* in large populations of dogs should be the detection of copro-antigens. This is highly sensitive, fast and cheap.

Detection of *E. granulosus* by ELISA has a very high negative predictive value. However positive prediction using this method becomes poor when the prevalence of the parasite in dogs is very low (Christofi *et al.*, 2002). In this epidemiological situation, copro-antigen positive dogs could be screened with a PCR-based technique to distinguish between true and false positive results. In the future, it may be possible to diagnosis copro-antigens using highly specific MAbs for the through ELISA test. This could be used for large scale screening in control programs and epidemiological studies.

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Communications

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Human echinococcosis in the Emilia-Romagna Region (northern Italy) in the years 1997 to 2002: an updating

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The present survey is an updating of the analyses of human cases of echinococcosis in the Emilia-Romagna Region (ERR) reported in the period 1997 to 2002 using data drawn from the hospital discharge records (HDR) and collected in a regional database. The aim of this study was a detailed analysis of the 249 cases of patients residing in the ERR and considered Italian citizens according to the original codifications of the different hospitals (Battelli *et al.*, 2004, Parassitologia 46, Suppl. 1, 25). The data are also referred to citizens residing in the ERR discharged from extra-regional hospitals with diagnosis of echinococcosis. To assess the incidence, considering that each patient might have been hospitalised more than once during the above period, only the first pathology-specific hospitalisation was taken into consideration; the denominator was chosen as the mean yearly population of Italians residing in the territory during the period considered. Table 1 shows the cases divided according to the provinces of residence and the geographic areas where the patients were born.

On the whole, the mean yearly incidence of echinococcosis in Italian citizens residing in the ERR was 1.07/100,000; the average incidence of persons born in Italy alone was 0.97/100,000. Out of 249 cases, 225 (90.4%) were persons born in Italy; 61.3% and 38.7% of these were persons born in the ERR and in other Italian regions, respectively. The cases of residents born in other regions were divided as follows: 3 Piedmont, 3 Lombardy, 4 Veneto, 2 Tuscany, 1 Umbria, 5 Marche, 1 Latium, 3 Abruzzo, 3 Molise, 15 Campania, 12 Apulia, 7 Basilicata, 10 Calabria, 14 Sicily, and 4 Sardinia. The cases of residents born abroad were divided as follows: 1 Belgium, 1 France, 1 Greece, 5 Morocco, 1 Portugal, 1 Rumania, 1 Serbia-Montenegro, 5 Tunisia, and 8 with unspecified birthplace. The province of Reggio Emilia (RE) showed the statistically highest incidence ($P<0.05$). Considering the 225 cases from persons born in Italy, the existence of an area with a radius of about 30 km at statistically higher risk ($P<0.01$) of echinococcosis (cluster 1) located in the Apennines between the provinces of RE and Modena (MO) was again confirmed using the spatial scan statistic (Ostanello *et al.*, 2004, Parassitologia 46, Suppl. 1, 57). Two other areas with non-significant ($P> 0.05$) increased risk were identified: the former (cluster 2), adjacent to cluster 1, including some municipalities of the Apennines in the Bologna province; the latter (cluster 3), was located in the Ferrara province (Fig. 1). Regarding cluster 1, the incidence cases were 15, 12 of which in persons born in the provinces of RE and MO, and 3 in persons born in other Italian provinces (Pistoia, Avellino and Agrigento). The average yearly incidence was 7.1/100,000, namely 9.4 and

Table 1. Hospitalised echinococcosis cases of Italian citizens residing in the ERR (1997-2002).

Province	No. cases (In)	No. born in Italy			No. born abroad
		Total (In)	Born in ERR	Born in other Italian regions	
Bologna	63 (1.18)	54 (1.01)	26	28	9
Forlì-Cesena	18 (0.86)	17 (0.81)	12	5	1
Ferrara	24 (1.15)	22 (1.06)	18	4	2
Modena	43 (1.19)	42 (1.16)	29	13	1
Piacenza	5 (0.32)	4 (0.26)	3	1	1
Parma	17 (0.74)	13 (0.56)	8	5	4
Ravenna	23 (1.12)	22 (1.07)	18	4	1
Reggio Emilia	42 (1.62)	38 (1.47)	20	18	4
Rimini	14 (0.88)	13 (0.82)	4	9	1
Total	249 (1.07) (CI: 0.75-1.40)	225 (0.97) (CI: 0.66-1.28)	138	87	24

In: average yearly incidence/100,000; CI: Confidence Interval 95%.

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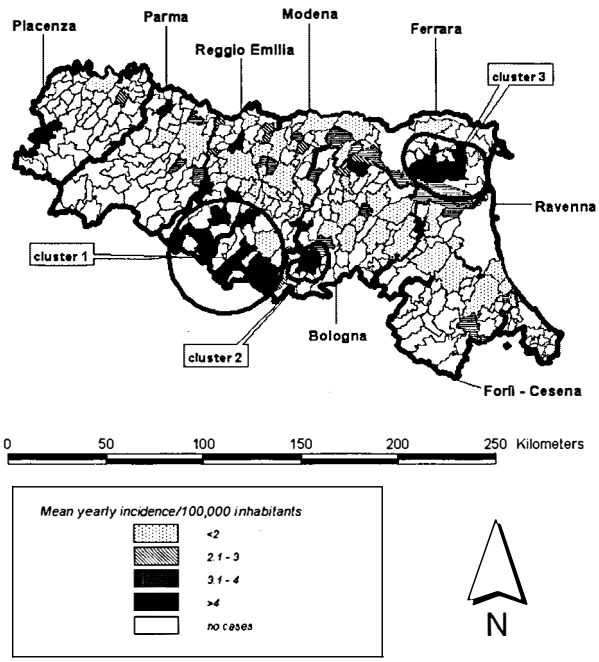

Fig.1. Spatial clusters and average yearly incidence of human cases of echinococcosis per each municipality in the ERR (1997-2002).

5.6/100,000 in the municipalities belonging to the cluster RE and MO, respectively. The total number of hospitalisations was 80. Two patients were surgically treated. Subdivided by age classes at the first hospitalisation, the patients were: 1 (20 to 29 years); 1 (40 to 49); 2 (50 to 59); 5 (60 to 69); 1 (70 to 79); 5 (80 to 89).

The present results confirm that: (1) the incidence of echinococcosis is not homogeneous over the ERR territory, even taking into consideration the citizens residing in the region and born in Italy, only; (2) the statistically highest incidence was found in the RE province; (3) there is an area in the Apennines between the provinces of RE and MO at a significantly higher risk, with an incidence to be considered high and similar to that encountered in areas considered endemic; (4) as an information source, the HDR has the following major advantages: (i) the detection of cases irrespective of the region of hospitalisation; (ii) the detection of a number of cases higher than that drawn from the operating room and *post mortem* records; (iii) the possibility, through a quick access to the regional databank, of readily conducting a collection of information, including the number of hospitalisation days and costs. Major disadvantages are: (i) the impossibility to determine the patients' professions; (ii) difficulties in assessing the diagnostic procedures.

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Evaluation of different diagnostic methods to detect *Echinococcus multilocularis* in the final host

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Echinococcus multilocularis is a cestode that occurs in the northern hemisphere within a large belt stretching from the northern tundra zone southward to some regions around 40°-45°N. The currently known endemic areas include regions in Europe, in Asia, and in North America. A few cases were also reported from northern Africa (Rausch, *Echinococcus* and hydatid disease, RCA Thompson and AJ Lymbery Eds, 1995; Schantz *et al.*, Alveolar echinococcosis, Uchino J and Sato N Eds, 1996). As far Europe, the parasite was known to occur in 10 countries (Belgium, France, Luxembourg, Germany, Switzerland, Liechtenstein, Austria, Poland, Czech Republic, Turkey), but recently it has been also reported in Italy (Manfredi *et al.*, 2002, Vet Record 150: 757-758). In these areas, red foxes (*Vulpes vulpes*) have to be regarded as the most important definitive hosts whereas domestic dogs and cats are far less frequently infected. The existence of this sylvatic cycle in Italy is alarming, because it suggests a potential infection risk for humans and other hosts exposed to the accidental contacts with food or soil contaminated by the parasite eggs, spread by foxes with droppings. Occupational and behavioural factors can influence risk levels, higher in people like hunters, trappers, workers in agriculture or with fur, walkers in wild areas, in which eggs swallowed develop in metacestodes. The severity of the larval lesions in humans makes the alveolar hydatidosis a cronic, cancer-like, disease with high mortality rates in untreated patients.

In order to understand the spread of this disease and to get ready suitable control measures, it is of basic importance to define the endemic areas and, therefore, to have very sensitive and specific diagnostic tools that allow a valid monitoring. Aim of this study is to investigate on the reliability of different methods to detect infected animals, by comparing both traditional (necropsy and microscopic analysis) and innovative (immunological, bio-molecular) techniques.

The study was carried out on 35 foxes found dead in Tuscan Appennines. In order to reduce/exclude an infection risk for laboratory personnel, intestines, after removed, were deep-frozen at –80°C for at least 5 days. The intestinal walls and contents were examined by traditional parasitological procedures aimed to detect eggs/proglottids in faeces and adults in mucosal scrapings, and by diagnostic approaches based on the egg/adult DNA detection in faeces/mucosal scrapings and, finally, on the coproantigens detection. Threrefore, faeces were collected, divided in 3 aliquots to allow the parallel macroscopic/microscopic, immunological and molecular analyses. The small intestine was opened, the mucous membrane was scraped using microscopic slides, and the mucosal squashes were divided in 2 aliquots to allow the identification of adults eventually present both by molecular methods and by microscopy.

Macroscopic search of proglottids and adult worms was carried out in stools and material from the intestinal scraping by the standard laboratory procedures, followed by the stereomicroscopic analysis at ×120 magnification, and completed by the identification of all found parasites. Microscopic search of eggs was carried out following a sedimentation-flotation treatment to concentrate faecal samples.

Immunological detection of antigens released by the parasite into the intestinal fluid was performed applying an *Echinococcus* specific ELISA kit based on rabbit polyclonal antibodies to *Echinococcus* to faecal material (Chekit Echinotest Monophasic, Bommeli Diagnostic, Switzerland).

Molecular detection of eggs/proglottids/adults of *E. multilocularis* in faecal samples/mucosal squashes and intestinal contents was carried out by PCR following a protocol previously described (Dinkel *et al.*, 1998, J Clin Microbiol 36: 1871-1876). The target sequence for amplification is part of the *E. multilocularis* mithocondrial 12S rRNA gene which has been used in phylogenetic studies (von Nickisch-Rosenegk *et al.*, 1999, J M Evol 48: 586-596). A preceding step of genomic DNA extraction from tissues was carried out using Wizard SV Genomic DNA Purification Kit (Promega, USA), and with QIAamp DNA Stool Kit (QIAGEN, Milan) from stool samples. The PCR was conducted in two steps. For the first PCR, the primer pair P60 and P373 rev that yield a 373 bp fragment recognized common, to date, to 12 cestode species (*E. multilocularis, E. granulosus, Taenia hydatigena, T. martis, T. taeniformis, T. crassiceps, T. mustelae, T. ovis, T. pisiformis, T. polyacantha, T. serialis, Mesocestoides leptothylacus*) was used. In the second step, positive

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specimens were analysed using the primer pair Pnest and Pnest rev for a nested PCR that selectively amplifies *E. multilocularis* DNA, with a characteristic band of 250 bp.

The results obtained with traditional and innovative techniques are summarized in the table.

Table 1. Results of traditional and innovative techniques applied to single out *E. multilocularis* in foxes

Method	Positive samples
Macro-microscopic	Cestodes (Mesocestoididae+ Dilepididae): 25/35 *Echinococcus*: 0/35
ELISA (Echinotest)	9/28
PCR for ≥12 cestodes	Faeces: 4/26 Intestine: 7/35
Nested PCR	0/35

No *Echinococcus* adult worm/egg was microscopically found in sampled foxes. Thirty two of 35 animals were heavily parasitized and 71.4% of them had cestodes mainly belonging to the genera *Joyeuxella* and *Mesocestoides*. Chekit Echinotest was positive in 9/28 (32.1%) of stool samples. At least *Joyeuxella* spp. was found in all the positive samples. Nested PCR was always negative while the "broad" PCR showed DNA fragments common, at least, to 12 cestode species in 11 foxes, in 4 stool samples and in 7 intestines particularly. Figure 1 is a picture of the pattern of 4 positive specimens, showing only the band of 373bp.

Fig. 1. Detection of cestode DNA by primers specific PCR amplification. M: marker; lane A: *E. multilocularis* positive control; lane B: positive to 'cestode' (*E. granulosus*) control; lanes C, D, E, F: 4 positive to "cestode" samples; lane G: negative control.

Preliminary results on the more reliable diagnostic strategy to single out *E. multilocularis* in the foxes are not satisfactory. In fact, search of metabolic coproantigens that is easy to be performed seems not enough specific. On the contrary, all methods that need of part of the parasite physically present (microscopic examination of stools/intestinal content, PCR) are not easy to perform, time consuming and might suffer of sensitivity due to the poor presence of faeces, to the low probability to find eggs or proglottids in faeces, and at least in these parallel microscopic and molecular analyses, to the fair share of available materials. Coproantigen detection could be improved. Nevertheless, at moment the strategies based on the direct parasite/DNA detection seem to be most reliable technique to study the presence of *E. multilocularis*. Finally, the microscopic analysis of intestinal content should be chosen on the basis of the lower costs and shorter time for results.

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Copro-diagnosis of *Echinococcus multilocularis* by a nested PCR in red foxes (*Vulpes vulpes*) from northern Italy

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Human alveolar echinococcosis is caused by the larval stage of the tapeworm *Echinococcus multilocularis*. It is considered one of the most pathogenic helminth zoonosis and endemic regions are in the northern hemisphere (Vuitton DA, Zhou H, Bresson-Hadni S, Wang Q, Piarroux M, Raoul F, Giraudoux P, 2003, Parasitology 127: 87-107). In Europe, the natural cycle of the parasite is predominantly sylvatic and mostly involves several rodent species as intermediate hosts and the red fox (*Vulpes vulpes*) as definitive host. This tapeworm is endemic in several regions of central-eastern Europe (Eckert J, Deplazes P, 2004, Clinical Microbiology Reviews 17: 107-135) and the evaluation of its prevalence in definitive host is an important parameter to estimate the potential risk of infection for humans.

E. multilocularis was recently found in foxes from north-east Italy (Manfredi MT *et al.*, 2002, Vet Record 150: 757) and it is present in countries neighbouring Italy (France, Switzerland and Austria). Therefore, this study was designed to assess the prevalence of infection in the red fox in northern Italy. A nested PCR analysis was used to investigate the presence of this tapeworm as this is the most specific diagnostic method available (not for prepatent infections and infections without egg excretion, see Deplazes P, Dinkel A, Mathis A, 2003, Parasitology 127: S53-S61).

A total of 318 faecal samples were collected and analysed: No. 7 and No. 10, from the Imperia and Savona provinces of Liguria region, respectively; No. 102, from the Bergamo province, Lombardy region; No. 146 and No. 30 from the Bolzano and Trento provinces, respectively, Trentino-Alto Adige region; and No. 23, from the Belluno province, Veneto region (Fig. 1). These were frozen at –80°C for 7 days for safety reasons and subsequently stored at –20°C until samples were analysed. From each faecal sample, approximately 0.5 g was used for DNA extraction. Samples were extracted, purified and concentrated in a 100µl volume using the "Wizard Magnetic DNA Purification System for Food" (Promega). This is based on paramagnetic particles which can be considered a mobile solid phase. Subsequently, samples were stored at –20° until DNA amplification. The target sequence of amplification is part of the *E. multilocularis* mitochondrial 12S rRNA gene (Dinkel A, Njoroge EM, Zimmermann A, Walz M, Zeyhle E, Elmahdi IE, Mackenstedt U, Romig T, 1998, J Clin Microbiol 36: 1871-1876). PCR was conducted in two steps. Initially, the primer pair P60.for and P375.rev was used (von Nickisch-Rosenegk M, Lucius R, Loos-Frank B, 1999, J Molec Evolution 48: 586-596) to amplify a 373-bp fragment. The thermal cycling of the amplification mixture was performed for 40 cycles, each cycle consisting of denaturation for 30s at 94°C, annealing for 60s at 55°C and elongation for 60s at 72°C. A total of 2µl of DNA was added to the 98µl reaction mixture. Subsequently, the primer pair Pnest.for and Pnest.rev was used to amplify a 250-bp fragment. The nested PCR was performed for 40 cycles, with each cycle consisting of denaturation for 30s at 94°C, annealing for 60s at 57°C and elongation for 60s at 72°C. A total of 2µl of DNA was added to the 48µl reaction mixture. Positive and negative controls were included in each amplification. In addition a spike (10 ng of DNA) for each sample was added in order to avoid false negatives due to PCRs inhibitors. For the detection, 10µl of PCR products were separated by electrophoresis in 1.5% agarose gel and stained by ethidium bromide. Positive PCR samples were sequenced. The sensitivity of the PCR assay was estimated using samples containing between 1 and 1000 *E. multilocularis* eggs. The specificity of the method was evaluated adding DNA samples extracted from 10 species of helminths (*Taenia hydatigena, T. crassiceps, T. solium, T. saginata, T. taeniformis, E. granulosus, Hymenolepis nana, Dypilidium caninum, Mesocestoides* sp., and *Uncinaria* sp.).

Positive amplified (21 samples) were analyzed by direct sequencing. Nineteen positive samples originated from foxes shot near the border with Austria, 1 positive sample was from a fox killed 13 km far from Bolzano city (Bolzano province) and 1 from a fox killed approximately 10 km near the Trento city (Trento province). For sensitivity purposes, 1 egg was found to be sufficient to give a specific signal.

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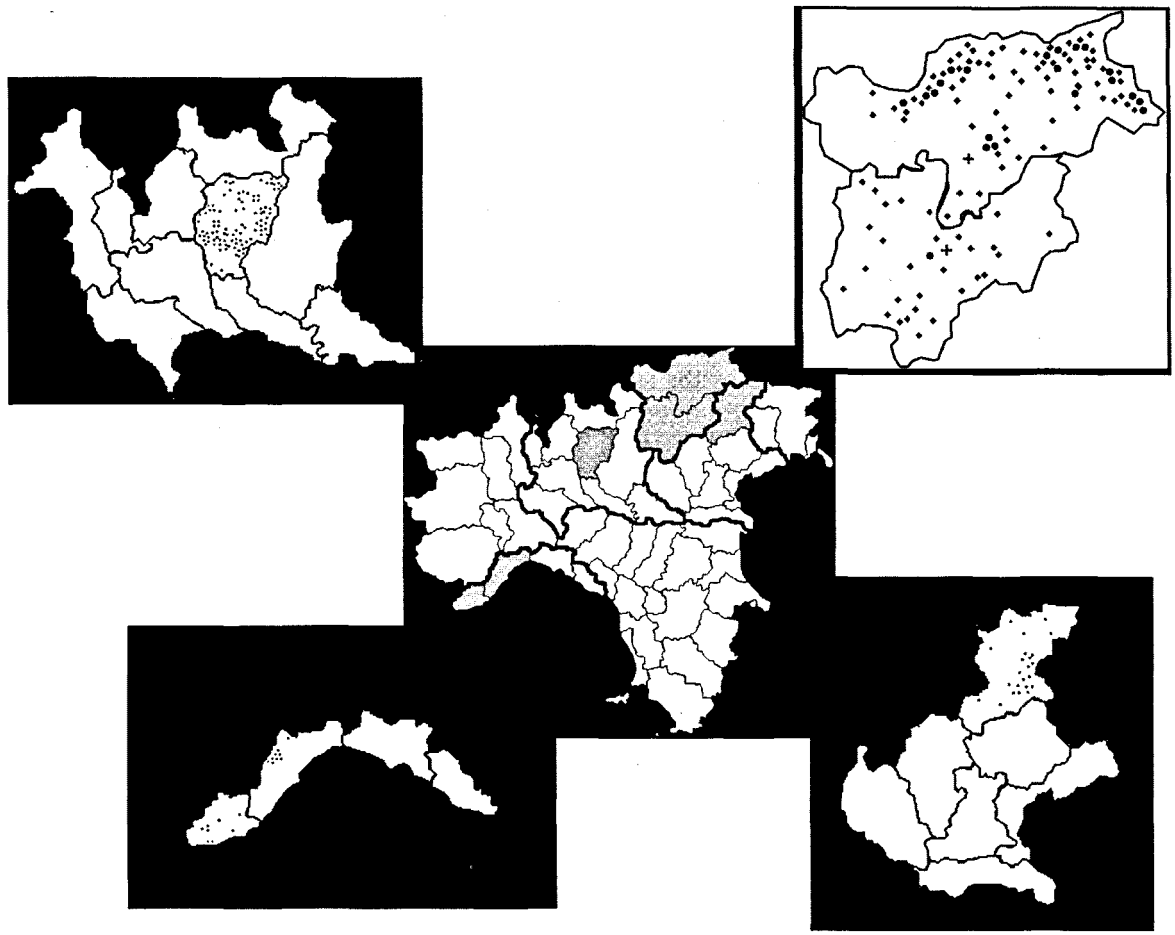

Fig. 1. Geographical distribution of red foxes (*Vulpes vulpes*) sampled (No.=318) for *Echinococcus multilocularis*. In grey, provinces of origin of examined foxes (Bergamo province, Lombardy region; Bolzano province and Trento province, Trentino Alto Adige region; Belluno province, Veneto region; Imperia and Savona provinces, Liguria region). Thick lines represent regional borders, thin lines province borders. Black dots represent positive foxes (No.=21); grey dots one or more negative foxes; plus symbols represent the cities of Trento and Bolzano.

About specificity, unspecific reactions were observed when high amount of *Mesocestoides* DNA was present in faecal samples and it was confirmed by sequencing.

These results confirm the presence the highly pathogenic tapeworm *E. multilocularis* in northern Italy. In addition foxes, the main definitive host, are becoming increasingly urbanised. Consequently an intensive surveillance programme is recommended to accurately assess the spread of this parasite in Alpine areas. Such information will indicate if control measures are necessary.

Acknowledgements

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Pilot vaccination project for the control of hydatid disease in Matera province (southern Italy)

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Cystic hydatid disease, caused by the larval stage of the cestode *Echinococcus granulosus*, is recognised as one of the most important parasitic zoonoses. This infection is endemic in many Mediterranean countries including Italy. The biology, aetiology and life cycle of this parasite have been exhaustively described by Thompson and McManus (in Eckert J, Gemmel MA, Meslin FX, Pawlowski ZS, Eds, 2001, WHO/OIE Manual on echinococcosis in humans and animals: a public health problem of global concern, World Organisation for Animal Health, Paris, France: 1-19). Control of transmission of this infection depends on the education of the public to prevent the access of the definitive host (i.e., the dog) to the entrails of infected intermediate hosts (mainly sheep) and on a regular treatment of dogs with antihelmintic compounds. However, control campaigns established upon those methods require intensive effort over many years and any downfall in infection control in definitive hosts may lead to new infections in intermediate hosts (Lightowlers MW, Jensen O, Fernandez E, Iriarte J A, Woollard D J, Gauci CG, Jenkins DJ, Heath DD, 1999, Int J Parasitol. 29: 531-534).

To develop EG95 vaccine, a cDNA library was produced from *E. granulosus* oncosphere mRNA and screened with antibody affinity-purified from a protein molecule. EG95 protein was selected and expressed in *Escherichia coli* transformed with pGEX-3-EX (Lightowlers MW, Lawrence SB, Gauci CG, Young J, Ralston MJ, Maas D, Health DD, 1996, Parasite Immunology 18: 457-462). EG95 clone was found to express a protein that was capable to induce protection against *E. granulosus* infection in vaccinated sheep. Indeed, vaccine trials carried out with *E. granulosus* isolates in New Zealand, Australia, Argentina and China have confirmed the effectiveness of this vaccine (Lightowlers MW, Flisser A, Gauci CG, Heath D D, Jensen O, Rolfe R, 2000, Parasitol Today 16:191-196).

In 2001 a pilot vaccination project, firstly supported by Istituto Superiore di Sanità (ISS) and then by the Basilicata Region in collaboration with ISS, was started to evaluate the efficacy of EG95 vaccine against *E. granulosus* affecting the ovine population of the Matera province (southern Italy). In the first year, the prevalence of this parasite and the fertility of cysts in sheep and goats were evaluated at the slaughterhouses. In the summer 2002, the first vaccination campaign with EG95 was carried out in this province, recruiting 12,000 animals belonging to 41 locks, which previously tested positive for the presence of *E. granulosus* cysts. Half of the animals present in each flock was vaccinated and the other half was used as a control. An antigen-dose of 50 µg was administered per animal with 1 mg of the adjuvant Quil A. The freeze-dried vaccine was daily reconstituted during the vaccination campaign. Animals were vaccinated subcutaneously in the neck, and after one month a recall was administered. No side effects have been observed in vaccinated animals. Since immunity persists after two injections for at least twelve months, the vaccine recall was given one per year in the following years.

In the course of the study, an Enzyme Linked Immuno Sorbent Assay (ELISA) will be used to evaluate the antibody response against the EG95 vaccine. Sera will be tested by ELISA using a peroxidase conjugated assay. Moreover, a copro-diagnosis for *E. granulosus* infection in sheepdogs, based on PCR-derived methods, will be performed (Abbasi I, Branzburg A, Campos-Ponce M, Abdel Hafez SK, Raoul F, Craig PS, Hamburger J, 2003, Am J Trop Med Hyg 69: 324-323).

Preliminary results show that lambs born from EG95-vaccinated ewes were immune to *E. granulosus* for the first few months of life due to the presence of specific anti-EG95 antibodies received from the mother via colostrum (Heath DD, Jensen O, Lightowlers MW, 2003, Acta Tropica 85: 133-143).

The aim of the project was to evaluate the protection level induced by the EG95 vaccine in sheep and goats breed in the Italian breeding system. If the expected results of protection will be achieved, this vaccine could be used to decrease the prevalence of the infection and the fertility of hydatid cysts in the intermediate hosts in endemic areas of Italy. This vaccination will be accompanied by integrated approaches on the control of infection in the final host and on health education of different categories of persons (veterinarians, school children, farmers and their families, shepherds, etc.).

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An epidemiological updating on cystic echinococcosis in cattle and sheep in Sicily, Italy

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Cystic echinococcosis (CE) is a zoonotic parasitic disease caused by larval stage of dog tapeworm *Echinococcus granulosus*. Transmission occurs predominantly in synanthropic cycles, involving sheep, goats, cattle and pigs as intermediate hosts. In Italy, according to sheep breeding, the infection rate increases from north to south regions including islands. Previous CE prevalence recorded in Sicily in cattle and sheep were 11.1% and 15.6%, respectively. The cyst viability, studied in sheep only, was 1,9% (Magliarditi D, Niutta PP, 1995, Atti Associazione Siciliana di Sanità Veterinaria: 165-167; Scala A *et al.*, 2001, 20th International Congress of Hydatidology, Kusadasi, Turkey: 303). This paper reports preliminary results on prevalence and viability of CE in cattle and sheep in Sicily and is part of a larger research focused on the epidemiological updating of *E. granulosus* infection.

Between May 2004 and October 2004, a total of 393 cattle and 411 sheep from northeast and midwest of the island were examined for CE at local abattoirs. Cysts from positive organs were examined for protoscoleces presence and classified as fertile (with viable protoscoleces) or sterile (without or with not viable protoscoleces). Aliquots of viable cysts (germinal layer and protoscoleces), were stored at –20°C in glass tubes. From these isolates and with the strain typing purpose, DNA was extracted with a commercial kit (High pure PCR template preparation kit, Roche) and PCR (Dinkel A *et al.*, 2004, Int J Parasitol, 34: 645-653) were carried out to discriminate the various strains (Eckert J *et al.*, 2001, WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. World Health Organization, Paris, France: 265). The strain obtained by PCR was confirmed by sequencing COI and NADH mithocondrial genes (Bowles J, McManus DP, 1993, Int J Parasitol, 23: 969-972; Bowles J *et al.*, 1994, Parasitology, 109: 215-221).

Cyst frequencies from the different organs and infection frequency in the different host age classes were compared by chi-squared test. The variances of CE prevalence within the host age were analyzed using logistic regression model. Statistical analyses were performed with the software SPSS 12.0 for Windows (Apache Software Foundation, Chicago).

The overall CE prevalence was 67.1% (264/393) in cattle and 57.6% (237/411) in sheep. In Table 1 are showed the infection rates by age classes of cattle and sheep. The logistic regression model for general trend of CE age-prevalence showed a positive correlation with an odds ratio per year of 1.23 (1.16-1.30) and 1.45 (1.19-1.76) for cattle and sheep, respectively. Additionally, sheep were found to be 2.2 time (1.4-3.3) more at risk than cattle for CE infection. Distribution of cysts in the internal organs (Table 2) showed different patterns in the 2 animal species.

Table 1. Age prevalence of *E. granulosus* infection in cattle and sheep.

Age (years)	Cattle rate (infected/examined)	Sheep rate (infected/examined)
0-1	0[a] (0/24)	0[a] (0/6)
1-2	0.33[b] (3/9)	0.42[b] (5/12)
2-3	0.38[b] (5/13)	0.43[b] (12/28)
3-4	0.23[b] (3/13)	0.69[c] (49/71)
4-5	0.59[c] (10/17)	0.56[c] (89/159)
5-6	0.77[c] (10/13)	0.82[d] (27/33)
6-7	0.58[c] (7/12)	0.71[c] (5/7)
7-8	0.65[c] (11/17)	0.86[c] (6/7)
8-9	0.78[c] (21/27)	–
9-10	0.72[c] (23/32)	1 (4/4)
>10	0.79[c] (171/216)	–
χ^2	91.64*	29.16*
Total	67.1 (264/393)	60.2 (197**/411)

* The differences in age groups with different letter in the same column are statistically significant ($p < 0.05$).
** Out of 411 sampled sheep only for 197 was possible the age determination.

Table 2. Distribution of *E. granulosus* cysts in cattle and sheep.

Infected organs	Cattle (n=264) Number	%	Sheep (n=237) Number	%
Liver	32	12.1[a]	88	37.1[a]
Lung	40	15.1[a]	27	11.3[b]
Liver+lung	190	72[b]	121	51[c]
Liver+lung+other	2	0.7[c]	1	0.4[d]
χ^2	430.38*		204.17*	

* The CE percentages among internal organs with different letter in the same column are statistically significant ($p<0.05$).

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Table 3. *E. granulosus* strains found from cattle and sheep.

Species (organ)	Number of isolates	G1 PCR (Dinkel 2004)	G5/G6/G7 PCR (Dinkel 2004)	NADH sequence	COI sequence
Sheep (lungs)	13	Positive	Negative	G1	G1
Sheep (liver)	5	Positive	Negative	G1	G1
Cattle (lungs)	5	Positive	Negative	G1	G1
Cattle (liver)	6	Positive	Negative	G1	G1

While in cattle most infections were characterized by the presence of cysts both in the liver and the lung followed by lung only and liver only, in sheep cysts were found mostly in liver. Overall cysts fertility rate was 4% (16/393) in cattle and 9.2% (38/411) in sheep. A positive correlation between cysts viability and sheep ages was found with rates ranging from 2% to 28% in 1-2 years and 8-9 years age classes, respectively. Furthermore, concerning the site of fertile cysts, significant differences were found in sheep where most viable cysts were found in lung (28/148; in liver 23/209). Strain typing by PCR and mithocondrial genes NADH and COI sequencing showed the presence of G1 or Sheep Strain (Table 3) from both sheep and cattle isolates.

Although in this paper are presented the preliminary results, the data have important implications. The positive trend recorded for CE age-prevalence, in agreement with other studies, shows that there is no evidence of parasite-induced host immunity or at least that the natural immunity response in cattle and sheep has no efficacy in CE infection control (Roberts MG *et al.*, 1986, Parasitology, 92: 621-641; Cabrera PA *et al.*, 1996, Int J Parasitol, 25: 807-813; Dueger EL, Gilman RH, 2001, Trans R SocTrop Med Hyg, 95: 379-383; Torgerson PR *et al.*, 2003, Vet Parasitol, 114: 143-153). Furthermore, the results show that sheep tend to be more at risk than cattle for CE infection, confirming their role as most important intermediate host in Sicily. The high number of positive animals and the cysts viability rates recorded, especially in old cattle, showed that these ruminants could act as active intermediate hosts in *E. granulosus* infection maintenance in the island. Additionally, although the high number of viable cysts found in sampled cattle no G5 or cattle strain (Thompson RCA, MacManus DP, 2002, Parasitol Today, 18: 452) was found. The finding of the G1 strain, only. It suggests that factors due to the strain type might influence the cyst viability in cattle. Compared to previous surveys in Sicily, our data show higher prevalence values. This could be as a consequence of several factors such as the ages of the sampled animals, the differences of the geographical prevalence and the methods of study. Thus, concerning the age, in the last survey carried out on sheep CE (Scala A *et al.*, 2001, 20th International Congress of Hydatidology, Kusadasi, Turkey: 303), overall prevalence and viability were 15.5% and 1.9% respectively, but all the sampled sheep were aged 2-3 year-old. Regarding the differences in geographic prevalence, Poglayen G *et al.* (2003, WAAVP 19th International Conference, New Orleans, USA: 164) well described the importance of climatic and environmental factors that could influence the CE prevalence in Sicily. About the method of study we preferred to personally check animals at abattoirs. In fact, it is important to note that surveys based on records from slaughterhouses usually showed lower prevalence respect to the real infection rate (Umur S, 2003, J Vet Med, 50: 247-252). In conclusion, according with Scala *et al.* (2001, 20th International Congress of Hydatidology, Kusadasi, Turkey: 303) "echinococcosis-hydatidosis continues to be a public health problem in the 2 biggest Italian islands" and these results, at least for the Italian endemic regions, should be used to stimulate the requirement of a continuous control program of this important parasitic zoonotic disease.

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Echinococcus granulosus in the wolf in Italy

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Abstract. During the period 1987-1999, 119 wolf cadavers were examined and checked for the presence of *Echinococcus granulosus*. All the animals were retrieved along the whole Apennines range of distribution of the species in Italy and most of them were illegally killed. Eighteen wolves resulted positive (15%). The mean intensity was 697.5. The force of infection for prevalence was 8.2 $^{year-1}$. The prevalence of the parasite was significantly and positively influenced by the local prevalence of cystic echinococcosis (CE) in sheep. Mean intensity was significantly and positively influenced by both the age of the wolf and the prevalence of CE in sheep. A deterministic model was used in order to simulate a purely theoretical sylvatic cycle of the parasites having the wolf as the only definitive host with 15% of prevalence. The expected prevalence of CE in wild intermediate species ranges between 10% and 25%. This prevalence overlaps the one observed in sheep. Even if both the wolf and the wild ungulate populations are increasing, the wolf still acts as a part of the main dog-sheep cycle of the parasite.

Key words: *Echinococcus granulosus*, cystic echinococcosis, wolf, wildlife, Italy.

According to Rausch (1995), the European biotype of *Echinococcus granulosus* involves many cycles. In the Mediterranean sub-region, where domestication of animals has an ancient history, the domestic cycle (dog-sheep) is dominant, whereas wildlife is considered irrelevant in the epidemiology of the infection. Nevertheless, in some environments both domestic and sylvatic cycles largely coexist and overlap (Schantz *et al.*, 1995). In Italy, the wolf and its wild prey species survived to a dramatic bottle neck observed in the early '70 (Boitani and Ciucci, 1992). Nowadays, the number and the distribution of the wolf benefit of the increased roe deer and wild boar populations. Both these ungulate species are fully susceptible to the parasite (Thompson and Allsopp, 1988; Rausch, 1995) and they represent the main wild source of food for the wolf (Meriggi and Lovari, 1996; Ciucci *et al.*, 1996). Predation of domestic animals still occurs, being sheep the most preyed domestic animal (Ciucci and Boitani, 1998). The increasing of fully susceptible wild species can enhance the probability that the parasite shifts towards a true sylvatic cycle. The parasite might enlarge its ecological niche utilising the susceptible wild species. If so, eradication or control plans should also consider the susceptible wild species.

Aims of the present study were: (1) to describe the epidemiology of *E. granulosus* in the wolf; (2) to assess which risk factor can affect the presence and the number of *E. granulosus* in the Italian wolf population; and (3) to determine the minimum prevalence range of cystic echinococcosis (CE) in wild intermediate hosts for maintaining a theoretical exclusive sylvatic cycle of *E. granulosus*, having the wolf (and its observed prevalence) as the only definitive host.

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Material and methods

During the period 1987-1999, 119 wolves were autopsied at the Istituto Nazionale per la Fauna Selvatica for forensic purposes. Necropsies were performed according to standard techniques. The animals originated from the whole Apennines range of the species. For each animal, locality and gender were registered. The age was determined by the *cementum annuli* count (Ballard *et al.*, 1992). *E. granulosus* individuals were isolated, identified and counted according to the standard method (Eckert *et al.*, 2001). Prevalence and mean intensity were calculated according to Bush *et al.* (1997). The age stratified force of infection (*per capita* rate at which susceptible individuals acquire infections) was calculated according to the polynomial catalytic infection model (Grenfell and Anderson, 1985), where immunity was replaced by the *E. granulosus* adult life span.

The importance of the following variables in influencing the epidemiology of the parasite has been checked: (a) wolf age, (b) wolf gender, (c) locality of retrieval; referring to the locality of retrieval: (d) sheep number (ISTAT, National census data: http://www.census.istat.it); (e) prevalence of CE in sheep (Lorenzini and Ruggeri 1987; Garippa *et al.*, 2004); (f) number of hunted wild boars; (g) roe deer population estimates; (h) red deer population estimates; and (i) total deer hunted. Data regarding points (f), (g), (h), and (i) were obtained from Anonymous (2002). Provincial or regional data were used when local data were unavailable. To assess which of the above variables could affect the

presence of the parasite (the dependent variable), a multivariate logistic regression analysis has been performed. Logistic regression models were selected by forward likelihood ratio selection. The logistic regression model fit was determined by the Hosmer-Lemeshow (HL) test statistic (Hosmer and Lemeshow, 2000). For assessing which of the independent variables could affect parasite number (the dependent variable), a multivariate regression analysis was performed transforming in the natural log (+1) the number of *E. granulosus* isolated in each wolf. Linear regression models were selected by stepwise method. The linear regression model fit was determined by the ANOVA test. Significance was set at alfa <0.05.

To calculate the prevalence of CE in the wild prey species leading to the observed prevalence in the wolf a deterministic "Susceptible-infected-removed" model (SIR) was used (see Anderson and May, 1991). For simplicity a "closed" wolf population of 100 heads and of 10.000 ungulates has been modelled. The SIR model was linked with a predator/prey density dependent model (Renshaw, 1991; Messier, 1992). According to the basic framework of the SIR models, a wolf becomes infected when preys an infected intermediate wild host. The annual percentage of the newly infected wolves is represented by the yearly force of infection. The latter is determined by beta (coefficient of transmission) time the number of infected intermediate hosts preyed. The observed yearly force of infection was used and the beta parameter was replaced by a predation rate. The equation was then solved for the number of infected intermediate wild hosts. Finally, the theoretical prevalence of CE leading to the steady prevalence observed in the wolf population was calculated (Guberti *et al.*, 1998). Many of the parameters used in the model were obtained by the literature. For the demography of the wolf from Ciucci and Boitani (1991), for the demography of the preys from Focardi *et al.* (1996) and Focardi *et al.* (2002), and finally for the main epidemiological parameters regarding *E. granulosus* from Gemmel and Roberts (1995). Due to unpredictability, the parameter "wolf probability of getting the infection when preying an infected intermediate host" was set equal to 1. This will underestimate the prevalence of CE in prey species, but it avoids the use of unrealistic figures.

Results

Out of 119 wolves examined, 18 (15%; SE 3.3%) resulted positive. The mean intensity was 697.5 (parasite range in positive animals 2-3862). The yearly force of infection for prevalence was 8.2^{year-1}. The mean age of the sampled wolves was 24 months (range 6-108 months). The male/female ratio was 1:1.1. The prevalence of *E. granulosus* in wolves is positively influenced by the prevalence of CE in sheep. The results of the multiple logistic regression analysis are shown in Table 1. The number of *E. granulosus* in the wolf was positively affected both by age (in months) and prevalence of CE in sheep. The results of the multiple linear regression analysis are shown in Table 2. The prevalence of CE in intermediate wild hosts allowing a theoretical exclusive sylvatic cycle of *E. granulosus* ranged between 10% and 25%, according to a different predation effort (60-30 heads per year per wolf, respectively).

Considerations

The role of the wolf as definitive host for *E. granulosus* is confirmed at least for the Apennines population of the species in Italy. The prevalence and the mean intensity reported in the present study are similar to those observed in the wolf (Raush, 1993; Hirvela-Koski *et al.*, 2003). The steady force of infection indicates that the wolf inhabits an endemic area. In this area, the probability that a wolf become infected is 8.2% for each year of life of the animal. Because the mean age of the examined wolves was about 2 years, the overall prevalence

Table 1. Multiple logistic regression analysis. Dependent variable: prevalence of *E. granulosus* in wolf.

Independent variable in the equation	B	ExpB	SE ExpB	% correct classification	LH statistic (P)	R^2 Nagelkerke
Prevalence of CE in sheep	0.106	1.112	0.081	85	6.944 P = 0.139	0.69

Table 2. Multiple linear regression analysis. Dependent variable: individuals of *E. granulosus* [$Log_n(N+1)$].

Independent variables in the best model	B	Beta	t	R^2	F	P
Months of age of wolves	0.00183	0.311	3.678	0.71	13.869	0.00
Prevalence of CE in sheep	0.02267	0.27	3.194			

reflects the age structure of the sample. According to the present work, a simple and robust scenario might be proposed. The prevalence of *E. granulosus* in a wolf population will result from the actual prevalence of CE in sheep. For each percentage point of CE in sheep, the prevalence of *E granulosus* in the wolf population will increase of 1.4% (95% CL: 0.4%-2.3%). The number of parasite harboured will depend again from the prevalence of CE in sheep and also from the age of the wolves. The proposed scenario is strengthened by the results of the theoretical model. The expected prevalence of CE in wild ungulate hosts is the same observed in sheep. The definitive prevalence and mean intensity of *E. granulosus* in a wolf population will depend by the wolf-sheep, predator/prey interaction and the age structure of the wolf population. According to the results of both the logistic and linear regression models, the explained variability of prevalence and mean intensity is about 70%. It means that other variables, not measured in the present study, can enter in the epidemiology of *E. granulosus* in the wolf, but they will affect only 30% of the host-parasite relationships.

The present work suggests that in Italy the wolf is still part of the classical dog-sheep cycle and thus a true wild cycle has not evolved. Records of CE in wild ungulates are still sporadic and not mentioned in the recent scientific literature. In the framework of the actual legislation, it could be worth to monitor wild ungulate populations in order to precisely estimate the prevalence of CE, at least in those areas where the presence of the wolf has been confirmed.

Acknowledgments

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Parassitologia 46: 429-430, 2004

Casual finding of a hydatid cyst during an autopsy in Veneto region (NE Italy)

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In this case-report we describe the finding of a large sized hydatid cyst in the liver of an immigrant from Morocco, born in the city of Beni Meskine, 45 years old, found drowned-dead in the "Idrovia", a channel between Padua and Venice (NE Italy).

The cyst, localized in the hepatic parenchyma of the left lobe, had an uniform spheroidal shape, 10-12 cm diameter (Fig. 1). The liver was normal in size and weight; the shape was strongly modified, as the left lobe looked very hypotrophic, almost completely occupied by the cyst, that protruded from the surrounding parenchyma. Between the liver surface and the cyst, many little adhesions were present. The right lobe and, partially, the quadratum and the caudatum lobes were enlarged, but didn't show any peculiar alteration.

Figure 1. The liver, isolated during the autopsy, clearly showed the presence of a large spheroidal cyst. (Photo: S. Lafisca).

After the removal, the cyst was preserved in formalin for some days and then, opened, to observe its macroscopic feature and to perform histological samples. The opened cyst was a transitional form between the "hyperlaminated caseous" and the "hyperlaminated granular" showing packed sheets of laminar tissue intervalled with yellowish degenerative matrix (Fig. 2) according to Bortoletti *et al.* (Bortoletti G, Cagetti M, Gabriele F, Conchedda M, 2002, Parassitologia 44: 159-171), typical of the inactive and degenerative stage of the 3th activity group (CE4/CE5 OMS). The microscopic investigation, performed by emathossilin-eosine staining, showed laminar tissue enveloping a widespread degeneration, although some scoleces were still intact (Figs 3 and 4). The preservation of the finding under formalin could not, unfortunately, allow biological, biomolecular and genetic analyses.

Figure 2. The dissected cyst, preserved in formalin, shows sheets of laminar tissue and walls partially filled with yellow degenerative matrix. It may be classified as a transitional form between "hyperlaminated granular" and "hyperlaminated caseous". (Photo: A. Lafisca).

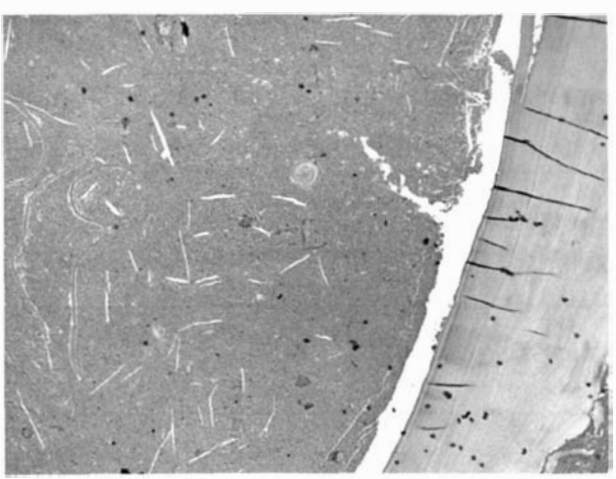

Figure 3. The histological appearance of the content showed a widespread degeneration, indicating that the cyst was very old. (Photo: Murer).

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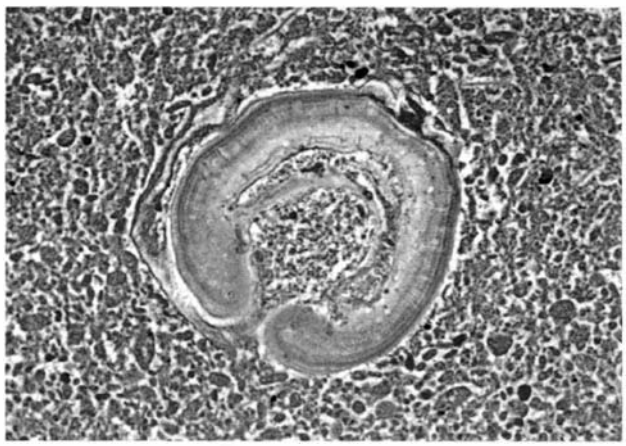

Figure 4. Even the widespread degeneration of the cystic mass, some scoleces were still intact (magnification of Figure 3). (Photo: Murer).

The autopsy, although complete and accurate, showed no other cysts nor other peculiar alteration beyond the hepatic mass (for a 45-years-old man, born in North Africa), nor at cephalic level, to explain the frequent headaches (probably linked to neurological peripheral phenomena), nor in any other district. The only pathological findings were multifocal pleurodiaphragmatic and interlobal adherences.

This finding was absolutely fortuitous as the host never reported symptoms that could let imagine such a parasitic syndrome. The only recurring symptom was frequent and painful headaches. Relatives and friends told that during these crises he suffered for everything was at contact with the skin and often undressed himself (Lafisca S., personal communication).

Several times during his life in Italy, he went to the hospital to cure these headache crises or after accidents, that occurred during the work. Doctors who visited him never reported the presence of the hydatid cyst, although the shape of the liver was strongly modified. If a semiotic visit was properly done, it would have easily identified the anatomical alteration. The finding of the hydatid cyst was, we ought repeat it, absolutely fortuitous. It represents a rare case in the Po River Plain (few cases have been reported in the Alpine region, where sheep breeding is more common).

The average low diffusion of parasite infections in Italy together with the low knowledge of parasitological diagnostic methods by most of non specialistic clinical doctors (the exam of parasitology is, in many Italian faculties of medicine, optional), makes so that these pathologies, more frequently present in persons coming from other countries, can be misidentified or not identified at all.

Parassitologia 46: 431-433, 2004

An updating on the epidemiological situation of *Echinococcus multilocularis* in Trentino Alto Adige (northern Italy)

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Human alveolar echinococcosis (AE) is a zoonosis caused by the metacestodes of *Echinococcus multilocularis*. The life cycle of the cestode includes mainly the red fox as definitive host, and rodents (i.e. *Arvicola terrestris, Microtus arvalis, Clethrionomys glareolus*) as intermediate hosts. Domestic dogs and cats could be accidentally included in the sylvatic cycle of the cestode, playing a role in the human infections (Eckert J, Conraths FJ, Tackmann K, 2000, Int J Parasitol 30: 1283-1294). In Central Europe, *Echinococcus multilocularis* has a wide geographical distribution and the parasite occur in red foxes of at least 11 countries (Eckert J, Deplazes P, 2004, Clin Microb Rev 17: 107-135). In Italy, several studies were carried out on helmintofauna of *Vulpes vulpes*. Yet, the presence of *E. multilocularis* in host red fox was proved only in 2002, when adult specimens of the cestode were found in two foxes, hunted in 2000, from Trentino Alto Adige (Manfredi MT, Genchi C, Deplazes P, Trevisiol K, Fraquelli C, 2002, Vet Rec 150: 757).

In this note we show the results limited to Trentino Alto Adige of a survey on the presence of this cestode in Italian Alps.

During 1997-2004, a total of 1018 foxes, of which 816 faecal samples and 433 small intestines, have been examined. Specimens were from Trentino Alto Adige (both Trento and Bolzano provinces) and were found dead or have been hunted (according to national law n. 157/92) in localities situated between 100 and 2200 m a.s.l. The carcasses were carried to the provincial sections of Zooprofilattici Institutes, where the intestine and the faeces were drawn by each sample and all the material was sent to the Department of Veterinary Pathology of Milan University. In order to reduce the risk of infection for laboratory personnel, the samples were deep-frozen (at least two weeks at –80°C and then at –20°C) and they were left overnight to defrost before they were examined. The main data (age, sex, locality, municipality, altitude of origin and date) were collected for each animal.

The infection by *E. multilocularis* was firstly assessed by the detection of parasite coproantigens (CA-ELISA, CHEKIT-Echinotest, Bommeli Diagnostic). Subsequently, samples with positive or ambiguos results to CA-ELISA, were tested by the parasitological examination of the small intestine to detect adult stages of the parasite, applying the analysis of the whole sediment and counting technique (SCT), as described in Hofer *et al.* (Hofer S, Gloor S, Muller U, Mathis A, Hegglin D, Deplazes P, 2000, Parasitology 120: 135-142). Further those samples were also analyzed by nested-PCR (see Casulli A, La Rosa G, Manfredi MT, Di Cerbo AR, Dinkel A, Romig T, Deplazes P, Genchi C, Pozio E, 2004, Parassitologia, in this volume). Overall *E. multilocularis*-CA were detected in 15.2% of faecal samples and 1.15% of the intestines had cestodes (Table 1). In Table 2 are shown details of foxes resulted infected by SCT. The results of this survey show that the infected foxes by SCT and those positives to *E. multilocularis* by nested PCR (Fig. 1) were from localities of two Alpine valleys (Vipiteno and Val Pusteria), in Bolzano province. These areas are very close to the Austrian regions (Voralberg and Tyrol) where highest prevalences of *E. multilocularis* infection in foxes and of human AE have been recorded (Auer H, Aspöck H, 1990, Zbl Bakt 272, 498-508; Stellnberger K, Pechan P, 1996, Vet J 48: 12). Previously, we have supposed that the parasite was introduced into Trentino Alto Adige by

Table 1. Summary of data on *Echinococcus* coproantigens and parasitological analyses on red fox from Trentino Alto Adige.

		CA-ELISA			Parasitological examination		
Province	No. of foxes	No. of negatives or ambiguos	No. of positives	Total	No. of negatives	No. of positives	Total
Trento	578	494	47	541	181	0	181
Bolzano	440	322	99	421	247	5	252
Total	1018	816	146	962	428	5	433

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Table 2. Data on *Vulpes vulpes* infected by adults of *E. multilocularis* detected in the intestine

Locality Altitude, m a.s.l.	Alpine valley	Distance (km) from the border	Year	Worm burden	Coproantigen level	Fox sex
Prags, 1300	Pusteria	15	2000*	>100	>90%	Male
Gsies, 1250	Pusteria	7,5	2000*	>100	>90%	N.R.
Racines, 1500	Vipiteno	13	2001	13	>90%	Male
Rasun Anterselva, 1000	Pusteria	16.5	2001	8	77%	Male
Dobbiaco, 1600	Pusteria	12	2003	>60	–	Female**

* Manfredi MT, Genchi C, Deplazes P, Trevisiol K, Fraquelli C, 2002, Vet Rec 150: 757.
** Adult female affected by mange and in cachectical state.

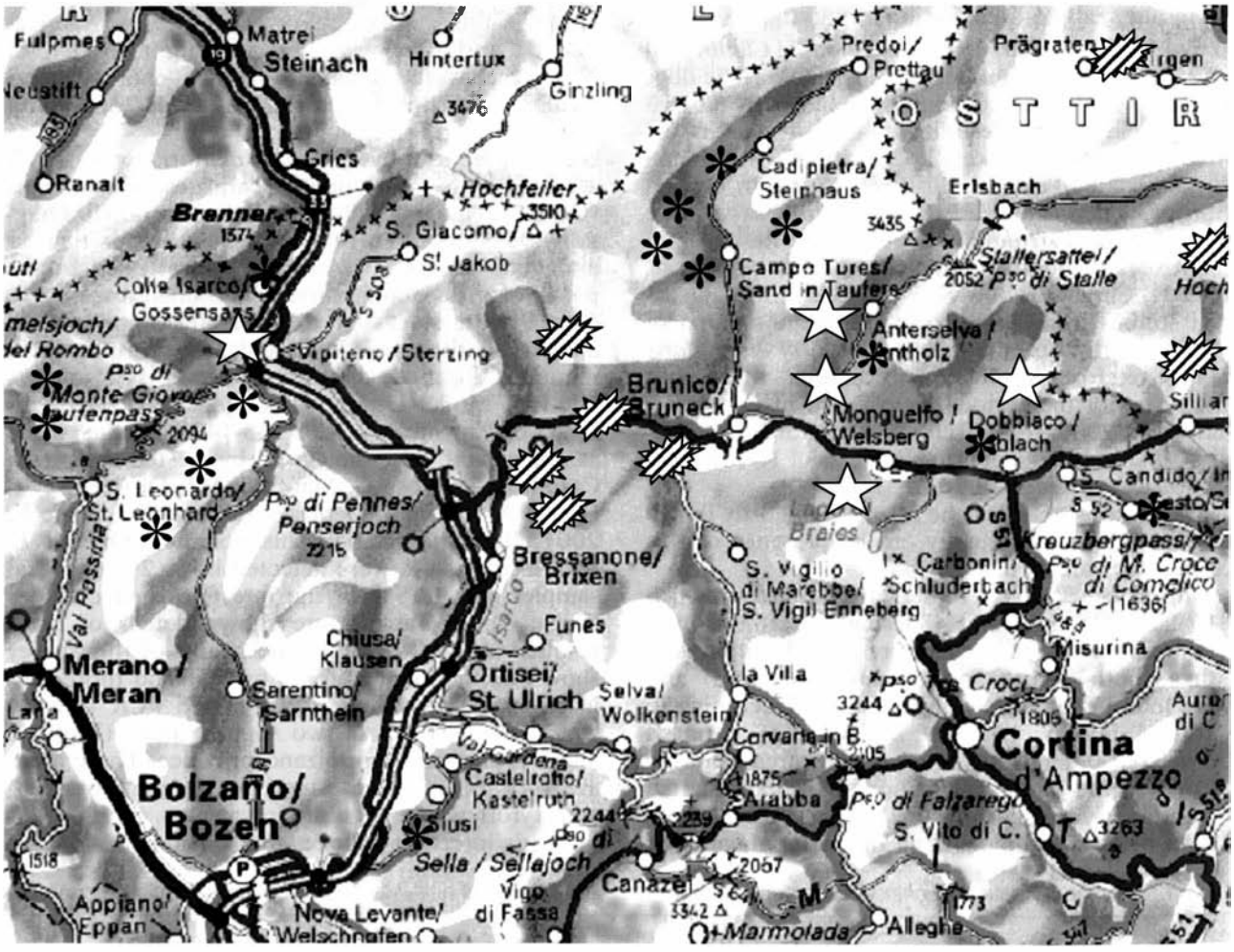

Fig. 1. Distribution of cases of *E. multilocularis* infection diagnosed in foxes by SCT (☆) or PCR (✱). We showed the localities where human AE cases occurred (▨) as reported by Posselt.

roaming foxes moving from the bordering Austrian regions (Manfredi *et al.*, 2002). This hypothesis could be convincing because a large number of infected foxes were young males and it is known that after six months of age they migrate with a dispersal distance of between 10 km and 50 km (Eckert J, Rausch RL, Gemmell MA, Giraudoux P, Kamiya M, Liu FJ, Schantz PM, Romig T, 2001, WHO/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern, Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS, Eds, World Organisation for Animal Health and World Health Organisation, 162-182). However, the Alpine valleys where infected foxes are from are similar to the Austrian Tyrol by geographical point of view and they are widely inter-communicating with this region through the Brennero Pass. Then, this area of Italy could represent part of the

Table 3. Personal data, locality of origin and occupational status of patients with confirmed or presumed AE in Tyrol (Val Pusteria), as classified by Posselt.

	Patient	Sex	Age at diagnosis	Year of diagnosis/death	Occupational status	Locality of origin
Group 1. Diagnosis made at necropsy.	S.A.	M*	50	1892	Farmer	Pfunders
	K.A.	F**	49	1892	Farm labourer	Franzensfeste
Group 2. Cases treated at the Innsbruck hospital. Symptoms compatible with EA.	–	M	32	1880	Railwail worker	Franzensfeste
	B.L.	M	53	1892-1896	Farmer	St. Andrä
	M.A.	M	34	1895	Farmer	Spinges
Group 3. Cases with EA presumed Posselt included cases from Bressanone and the beginning of the Val Pusteria.	S.P.	M	–	1884	Sheperd	Vals
	K.T.	F	48	1894	Farmer	Rodengo
	–	M	–	1894	Butcher	Lüsen
	–	M	23	–	Farmer	Prägraten
	–	M	36	–	Tailor	Lavant (Lienz)
	–	M	–	1897	Railwail worker	Sillian
	–	M	65	1896	Butcher	Bressanone

* Male. ** Female.

central European range of *E. multilocularis* because of the climate, the geological characteristics and the availability of the intermediate and final hosts supporting the parasite life cycle (Lucius R, Bilger B, 1995, Parasitol Today 11: 430-434; Veit P, Bilger B, Schad V, Schäfer J, Frank W, Lucius R, 1995, Parasitology 110:79–86). Further, we stress that the tendency of the study area for the life cycle of *E. multilocularis* could be demonstrated by the occurrence at the end of 1800 of cases of the human AE as reported by Posselt (Posselt A, 1900, Die Geographische verbreitung des blasenwurmleidens, Verlag von Ferdinand Enke, Stuttgart). The author reviewed all AE cases from Tyrol (both Austrian and Italian parts) and recognized the existence of two "foci" of AE in Val Pusteria (Table 3).

Acknowledgements

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Recombinant antigens of *Echinococcus granulosus* recognized by IgE and IgG4 of sera from patients with cystic echinococcosis

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A characteristic feature of the immune response in helminth infections is an increased level of specific IgE. This high IgE level is not always associated with an allergic response, probably because the high IgG4 level produced during helminth infections blocks the antigenic epitopes responsible for the allergic response (Vercelli D, 2000, Am J Resp Crit Care Med 162: S86-S90).

Cystic echinococcosis (CE), caused by *Echinococcus granulosus,* shares with other helminthiases three typical aspects of immediate hypersensitivity reactions: elevated IgE/IgG4 production, eosinophilia and mastocytosis (Pawlowski ZS, 1997, in FL Andersen, H Ouhelli, M Kachani, Eds, Compendium on Cystic Echinococcosis. Brigham Young University Print Services, Provo, Utah, USA: 199-35).

Despite the high percentage of CE patients presenting specific serum IgE, in our experience only 20% of these has allergic manifestations. IgE decrease rapidly in serum of patients after surgery or successful chemotherapy, and could be a useful marker of the outcome of CE (Riganò R, Profumo E, Ioppolo S, Notargiacomo S, Ortona E, Teggi A, Siracusano A, 1995, Clin Exp Immunol 102: 281-285). Aim of this study was the identification and immunological characterization of recombinant antigens by the immunoscreening of a cDNA library with IgE or IgG4 of sera from CE patients with acute allergic manifestations. Further, the association between the presence of IgE and IgG4 specific to these antigens and allergic reactions or disease feature was evaluated.

Patients with clinically proven CE were divided according to the presence of allergic manifestations (itching and urticaria) and disease feature evaluated on the basis of the type of cyst (active disease: cyst type CE1 CE2; inactive disease: cyst type CE4, CE5). The cDNA library was prepared as previously described (Margutti P, Ortona E, Vaccari S, Barca S, Riganò R, Teggi A, Muhschlegel F, Frosch M, Siracusano A, 1999, Parasite Immunol 21: 485-492). The cDNA clones were screened with sera from confirmed positive CE patients showing strong allergic manifestations at the time of serum sampling. The nucleotide sequence of the cloned cDNA insertion was sequenced with automated sequencer ABI prism 310 Collection (PE). Sequences were then compared with the GenBank sequence database using both Fasta and Blast analysis (Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ, 1990, J Mol Biol 215: 403-410). The selected cDNA clones were subcloned into the *Bam* HI/*Kpn* I site of the QIA express vector, pQE31. The 6X fusion proteins were expressed in *Escherichia coli* SG130009 cells, purified by affinity of NI-NTA resin for the 6Xhis tag and eluted under denaturing conditions (urea) according to the supplier (Qiagen) instruction. The immunoreactivity with patient sera was determined by immunoblotting (IB) technique using peroxidase goat anti-human IgE or mouse anti-human IgG4 as second antisera and goat anti-mouse IgG as third antiserum as previously described (Margutti P *et al.,* 1999, Parasite Immunol 21: 485-492).

Immunoscreening of the *E. granulosus* expression library with sera of patients with CE and allergic reactions identified four strongly reactive clones. Basing on the amino acid sequences, we named the clones: EgEF-1β/δ (Margutti P *et al.,* 1999, Parasite Immunol 21: 485-92), EA21 (Ortona E, Vaccari S, Margutti P, Delunardo F, Riganò R, Profumo E, Buttari B, Rasool O, Teggi A, Siracusano A, 2002, Clin Exp Immunol 128:124-130), Eg2Hsp70 (Ortona E, Margutti P, Delunardo F, Vaccari S, Riganò R, Profumo E, Buttari B, Teggi A, Siracusano A, 2003, Parasite Immunol 25:119-126) and EgTeg. Qualitative analysis by IB of IgE response in patient sera showed significantly higher binding reactivity to EgEF-1, EA21 and EgTeg in sera from patients with allergic reactions than in those without ($P<10^{-4}$). IgG4 response to EA21 was significantly higher in sera from patients without allergic reactions than in those with allergic reactions ($P<10^{-4}$) (Figure 1). Dividing the patients according to the activity of the disease, we found that both IgE and IgG4 responses were significantly higher in patients with inactive than in those with active disease (Figure 2).

Our data demonstrate that the new isolated *E. granulosus* proteins recognized by serum IgE of a high percentage of CE patients, are parasite allergens with a relevant role in CE pathology. In particular, we found a significant association between IgE specific to EgEF-1β/δ, EA21 and EgTeg and allergic

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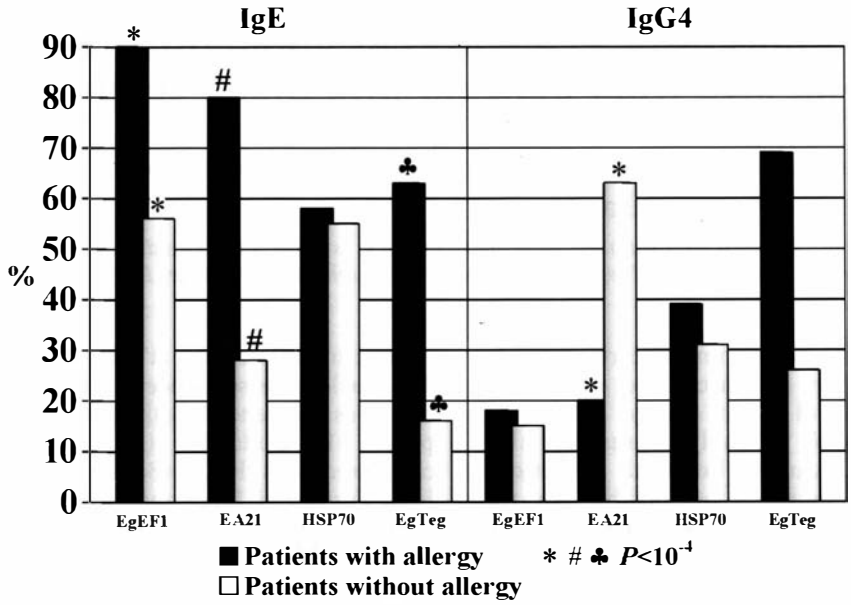

Figure 1. Percentages of IgE and IgG4 positivity to EgEF-1β/δ, EA21, Eg2HSP70 and EgTeg in sera from patients with cystic echinococcosis with and without allergic reactions.

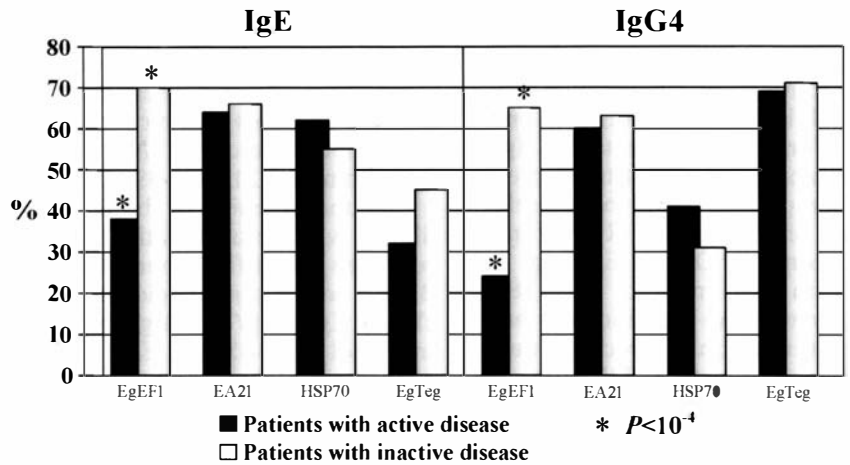

Figure 2. Percentages of IgE and IgG4 positivity to EgEF-1β/δ, EA21, Eg2HSP70 and EgTeg in sera from patients with cystic echinococcosis divided on the basis of the activity of the disease.

disorders related to CE. The 2 contrasting immunoglobulin associations between IgG4 specific to EA21 and protection against allergic reactions, and between IgE to EA21 and allergic manifestations, suggest that in CE, as in other parasitic infections, IgG4 acts to block pathogenic processes. The role of Eg2Hsp70 in the host-parasite relationship and in allergic reactions needs further investigations. The IgE and IgG4 reactivity to EgEF-1β/δ is associated to the inactivity of the disease. Probably calcified, degenerated cysts release the intracellular protein that could be captured by the antigen presenting cells becoming antigenic.

Overall the construction of a panel of molecular allergens could be used to monitor the CE related allergic reactions and the development of infection.

Acknowledgments

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Parassitologia 46: 437-438, 2004

Distribution of hydatidosis in slaughtered animals in Umbria Region from 1995 to 2004: a retrospective analysis

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Cystic echinococcosis is one of most important and widespread parasitosis in the Mediterranean Region. It is a considerable problem not only in animal breedings because of economic losses, but also for public health because considered as a re-emerging zoonotic disease (Garippa G, Battelli G, Cringoli G, Giangaspero A, Giannetto S, Manfredi MT, 2004, Parassitologia 46: 33-38).

Although the parasitosis is reported as usual finding at the slaughterhouse inspection in Umbria Region, nowadays no recent epidemiological data are available of its diffusion and distribution (Pellegrini D, 1955, Ann San Pubbl 16: 81-103).

In this paper a first study on the distribution of hydatidosis in Umbria by a retrospective analysis of data reported in the official records from 1995 to 2004 is presented. The survey has been done in a large area (Valnerina) where open farming is practised and a close association between man, sheep and dogs and between stray dogs, cattle, sheep and wild animals has been observed.

Data were collected from the official records of slaughterhouse of Norcia mainly, being the epidemiological observatory of the territory for all the slaughtered sheep, goats, pig and 85% of cattle. Regarding the remaining 15% of cattle, data recorded at slaughterhouses of Spoleto, Foligno, Terni, Ascoli Piceno were used. The study was carried out between June and September 2004. Only animals with hydatidosis diagnosed during the *post mortem* examination were considered positive.

For each positive animal, these data were recorded: animal species, sex, age, year of slaughtering, cyst location.

From 1995 to 2004, a total of 5677 cattle, 11908 sheep, 173 goats, 19459 pigs were slaughtered. An overall infection rate of 81.18, 71.97, 7.34 and 0.82 percent was found in sheep, goats, cattle and pigs, respectively. Regarding the distribution of infection rates during the period 1995-2004 (Fig. 1), no significant difference in the different host species was found between the first and the second 5-year period ($p<0.001$) (by Proc GLM, Statistical Software SAS). Regarding the age factor, the prevalence distribution showed increasing values in older animals as dairy cattle, tups, old kibs, female goats and billy goats (Figs 2-4). However, since very few old animals are

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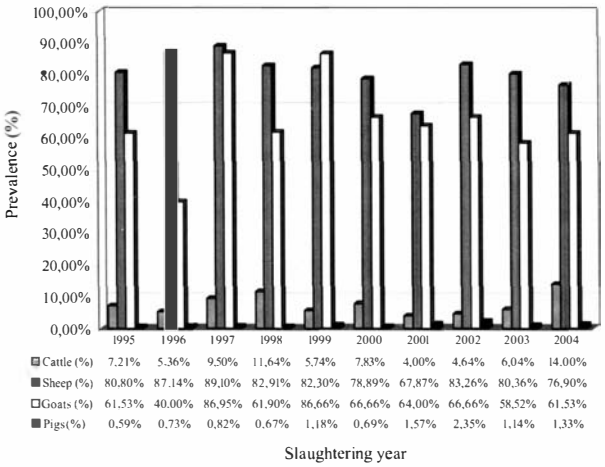

Fig. 1. Hydatidosis prevalence in slaughtered cattle, sheep, goats and pigs.

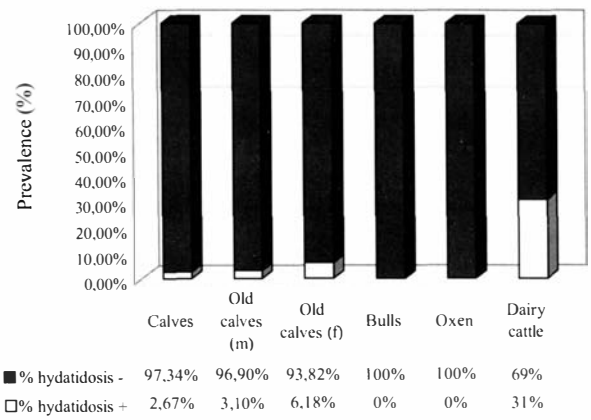

Fig. 2. Hydatidosis distribution in cattle according to different age/productive classes.

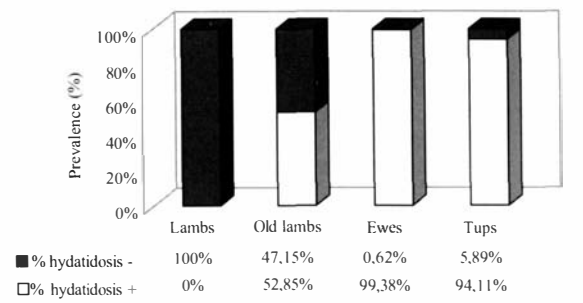

Fig. 3. Hydatidosis distribution in sheep according to different age classes.

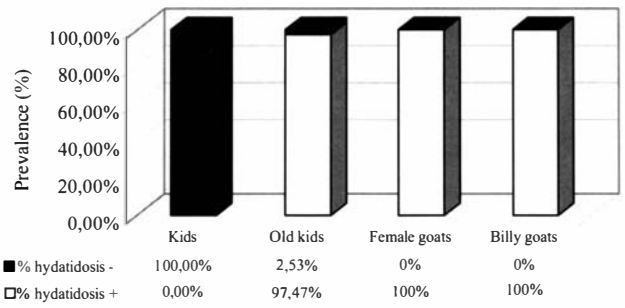

Fig. 4. Hydatidosis distribution in goats according to different age/productive classes.

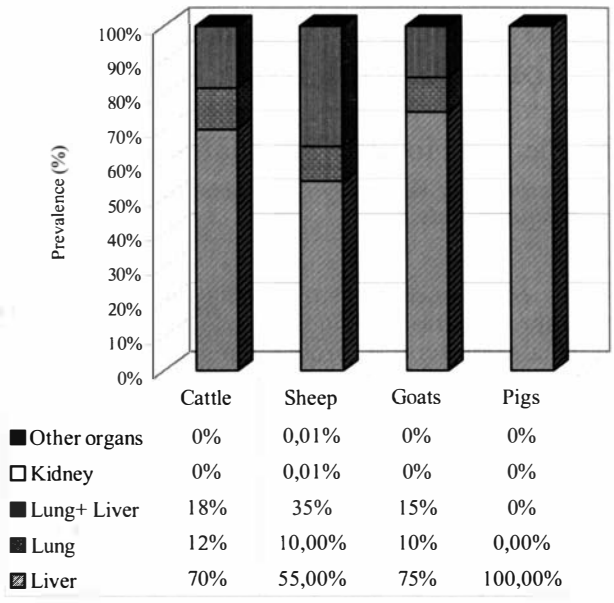

Fig. 5. Distribution of cystic lesions in the different animal species.

slaughtered and in young animals the identification of cysts was very difficult because of the small size of cysts, the reported prevalence is certainly underestimated.

The study done pointed out that the liver was the only parasited organ in the 100% of pigs, 75% of goats, 55% of sheep, 70% of cattle while in the 34.97% of sheep and 17.98% of cattle the infection was present both in liver and lung (Fig. 5). No data of cyst fertility were reported.

Our present survey, that represents the first one carried out in the Umbria Region after 50 years, has shown a wide diffusion of hydatidosis with prevalence values similar to those reported in endemic areas. The data obtained cannot be wholly relied upon as accurate but it can be used as a baseline for the proper evaluation of the status of this zoonotic disease in future coordinated surveys. This type of study will make possible to control the infection and ensure effective protection non only for animal population but also for humans at risk of contracting the infection.

Parassitologia 46: 439-440, 2004

From Nairobi to Sassari, a realistic role for Italian Hydatidology. Thoughts from the XXI International Congress of Hydatidology

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The XXI International Congress of Hydatidology was held in Nairoby (Kenya) from 15 to 21 August 2004 and was organized by the Kenya Society for Hydatidology under the auspices of the International Association of Hydatidology and with the help of the African Medical and Research Foundation. The Congress had a great success, as demonstrated by the number of participants (110 delegates from 32 countries) and presentations (three plenary sessions and 18 sessions with 124 oral papers and 22 posters). All the summaries are published in the 35th volume of International Archives of the Hydatidosis and the presentations are collected in a CD.

Peter M. Schantz (CDC, Atlanta) made the opening session highlighting the global situation of cystic echinococcosis (CE) in the world. Only three countries were considered to have eradicated CE: Iceland, New Zealand and Tasmania. National and regional programs in Uruguay, Argentina and Chile have documented the reduction of prevalence in dogs, livestock animal intermediate host and humans. On the contrary, appears tragic the situation of some areas of China, Asia and Africa where the documented rates of infections in humans are dramatically rising. Political, social and economic conditions in these populations are far different in comparison with those where successful control of CE has been achieved. The goal of the intervention programs demonstrates that we are able to manage adequate technology to control and eliminate CE but further progress, building on these positive experiences, should be done to reduce the period for the eradication that now is unacceptably long (15-25 years). This will be one of the big challenges for public health in the near future.

The main topics of the sessions on CE were: epidemiology and economics, epidemiology and diversity, host/parasite interaction, diagnosis and follow up in humans, diagnosis and control in animals, chemotherapy. A large part was dedicated to *Echinococcus multilocularis* and its environmental relationship studied also by new approaches such as spatial epidemiology and remote sensing techniques (Bowyer *et al.*, UK) that were also applied to CE (Poglayen *et al.*, Italy; Macpherson *et al.*, Uganda). Diagnostic techniques were discussed and matched with different approaches; from a quantitative ELISA to the traditional fox gut scraping (Romig, Germany). For this parasite that is dramatically increasing also in Europe, where raccoon dog (*Nyctereutes procyonoides*) was found to be a new suitable definitive host (Tackmann and colleagues, Germany), therapy and diagnosis in man, fox treatment by anthelmintic baiting (Heggling, Switzerland) were focused. The success of this particular kind of intervention in fox was assured by the rapid occupation of the parasite ecological niche by *Taenia teaniaeformis*.

The basic approach to CE produced fascinating results that may result, in the future, of enormous practical use. The demonstration of common cell signal transduction system between cestodes and mammals forecasts interesting therapeutic implication (Konrad *et al.*, UK). Gottstein (Switzerland) investigated the role of cyst laminated layer in the host/parasite interaction while Peng and colleagues (China) used the membrane "exo-adventita" for a new successful surgical approach towards liver CE: the "subadventital pericystectomy". Rogan (UK) presented another interesting result with future perspectives in term of host/parasite relationship, natural history, and treatment: he hypothesized the origin of daughter cysts, expression of suffering (damage) in the primary cyst wall. Finally a new strain, G10, of *Echinococcus granulosus* in reindeer (closely related to camel strain G6) reappeared in Fennoscandia but with a small risk of human infection (Oksanen and Lavikainen, Finland) while a new species, *E. shiquicus*, was reported in plateau pika (*Ochotona curzoniae*) from the Tibetan plateau (Xiao *et al.*, Japan).

Also the applied contributions resulted of great interest, for instance the recent worrying data on animal and human CE in Romania, where the 10% of human mortality in surgical cases were observed (Ionescu *et al.*). The picture on ecological and behavioural aspect of taeniid parasites, that promote transmission in rural environment, was particularly intriguing (Shaikenov *et al.*, Kazakhstan).

These were the main subjects discussed and in this context a general comment should be done. Also in the hydatidology group of scientists the world is clearly divided in two parts: rich and poor countries with scarce communication channels. The rich, western world where the impact of echinococcosis

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appears to be limited, mainly in term of human suffering, is working in hard biotechnology and is proposing very complicate epidemiological models that fit well only where health services are organized and efficient. This means that data recording and specimen collection must be possible, reliable accurate and, obviously, expensive. In the other bank of the terrestrial globe: the estimated prevalence in some human communities approaches 8% (Zeyhle *et al.*, Kenya); the use of ultrasound screening in domestic animals assumes the only significance of health education (Njoroge *et al.*, Kenya); disease in man became the unique epidemiological indicator; non verbal communication is part of health education programmes (Iriarte, Argentina); and herbal medicine is considered a promising chance (Tanveer, Pakistan; Sadjjadi *et al.*, Iran). Someone asked to the hyper-technocracy to consider "more realistic situation". Inside this scarce interactivity the role of Italy should be of great interest for a lot of real situations present in our country. In the Mediterranean basin hydatid disease is highly prevalent and Italy is in the centre of the area. We have the coexistence of a lot of scattered environmental and socio-economic variety that reproduce, in the same context, situations that are similar to north Africa and to north Europe ones. Also our breeding units range from traditional animal farming to modern, greatly specialized husbandry with an health support that may be of high level. We have a documented historical experience on echinococcosis, we are able to manage molecular approach, sophisticate epidemiological model, socio-economic analyses and to excel in less invasive surgical techniques such as PAIR; our scientists have collaborations all over the world. Finally, emerging countries, with different motivations, show interest to Italy. For all these reasons it is of great importance to refound the Italian Society of Hydatidology that may represent a bridge between two parts of the same scientific world. The Italian Society of Hydatidology may play an important role in harmonizing different professionalisms for a common project of echinococcosis control/eradication to export to less lucky lands.

Parassitologia 46: 441-442, 2004

Cytokine expression in the follow-up of patients with cystic echinococcosis

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Cystic echinococcosis (CE) is a severe parasitic disease caused by the cestode *Echinococcus granulosus* which stimulates in humans both humoral and cellular immune responses. It has been shown that the two arms of the immune system, cellular and humoral, are regulated by different groups of cytokines produced by two CD4+ T cell populations termed Th1 and Th2 (Romagnani S, 1992, Immunol Today 12: 256-257). Th1 cells primarily control cytolytic T cells and macrophages whereas Th2 cells are mainly responsible for B-cell regulation. Chronic helminth infections consistently generate a pronounced Th2 response (production of IL-4, IL-5, IL-6 and IL-10), accompanied by a concomitant decrease in the Th1 response (production of IL-2 and IFN-γ) (Pearce EJ *et al.*, 1991, J Exp Med 173: 159-166). Ample evidence shows that in parasitic infections a strong Th2 response correlates with susceptibility to disease, whereas a Th1 response correlates with protective immunity (Grau GE, Modlin RL, 1991, Curr Opin Immunol 3: 480-485). We have previously demonstrated that in human CE, as in most parasitoses, Th1 cell activation is related to protective immunity, Th2 cell activation to susceptibility to disease (Siracusano A *et al.*, 2002, Endocr Metabol Disord 2: 235-245). We have then investigated serum cytokine production for use in the clinical follow-up of patients with CE. This *in vivo* study, despite confirming our previous *in vitro* findings, demonstrated the scarce utility of serum cytokine monitoring in the follow-up of patients because of its low sensitivity (Siracusano A *et al.*, 2002, Endocr Metabol Disord 2: 235-245). Seeking better immunological markers indicating the long-term outcome of CE after chemotherapy, the aim of the present study was to assess the prognostic role of intracellular Th1 and Th2 cytokine monitoring during the follow-up

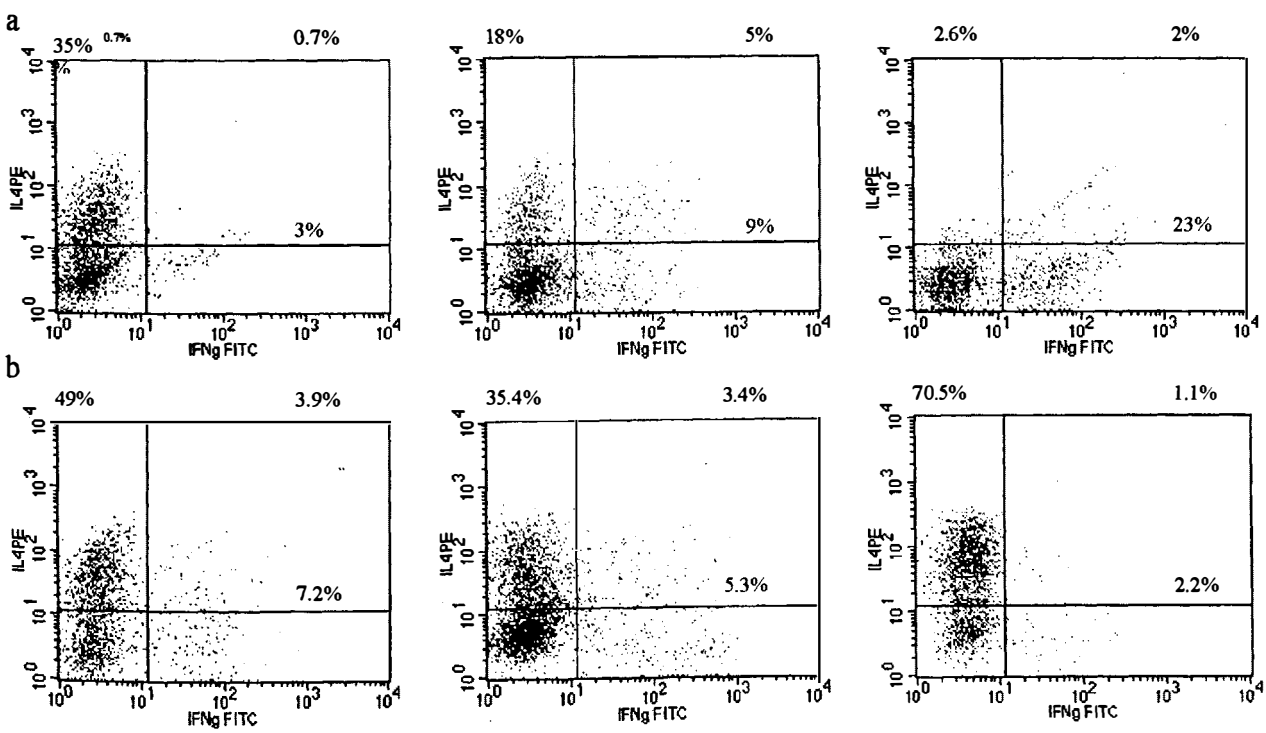

Figure 1.

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of patients with pharmacologically treated CE. For this purpose we evaluated intracellular expression of IFN-γ and IL-4 in T lymphocytes obtained from patients with success or no success of a three month cycle of chemotherapy with albendazole at baseline, at 1 month and at the end of chemotherapy.

Intracellular cytokine expression was evaluated by three colour flow cytometry using PerCP-conjugated CD3 specific monoclonal antibody (MoAb), PE-conjugated CD69 and IL-4 specific MoAbs and FITC-conjugated IFN-γ specific MoAbs (BD Biosciences) as previously described (Profumo E *et al.*, 2003, J Cardiovasc Surg 44: 237-242)

In patients with success of therapy we observed that the percentage of cells positive for the Th2 cytokine IL-4 dramatically decreased in comparison to baseline already at 1 month post chemotherapy whereas the percentage of cells positive for the Th1 cytokine IFN-γ increased (Fig. 1a). In contrast, in the 80% of patients with no success of chemotherapy, we observed at the end of chemotherapy the increase of the Th2 cytokine IL-4 and the simultaneous decrease of IFN-γ (Fig. 1b). In the remaining 20% of patients with no success of therapy both cytokines did not show relevant variations during the follow-up (data not shown).

The response to treatment is unpredictable; it also entails constant medical supervision and regular monitoring of imaging findings and serologic responses. The incidence of relapse increases with the length of follow-up. Early diagnosis of progression of disease before it becomes apparent could lead to earlier treatment with another cycle of therapy or to the adjustment of it. Monitoring imaging findings during follow-up can be difficult because cysts often undergo relatively small changes that imaging cannot visualize. The viability and presence of all foci is also difficult to assess. As a method for clinical follow-up, serologic testing also has drawbacks because specific antibodies may persist in patients' sera for several years after recovery and combined serologic testing provides scarce information on the long-term outcome of CE (Riganò R *et al.*, 2002, Clin Exp Immunol 129: 485-492). Furthermore cytokine production assays in PBMC culture supernatants and cytokine expression at gene level, technically complex and expensive to perform, are unlikely to become part of a routine diagnostic protocol and serum cytokine monitoring was proved equally unsuccessful (Siracusano A *et al.*, 2002, Endocr Metabol Disord 2: 235-245).

In conclusion intracellular expression of Th1 and Th2 cytokines in whole blood of patients with CE seems to be promising to monitor the outcome of pharmacologically treated disease and our findings here, even though very preliminary, open new perspectives in the immune surveillance of CE.

Parassitologia 46: 443-444, 2004

An epidemiological and biomolecular survey of cystic echinococcosis in cattle in Sardinia

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Although Cystic Echinococcosis (CE) is still a health, economic and social problem of great importance in Sardinia today, not all aspects of it have been studied, and in particular its epidemiology in cattle. This note updates the epidemiological data on Bovine CE in Sardinia, and with the help of advances made in biomolecular taxonomy identifies the strains of *Echinococcus granulosus* in cattle.

Between January and June 2004, 392 cattle of various breeds were examined. These were mainly raised extensively and were slaughtered in Sardinia. Their ages were determined from slaughterhouse data. The number, locality and types of hydatids found were then classified as fertile, acephalocysts, caseous and calcified.

Samples were taken of the proligerous membranes and the cystic liquid (0.1 g) and the DNA was extracted with a commercial kit (Roche, DNA Template extraction kit).

A fragment of the mithocondrial gene codified for NADH dehydrogenase (ND1) was then amplified with PCR using the primers suggested by J. Bowles and D.P. McManus (1993, Int J Parasitol 23(7): 969-972).

The amplicons obtained were then sequenced with capillary sequencers (Applied Bio-systems). The sequences were then compared with those in the web data banks to determine the level of agreement of each sequenced sample, using BLAST (NCBI) and Bioedit software.

CE was found in 19.6% of the samples, although only 0.76% of the cattle had fertile hydatids. CE was found in 17% of cases in the liver and in 12.5% of cases in the lungs (χ^2 =3.28; P=0.070). A greater percentage of viable hydatids were found in the lungs (2.5% against 0.25% in the liver - χ^2=10.09; P=0.0014).

With respect to the typology of the cysts, caseous/purulent hydatids were found more frequently in the liver (50%) and then calcified cysts (45.4%), while acephalocysts were the most frequent type in the lungs (40.8%).

Abundance (number of hydatids/animals sampled) was 2.2, while average intensity (number of hydatids/positive animals) was 11.2.

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9.9% of the animals examined were massively infested (>10 hydatids). In these cases both the liver and lungs contained hydatid cysts.

The animals were divided into 5 age groups. This allowed us to highlight the following prevalence and the respective Odds Ratio values. These are shown in Table 1.

Table 1. Prevalence and Odds Ratio for the cattle divided by age.

Age	No. of examined animals	No. of positive animals	Prevalence	Odds Ratio
≤2 years	229	6	2.6%	1.00
>2-≤ 4 years	109	35	32.1%	17.58
>4-≤ 6 years	8	3	37.5%	22.30
>6-≤ 8 years	20	12	60%	55.75
>8 years	26	21	80.8%	156.10

The differences in prevalence by age were statistically significant (χ^2 trend=131.03; P<0.0001).

Sequence analysis determined that the strain of *E. granulosus* in the processed isolates was exclusively G1 (*sheep strain)*.

The prevalence rate was 19.6%. This confirms that there is still strong parasitic pressure from the G1 strain CE in Sardinia. This is not the specific strain for cattle (G5 or *bovine strain*), which could possibly explain the low fertility values found (0,25%).

The sheep strain (G1) is the genetic variant which has been found most often in Sardinia in the most recent works on genotyping the parasite and thus can be considered endemic on the island (Idini G *et al.*, 2000, Atti SIPAOC 14: 33-36; Varcasia A *et al.*, 2004, Parassitologia 46 Suppl 1: 193), as it is also in Spain, Tunisia and Eastern Europe (Gonzales LM *et al.*, 2002, Exp Parasitol 102(1): 46-56; Thompson RCA, McManus DP, 2002, Trends Parasitol 18(10): 452-457).

It should be reiterated that the G1 strain of *E. granulosus* in cattle behaves in exactly the same way as it does in sheep. In fact in Sardinia it was found in cattle more frequently in the liver and with higher fertility in the pulmonary cysts (Scala A *et al.*, 2001, XX International Congress of Hydatidology 34: 303).

The prevalence and level of fertility in cattle confirms the results of a similar survey conducted in

Sardinia 20 years ago by Nieddu *et al.* (1980, Atti Tavola Rotonda Echinococcosi/Idatidosi, Alghero 22/5/1980, 33-35). However this previous survey was conducted in a pre-biotech period and the low fertility level (0.99%) was attributed to the parasite, not being or being less suitable to develop in a host which was found less widespread in the island. It may be that in this intermediate host there is a strong immunitary response to a strain of CE (G1) not species-specific for cattle, that inhibits the hydatids which are generally found in the liver from developing a caseous-purulent reaction and then calcifying (50% and 45% respectively of the cysts found). In the lungs this phenomenon was observed less often and acephalocysts were found more frequently.

CE tended to progressively accumulate in adult cattle in Sardinia.

In conclusion we can state that cattle, although they are not considered an important host for the persistence of CE in Sardinia, may be considered a further indicator of infestation and that even though sometimes the parasitosis could be limited to a single calcified hydatid, it damages the organs and thus reduces the value of the animal when it is slaughtered, especially in the case of older animals.

Acknowledgements

This study could be performed thanks to the invaluable collaboration of Mr Mario Salvatore Nieddu and financed by MIUR PRIN 2003 prot. n. 2003070410_001 and Finalized Sanitary Search IZS Sardinia (prot. n. DGRSVE/CRS/RF-2003/90).

Parassitologia 46: 445-447, 2004

Preliminary data on *Echinococcus granulosus* (Batsch, 1786) in dogs from Lombardia and Marche regions (Northern and Central Italy)

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In Northern and Central Italy, only a limited amount of data is available on spread of *Echinococcus granulosus* (Batsch, 1786) in definitive and intermediate hosts. Concerning Lombardia and Marche regions, epidemiological investigations on the parasite were carried out mainly among slaughter-houses, where only information on intermediate hosts (cattle, sheep) has been recorded (Garippa G, Battelli G, Cringoli G, Giangaspero A, Giannetto S, Manfredi MT, 2004, Parassitologia 46:33-38).

The present research aims to assess by CA-ELISA the presence of *E. granulosus* in definitive host from these two regions, and it takes part in an ample project on echinococcosis in Italy.

One hundred twenty-eight faecal samples of dog (of which 62.5% from farms and 37.5% from owners) were collected from Marche (n=106 shared between the provinces of Macerata and Ancona) and Lombardia (n=22, shared between the provinces of Milan, Pavia and Lecco). Data on life style of dogs (owned, kennel or sheep-dog), sex and age were also recorded (Table 1).

Faecal samples were deep-frozen (at least two weeks at –80°C and then at –20°C) and they were left to defrost before they were analysed. The *Echinococcus*-specific coproantigens were detected by a commercial ELISA test (CHEKIT-Echinotest, Bommeli Diagnostic, Switzerland) and the results were expressed in value %, as described by the manufacturer (values <30% were considered as negative, those within 30-40% as ambiguous and values >40% as positive). Further, the faeces were examined for parasite eggs by flotation method with sodium tiosulphate (SG=1.450), in order to identify possible cross reaction with the ELISA test, caused by other helminths.

Out of 128 samples, 107 dogs were negatives (83.6%), 9 samples were ambiguous (7%), 12 resulted positives to *Echinococcus* by CA-ELISA test (9.4%), of which 5 dogs were from Macerata and Ancona provinces and 7 dogs from Milan and Lecco provinces (Table 2).

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The coprological test by flotation technique recorded 61.7% of faecal samples positive to one or more parasite species (79/128). The frequence percentage of infected animals varied in the provinces as follows: 61.8% in Macerata (34/55), 51% in Ancona (26/51), 92.8% in Milan (13/14), 33.3% in Lecco (2/6) and 100% in Pavia (2/2). The collected species belonged to Nematoda (*Toxocara canis*, *Trichuris vulpis*, *Ancylostoma caninum*, *Capillaria* spp, *Strongyloides* spp), Cestoda (Taenidae, *Dipylidium caninum*, *Spirometra* spp), Protozoa (*Sarcocystis* spp, *Isospora* spp, *Giardia* spp.). In 43.7% of dogs were recorded only nematodes (56/128), in 9.37% only cestodes (12/128), in 5.47% were found both *taxa* (7/128), while in 3.12% of samples protozoan parasites have been seen (4/128). The presence of Cestoda (in association or not with other *taxa*) resulted significantly higher in positive samples to CA-ELISA rather than in the other categories (Kruskal Wallis Test, χ^2=7.485; p=0.024). In particular, eggs have been found in 12.5% of negative samples (13/107), in 11.1% of samples with ambiguous result (1/9) and in 41.7% of positive samples (5/12) to CA-ELISA for *Echinococcus* (Table 3); besides *Dipylidium caninum*, considered responsible for crossing reactions with ELISA was collected only in one positive and in two negative faecal samples to CA-ELISA. Data on style life of dog (owned, sheep and kennel dog) were compared both with the CA-ELISA and copromicroscope analyses for samples from Macerata and Ancona provinces, while not in Lombardia, as here only sheep-dogs have been tested. Both in Macerata and Ancona, data on CA-ELISA were similar among the dog categories. Concerning parasitological survey, no differences were recorded in dogs from Ancona. In those of Macerata highly significative differences have regarded number of infected animals and composition of helminths (Kruskal Wallis Test, χ^2=14.021; p=0.001 and χ^2=22.474; p<0.001, respectively). A higher frequence of owned dogs with parasites was observed rather than in the other dog categories (owned dog: P= 100%, sheep-dog: P=27.8% and kennel dog: P=75%), and nematodes represent the most common *taxon* (P=45.5%).

If necroscopy is the "gold standard" to detect *E. granulosus*, there are objective difficulties, (included national laws n. 281/91 and n. 189 of 20 July 2004, art. 544 bis) to apply this technique for inves-

Table 1. Data on dog tested by CA-ELISA according to different study areas.

Provinces	No. sheep farms	No. dogs in farms	No. owned dogs	No. kennel dogs	No. dogs examined	No. positive dogs
Macerata	7	18	5	32	55	4
Ancona	16	41	1	9	51	1
Milano	2	14	0	0	14	6
Lecco	2	6	0	0	6	1
Pavia	0	0	2	0	2	0
Total	26	79	8	41	128	12

Table 2. Data on positive dogs to CA-ELISA test.

Sample	Life style	Province	Breed of dogs	Sex (age)	Dogs for farm	Sheep for farm
01	Owned	Macerata	Maremmano sheperd	Female (–)	–	–
02	Kennel dog	Macerata	Cross-breed	Male (–)	–	–
03	Kennel dog	Macerata	Cross-breed	Male (–)	–	–
04	Kennel dog	Macerata	Cross-breed	Male (–)	–	–
05	Sheep dog	Ancona	Cross-breed	Male (–)	3	90
06	Sheep dog	Milano	Maremmano sheperd	Female (1.4 years old)	14	2000
07*	Sheep dog	Milano	Maremmano sheperd	Male (1.4 years old)		
08	Sheep dog	Milano	Border collie	Male (5 months old)	8	2000
09	Sheep dog	Milano	Bergamasco sheperd	Male (11 years old)		
10	Sheep dog	Milano	Border collie	Female (4 years old)		
11	Sheep dog	Milano	Bergamasco sheperd	Male (4 years old)		
12*	Sheep dog	Lecco	Bergamasco sheperd	Female (2 years old)	3	44 sheep and 1 cattle, 100 goats and 1 donkey

* Dogs PCR-positives to *Echinococcus granulosus*.

Table 3. Comparison between CA-ELISA and copromicroscopic exam.

CA-ELISA	Copromicroscopic exam						
Classes	Negative	Only Nematoda	Only Cestoda	Nematoda+ Cestoda	Only Protozoa	Protozoa+ Nematoda	Total samples
Negative	42	48	9	4	2	2	107
Ambiguous	3	5	0	1	0	0	9
Positive	4	3	3	2	0	0	12
Total samples	49	56	12	7	2	2	128

tigations on a large scale. For routine diagnosis of *E. granulosus*, a coproantigen ELISA could be applied as a primary test, because the coprodiagnosis can only provide data on presence/absence of eggs and proglottides of Taeniidae in the faeces, but not on species identification, being eggs of *E. granulosus* similar to those of *Taenia* spp. (Eckert J, Deplazes P, 2004, Clin Microbiol Rev, Jan 107-135).

Through our investigations by CA-ELISA, we have obtained the first records on *E. granulosus* in definitive hosts, in Marche and Lombardia. The frequence percentages of positive dogs to *Echinococcus* coproantigen were 4.72% and 31.8% on total samples examined in Marche (106) and Lombardia (22), respectively. Moreover, it was possible to confirm the diagnosis of canine echinococcosis in two positive animals from Lombardia through nested PCR, that was carried out by the Parasitology Laboratory of the Istituto Superiore di Sanità (Rome). One dog was from a sheep farm in Milan province, where previously a cyst of *E. granulosus* was found in a slaugthered sheep; the other dog was from a farm in Lecco province, where cysts have been found in organs (liver/lungs) of sheep and cattle.

Certainly, the sampling method applied in the present study was also a contributing factor to the relative higher percentage of positive samples in Lombardia with respect to Marche. In fact, while a random sampling was carried out in Marche, in Lombardia we have chosen to test only sheep dogs coming from areas where *E. granulosus* was found in intermediate hosts. As preliminary investigations, we were more interested to verify the presence of *E. granulosus* in dogs in localities where it was previously signalled in intermediate hosts to understand if parasite's life cycle could be completed in these areas. Thus, the probability to detect more positive animals in this group was vitiated by our choices to select localities and animals. For next steps our purposes will be to extend the epidemiological study to the whole regional territory, such as to continue data collection in Marche region.

Finally, a particular attention has to be paid to helminths which could favor transmission of zoonoses, mostly when these parasites infect domestic animal closely connected with humans, as dog. Giardiosis, particularly, seem to be a re-emerging infection disease in the last decade (Capelli G, Paoletti B, Iorio R, Frangipane di Regalbono A, Pietrobelli M, Bianciardi P, Giangaspero A, 2003. Parasitological Research 90: S154-S155). The finding of this study (as *Giardia* sp. recorded in a kennel dog from Macerata) could support the hypothesis that confinement in a limited area is an importat risk factor, as reported also in Capelli *et al.*, 2003. Thus, this record together with data on dogs positive to *Echinococcus* coproantigen have to be considered in human health management, at least at regional level.

Our results lead us to stress that specific surveys in these areas should be necessary to better define diffusion of important parasite diseases, as echinococcosis and giardiosis.

Acknowledgements

Special thanks to Dr Pozio and Dr Casulli for PCR analyses on some samples. Research was partially financed by MIUR (PRIN 2003).

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Cystic echinococcosis in the Campania region (southern Italy)

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Echinococcosis is cosmopolitan zoonosis caused by adult or larval stages of tapeworms belonging to the genus *Echinococcus* Rudolphi, 1801. Within the genus *Echinococcus* four species are presently recognised, namely *Echinococcus granulosus, E. multilocularis, E. oligarthrus* and *E. vogeli*, and taxonomic revision of the genus is probably needed (Thompson RCA, McManus DC, 2002, Trends Parasitol 18: 452-457). *E. granulosus*, the major species of medical and public health importance which causes cystic echinococcosis (hydatidosis), has a global distribution.

The range of intermediate host species (domesticate ungulates) depends on the infecting strain of *E. granulosus*, regional or local differences in the availability of the various intermediate host species, and other factors. Laboratory and field observations have revealed considerable phenotypic variability among isolates of *E. granulosus* from different species of intermediate hosts (Thompson and McManus, 2002). In total, 10 distinct strains (genotypes) of *E. granulosus* have been described using DNA sequence data: G1 (common sheep strain), G2 (Tasmania sheep strain), G3 (buffalo strain), G4 (horse strain), G5 (cattle strain), G6 (camel strain), G7 (pig strain), G8 (cervid strain), G9 (human strain), and G10 (Fennoscandian cervid strain) (McManus DC, 2002, Trans R Soc Med Hyg 96: 151-157; Lavikainen A, Lehtinen MJ, Meri T, Hirleva-Koski V, Meri S, 2003, Parasitology 127: 207-215; Maravilla P, Andrew Thompson RC, Palacios-Ruiz JA, Estcourt A, Ramirez-Solis E, Mondragon-de-la-Pena C, Moreno-Moller M, Cardenas-Mejia A, Mata-Miranda P, Aguirre-Alcantara MT, Bonilla-Rodriguez C, Flisser A, 2004, Acta Trop 92: 231-236). Although the validity of the G9 genotype has been questioned (Snábel V, D'Amelio S, Mathiopoulos K, Turceková L, Dubinsky P, 2000, J Helminthol 74: 177-181) and it might correspond to the G7 genotype. The horse strain G4 and the cattle strain G5 have been also considered to represent distinct species, *E. equinus* and *E. ortleppi*, respectively (Thompson and McManus, 2002). Data regarding the presence and distribution of echinococcosis-hydatidosis in definitive and intermediate hosts are scant and fragmentary in Italy (Garippa G, Battelli G, Cringoli G, Giangaspero A, Giannetto S, Manfredi MT, 2004, Parassitologia 46: 33-38). Scant are also the studies regarding the number and type of *E. granulosus* strains present in Italy; researches performed by the sequencing of the mitochondrial genes NADH dehydrogenase and CO1, showed the presence of the G1 strain in sheep and cattle and of the G1 and G7 strains in pigs from Sardinia (Garippa *et al.*, 2004; Varcasia A, Nieddu MS, Scala A, Garippa G, 2004, Parassitologia 46: 193). In addition, Busi *et al.* (Busi M, Snabel V, De Liberato C, D'Amelio S, 2004, Parassitologia 46: 164) recently reported the presence of the G1 strain in sheep and of the G1 and G2 strains in cattle from Sardinia, as well as the presence of the G1 and G3 strains in sheep from Latium region. It is noteworthy that the G3 buffalo strain, detected for the first time in India, seems to be prevalent in the hydatid cysts obtained from human infections (Busi *et al.*, 2004).

Literature reports only three surveys on animal echinococcosis-hydatidosis in the Campania region of southern Italy. In the first one, Damiano, 1964 (Acta Med Vet 10: 397-401) reported prevalence values of 7.1% in cattle slaughtered in the Caserta province. The second one (Capurso A, Rivellini P, Guarino C, 1968, Atti SISVET 22: 725-729) reported prevalence values of 1% in dogs from Naples province and of 2.4% in intermediate hosts (cattle, sheep, goats, pigs and horses) slaughtered in the same zone.

In a recent survey, Cringoli *et al.* (Cringoli G, Capuano F, Landolfi MC, Esposito A, Veneziano V, Rinaldi L, 1998, Atti Giornate Scientifiche Campane: 238), as a result of a surveillance at 26 slaughterhouses in the Campania region, reported an average prevalence of cystic echinococcosis lower than 5% in cattle, sheep, goats and pigs. However, some zones were at high risk for cattle and sheep with prevalence values ranging between 16 and 21%.

The present paper reports the results of an abattoir-based survey aimed to enlarge the knowledge on cystic echinococcosis in the Campania region of southern Italy.

Starting in October 2003, cattle and water buffaloes were examined for cystic echinococcosis at 2 slaugtherhouses located in the Caserta province (north of the Campania region). Each animal was inspected in order to detect and collect hydatid cysts. The number of animals slaughtered each day was recorded, as well as the age, sex and origin of each animal. Parasitized organs were examined in

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order to evaluate the number of cysts, their sizes, their form (unilocular, pseudo-multilocular, or iperlaminated). Fertility was assessed by determining protoscolex viability by microscopic examination, observing protoscolices and their flame cells movements; degenerative modifications (calcification, caseation) were also determined.

Out of a total of 108 cattle examined, 16 (14.8%) were found to be infected, aged between 1 and 12 years, all were females. Out of a total of 494 water buffaloes examined, 43 (8.7%) were found to be infected. They aged between 2 and 19 years and were 42 females and 1 male. The average number of cysts per cattle was 15.7 (minimum 1, maximum 74). Six animals had hydatid cysts only in the liver (with an average of 9.7 cysts/liver), 1 only in the lungs (with an average of 2.0 cysts/lungs), and 9 cattle had cysts both in the liver and in the lungs. Fertile cysts were not found. The frequency, form and type of hydatid cysts recovered from different organs of cattle, are reported in Table 1.

With respect to water buffaloes, the average number of cysts per animal was 5.7 (ranging from 1 to 45 cysts/animal). Ten animals had hydatid cysts only in the liver (with an average of 8.0 cysts/liver), 20 only in the lungs (with an average of 1.7 cysts/lungs), and 13 buffaloes had cysts both in the liver and in the lungs. Fertile cysts were found in 7 (16.3%) out of the 43 positive buffaloes. The frequency, form and type of hydatid cysts recovered from different organs of water buffaloes, are reported in Table 2.

In order to display the presence and distribution of cystic echinococcosis in animals from the Campania region, provenience data of positive animals were utilized, combining the data from present survey with the data from the survey performed in 1998 (Cringoli *et al.*, 1998) and a distribution map (Fig. 1) was drawn using a Geographical Information System (GIS) (software Arc-View 3.2 GIS, ESRI, Redlands, CA, USA). This map uses the municipality as the geographic unit of reference and display the municipalities with animals (buffaloes, cattle, sheep, goats and pigs) parasitized by cystic echinococcosis. This map show that all the five provinces of the Campania region had municipalities with animals positive for cystic echinococcosis.

Cystic echinococcosis is known to be one of the most important parasitosis in livestock in the Mediterranean region and it is the most important parasitic zoonosis in this area (Garippa *et al.*, 2004). The findings of this survey are important to better know the distribution of cystic echinococcosis in the Campania region of southern Italy, mostly for the data reported for water buffaloes. In fact, until now, surveys aimed to evaluate the presence and distribution of bubaline cystic echinococcosis have been performed neither in Italy, nor in the whole Mediterranean region. In a recently published review of the epidemiological situation on echinococcosis in the Mediterranean region by the Mediterranean Zoonoses Control Centre, WHO (Seimenis A, 2003, Acta Trop 85:191-195), the Author reports incidence and/or prevalence values of echinococcosis/hydatidosis in humans, cattle, sheep, goats, camels, and dogs; there are no data on the water buffaloes. The prevalence values reported for buffaloes (8.7%) are noteworthy; they are higher than the average values previously reported in cattle, sheep, goats and pigs by Cringoli *et al.* (1998). Noteworthy is also the presence of fertile cysts in the 16.3% of positive buffaloes; this let to believe that the *E. granulosus* buffalo strain G3 – that seems to be prevalent in human infections too – is present in water buffaloes bred in Italy. The prevalence values reported for cattle in the present paper (14.8%) are double than the average values

Table 1. Frequency, form and type of hydatid cysts recovered from different organs of cattle.

Organs	No. of cysts	Form of cyst			Type of cyst			
		Unilocular	Pseudomultilocular	Iperlaminated	Fertile	Acephalous	Sterile	Calcified/ caseous
Liver	176	119	56	1	0	51	13	112
Lungs	64	58	3	3	0	35	0	29
Total	240	177	59	4	0	86	13	141

Table 2. Frequency, form and type of hydatid cysts recovered from different organs of water buffaloes.

Organs	No. of cysts	Form of cyst			Type of cyst			
		Unilocular	Pseudomultilocular	Iperlaminated	Fertile	Acephalous	Sterile	Calcified/ caseous
Liver	142	107	21	14	29	27	5	81
Lungs	111	81	11	19	30	14	6	61
Total	253	188	32	33	59	41	11	142

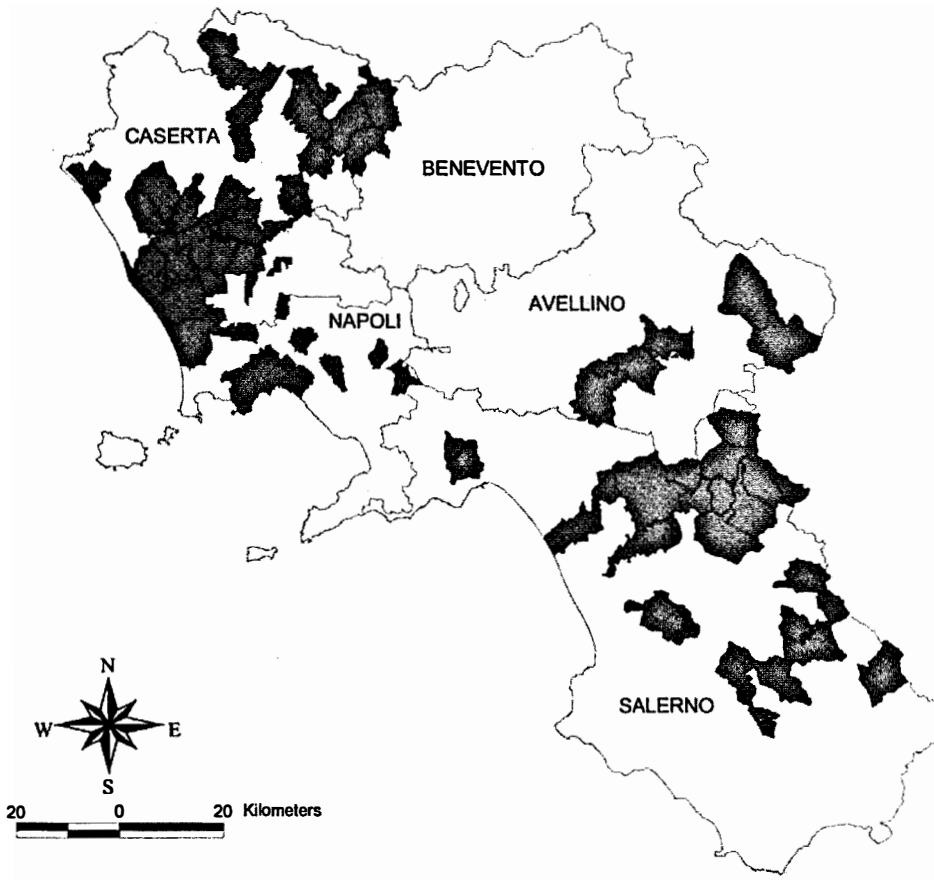

Fig. 1. Campania region: Municipalities with animals (buffaloes, cattle, sheep, goats and pigs) parasitized by cystic echinococcosis.

reported from the same province (Damiano, 1964) and they are in line with the values reported by Garippa *et al.* (2004) in southern Italy (13.3%). Further researches are necessary to explain the absence of fertile cysts in the cattle.

In conclusion, the findings of the present survey showed that cystic echinococcosis is widespread in the Campania region of southern Italy; they represent an important starting point for further studies aimed to the molecular characterization of *E. granulosus* strain(s) in buffaloes from Italy, evaluating also its/their zoonosic potential.

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Instructions to authors

Parassitologia is a medium for publication of research on: (i) systematics and biology of parasites and disease vectors; (ii) parasite-host-vector interactions; (iii) epidemiology and control of parasitic and/or vector-borne infections; (iv) history of parasitology; (v) techniques for the study of parasites and vectors. The journal publishes original papers (including short notes), review articles and proceedings/abstracts of meetings with special reference to those sponsored by the Italian Society for Parasitology. Three complete copies of the paper, typewritten double-space, together with the matching file in PDF or common word processor, should be sent to the Editor: Prof. Mario Coluzzi, Istituto di Parassitologia, Università di Roma "La Sapienza", Piazzale Aldo Moro 5, 00185 Roma, Italy, <mario.coluzzi@uniroma1.it>. Languages accepted for contributions are Italian or one of the most widely used in the European Union. The use of English is strongly recommended. The typescript should be structured as follows. *First page*: **title** (possibly not exceeding 120 letters and spaces); **authors' names** with exponential numbers referring to their **affiliation** to be reported under the names; **running head** (not exceeding 40 letters and spaces); **name and complete address** (with telephone, fax and e-mail) of the author to whom correspondence should be sent. *Second page*: **abstract** in English (possibly not exceeding 200 words); **key words** (3-5) in English. *Subsequent pages*: **Introduction** (although this heading will not appear in print); **Study area** (if required); **Materials and methods** (with subheadings if necessary); **Results** (with subheadings if necessary); **Discussion**. In the case of short notes, whose length should be less than two printed pages (about 1,200 words), the same text structure will consist of consecutive paragraphs without headings. The last headings will be **Acknowledgements** and **References**. Listed in alphabetical order and cited in the text under the "name-and-date" system, the references should contain: name and initials of authors, year of publication in parentheses, title of article, abbreviated name and volume number of periodical (for books: title and publisher), first and last page of the article. **Tables**, typed on separate sheets with their respective legends, should be self-explanatory. **Figures**, consisting of graphs, drawings or photographs of high quality, should be prepared and labeled in a suitable format to be printed with a base of either 80 mm (one column) or 165 mm (two columns) and a maximum height of 225 mm, including the legend space. Where appropriate, scale bars and units should be given while statements of magnification are not acceptable. It is recommended that authors evaluate on suitably reduced figures the final appearance and size of the explanatory labelling. The illustrations may be mounted as a group to fill the printed page and identified in a single figure legend as A, B, C, etc. Colour plates are printed by special arrangement only. Figure legends must be grouped together on a separate sheet. Tables and figures must be cited in the text and numbered separately with Arabic numerals. Generic and specific names will be printed in italics and should be underlined on the typescript. Taxonomic details, including author and year, should be given in the text, not in the title. Submission of a manuscript implies that it has been approved by all the named authors and that it is not considered for publication elsewhere. Once the paper is accepted, the authors are asked to provide the final version also on a diskette (IBM or compatible or MacIntosh systems), indicating which wordprocessor has been used. The corresponding author will receive the proofs to be checked and returned to the Editor within one week from receipt. Only essential corrections should be made, and authors will be charged for excessive alteration at the proof stage. A purchase order form for reprints will be sent soon after the proof reading.

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