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Dog echinococcosis in northern Spain: Comparison of coproantigen and serum antibody assays with coprological exam

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Abstract

A large sheep-dog population from the province of Álava (northern Spain) has been investigated in order to determine the prevalence of the cestode parasite *Echinococcus granulosus*. Worms were detected in 14.0% of 721 dog faecal supernatants by coproantigen ELISA, and in 9.1% of 754 dog serum samples by serum antibody ELISA. A weak but statistically significant correlation (Spearman's $\rho = 0.103$, 95% CI: 0.023–0.178) between the two immunoassay results was found. In addition, eggs of the family Taeniidae were detected in 10.3% of 726 faecal samples examined by coproparasitological (flotation and sedimentation) tests. The overall *E. granulosus* infection rate, based on a Bayesian latent class model that accounts for the imperfect sensitivities and specificities of all diagnostic tests used, was estimated to be 8.0% (95% credible interval: 5.4–11.4%), corroborating that sheep-dog is the dog class most vulnerable to acquiring the infection. Dog sex did not influence the prevalence of *E. granulosus*, independently of the diagnostic test used or the dog region of origin. No significant linear correlation was found between the coproantigen ELISA OD values and the dog age (Spearman's $\rho = -0.049$, 95% CI: −0.234 to 0.135), suggesting that there were no differences in prevalence of *E. granulosus* between old and young dogs. The obtained results highlight the importance of initiating a control program based on regular treatment of the sheep-dogs with praziquantel in the province of Álava.

Keywords: *Echinococcus granulosus*; Intestinal dog echinococcosis; Epidemiology; Coproantigen; Spain

1. Introduction

Cystic echinococcosis (CE) is an important zoonosis caused by the taeniid tapeworm *Echinococcus granulosus* with a considerable impact in both human and animal health in endemic areas (Schantz et al., 1995). The disease has a wide geographical distribution, with emerging and re-emerging regions mainly in Central Europe and China (Eckert and Deplazes, 2004). Spain, together other Mediterranean countries, is currently considered as hyper endemic area (McManus et al., 2003).

The parasite's domestic life cycle is maintained through dogs (which harbour the adult tapeworm) and a range of domestic livestock intermediate host species, generally sheep and cattle. Due to the high biotic potential of *E. granulosus*, infected dogs can excrete a

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large number of parasite's eggs with their faeces, contaminating wide extensions of soil, and spreading the disease (Gemmell, 1990). Because its pivotal role in the transmission dynamics of CE, detection of *E. granulosus* in the definitive host is a key point in developing of epidemiological studies and implementation of hydatid control programmes in endemic areas (WHO/OIE, 2001).

Necropsy of dogs and examination of the small intestine is the reference method for the detection of intestinal infections with *E. granulosus*, but this laborious and ethically questionable procedure is not suitable for mass screening. Thus, a number of antemortem methods have been developed for diagnostic purposes. Arecoline purging, although 100% specific, has a highly variable sensitivity (a negative result even after two or more treatments does not guaranteed the animal is *Echinococcus*-free), is labour intensive, biohazardous and some dogs suffer undesired side-effects (Wachira et al., 1990; Eckert et al., 1984, 2001). Coprological exams have low specificity and sensitivity, as eggs from different taeniid cestodes cannot be differentiated by light microscopy, and egg production may be erratic. Finally, ELISAs for detecting parasite-specific antibodies in serum have showed variable sensitivities, ranging from 40 to 90% (Benito et al., 2001; Gasser et al., 1994; Jenkins et al., 1990), and cross-reactivity with other parasite species is often detected (Gasser et al., 1988).

Currently the most practical approach for the diagnosis of the intestinal *E. granulosus* infection in dogs is the detection of parasite antigens in faecal samples (coproantigens) by ELISA using antibodies against adult somatic antigens (Allan et al., 1992), and excretory–secretory products from proglottids (Deplazes et al., 1992) or protoscoleces (Benito and Carmena, 2005). This method considerably improves both diagnostic sensitivity and specificity, permits the detection of the parasite during the prepatence period (Ahmad and Nizami, 1998), shows the current status of the infection (Jenkins et al., 2000), and ELISA results correlate well with the worm burden in the dog intestine (Craig et al., 1995). Coproantigen ELISAs have been successfully used in the field in Libya (Buishi et al., 2005), Cyprus (Christofi et al., 2002), Uruguay (Malgor et al., 1997), and Wales (Palmer et al., 1996), demonstrating their usefulness for epidemiological studies.

Recent field surveys have shown that sheep and stray dogs have the highest *E. granulosus* infection rates, most likely because of their greater access to offal or casualty animals (Buishi et al., 2005; Shaikenov et al., 2003). Therefore, those are the dog classes currently considered to be at highest risk of infection by this cestode. In the province of Álava (northern Spain) we have previously reported similar parasitological findings (Benito et al., 2003), although most of the dogs analyzed in that study came from urban environments. In this paper we present data on the detection of *E. granulosus* in a large population of sheep-dogs from the same region, by using ELISAs for the detection of parasite-specific coproantigens and antibodies, and coproparasitological examination.

2. Materials and methods

2.1. Sampling plan design

The province of Álava (northern Spain, 43°22′0N, 6°12′0W), extends over 3037 km^2, has an averaged annual temperature of 12 °C, and an annual precipitation in the range of 650–900 L/m^2. A census of sheep-dogs from livestock farms in the province was prepared in collaboration with the Epidemiology Unit of the Subdirection of Public Health of Álava (Department of Health, Basque Government, Spain) and the Department of Agriculture (Provincial Government of Álava, Spain) including data on dog age and sex, and number of head of cattle per farm. Livestock farms with less than 10 sheep were not considered in this study. In order to investigate whether geographical and climatologic parameters influence the infection rate of *E. granulosus* in dogs, livestock farms were distributed as follow (Fig. 1): (i) Northern region, with an Atlantic climate characterized by warm summers, mild winters, and abundant precipitations through the year; (ii) Central region, with a transition climate from Atlantic to Mediterranean; (iii) Southern region, with a typical Mediterranean climate, with long, hot, dry summers and mild, rainy winters. The dog sample size estimation was performed using the Epi Info™ V6 software (CDC, Atlanta, USA) for epidemiologic statistics, assuming a 1% precision with a 95% confidence interval. An *E. granulosus* theoretic frequency of 2.5% was estimated on the basis of an overall prevalence of 0.5% for this cestode found in dogs from the same area in a previous survey (Benito et al., 2003). The total sheep-dog population in the province of Álava was 1858 dogs. Of them, 978 dogs were initially registered for blood and faecal sampling: 422 form the Northern region, 487 from the Central region and 69 from the Southern region. However, due to inherent problems during the specimen's collection (unavailability of some dogs at the time of visit, or owner's refusal to cooperate),

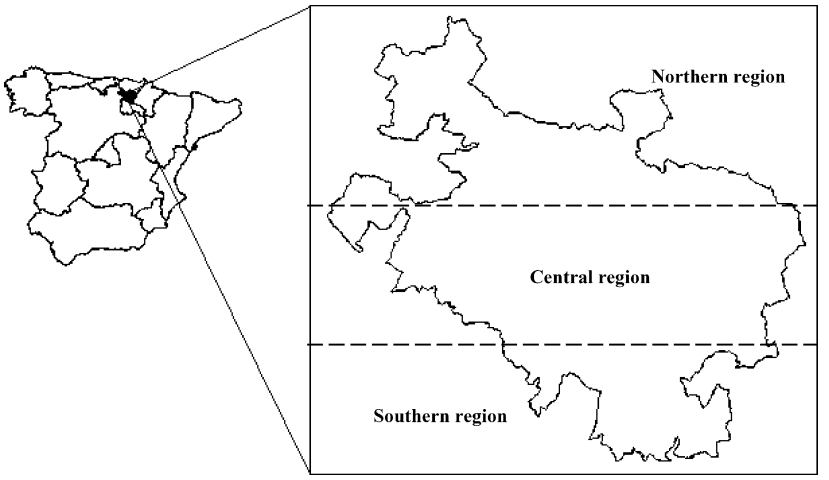

Fig. 1. Distribution of the sampling regions in the province of Álava (northern Spain) according to geographical and climatological criteria.

a smaller number of samples were available to perform this survey (see Section 3). Sampling took place during a 15-month period from April 1997 to June 1998.

2.2. Blood and faecal samples

Venous blood was taken from the cephalic vein of the dogs, and allowed to clot at room temperature for 90 min. Serum was separated by centrifugation at 2000 × *g* for 15 min, aliquotted and stored at −20 °C until tested. A faecal sample was taken from each dog either rectally using a plastic spatula, or collected from the ground after defecation. Faecal supernatants were obtained by mixing the samples at 1:2 ratio (v/v) with PBS buffer containing 5% formaldehyde and 5mM EDTA. Samples were shaken vigorously until slurry was formed, and this was centrifuged at 3500 × *g* for 20 min. Supernatants were aliquotted and stored at −20 °C. An untreated portion of each stool sample was kept and stored at −20 °C for coproparasitological examination.

2.3. Coproparasitological examination

In order to identify the presence of helminths of the family Taeniidae in the dog population, parasite eggs were isolated from the stool samples by using both sedimentation (Ritchie, 1948) and flotation (Sloss et al., 1994) techniques.

2.4. Serum antibody ELISA (Ab ELISA)

ELISAs for the detection of parasite-specific antibodies in serum samples were carried out as described by Benito et al. (2001). Briefly, polystyrene 96-well microtitre plates (MaxiSorp™, Nunc, Roskilde, Denmark) were coated with 10 μg ml^{-1} protoscolex somatic antigens in PBS buffer for 1 h at 37 °C. Plates were blocked with PBS–1% BSA (Sigma–Aldrich) for 1 h at 37 °C. Dog sera were assayed in PBS–0.5% BSA in duplicate, and incubated for 1 h at 37 °C. Sera serial dilutions ranging from 1:50 to 1:400 were tested. Rabbit anti-dog IgG peroxidase conjugate (Sigma–Aldrich) was used as secondary antibody at 1:1000 dilution in PBS–4% BSA–0.05% Tween 20 for 1 h at 37 °C. Binding was visualized with 5-aminosalicilic acid. The reaction was stopped by adding 25 μl/well NaOH 1N, and the absorbance value was measured at 450 nm. Positive-negative cut-off absorbance value of 6089 arbitrary units was based on two standard deviations above the mean optical density (OD) value for uninfected and heterologous helminth species infected dogs, previously diagnosed by post-mortem intestinal examination. Diagnostic performance of Ab ELISA was previously evaluated using necropsy as gold standard method. An overall sensitivity of 80.5% and specificity of 92.1% were obtained. Positive and negative predictive values were 59.2 and 97.1%, respectively, and the diagnostic efficiency was 90.7% (Benito et al., 2001). These estimates, however, do not account for the possibly imperfect sensitivity and specificity of necropsy. Below we describe the statistical methods used to account for this possibility.

2.5. Coproantigen ELISA (CpAg ELISA)

ELISAs for the detection of parasite excretory–secretory products (ES-Ag) in faecal samples (coproantigens) were performed according to Benito and Carmena (2005). In summary, polystyrene 96-well

microtitre plates (MaxiSorp™, Nunc) were coated with 30 μg ml^{-1} of the purified IgG anti-ES-Ag fraction, and incubated for 3 h at 37 °C. Blocking was carried out with PBS–0.05% Tween 20 overnight at 4 °C. Faecal supernatants were assayed at 1:2 dilution in PBS in duplicate, and incubated for 1 h at 37 °C. Biotinylated IgG anti-ES-Ag fraction was used as secondary antibody at 1:250 dilution in PBS–5% pre-immune rabbit serum for 1 h at 37 °C. Then, peroxidase-conjugated EstrAvidin® (Sigma) was added at 1:1000 dilution in PBS for 1 h at 37 °C. Binding was visualized with 5-aminosalicilic acid. The reaction was stopped by adding 25 μl/well NaOH 1N, and the absorbance value was measured at 450 nm. Positive–negative cut-off absorbance value was defined as the mean absorbance of the faecal supernatants from helminth-free dogs (previously diagnosed by post-mortem intestinal examination) plus two standard deviations. This value corresponds to an ES-Ag concentration of 0.24 μg ml^{-1}. Diagnostic performance of CpAg ELISA was previously evaluated using necropsy as gold standard method. The assay accredited an overall sensitivity of 78.4% and specificity of 93.3%. Positive and negative predictive values were 72 and 95%, respectively, and the diagnostic efficiency was 90.5%. In addition, the detection limit has been estimated in 5.12 ng ml^{-1} of *E. granulosus* ES-Ag (Benito and Carmena, 2005). As mentioned above, an alternative method is discussed below that does not assume necropsy to be a perfect gold standard.

2.6. Statistical analysis

A Bayesian statistical approach (Joseph et al., 1995) was used for estimating the prevalence of *E. granulosus* in the studied sheep-dog population and the properties of the diagnostic tests utilized in this field survey. Our estimation procedure consisted of two stages: at the first stage, we estimated the sensitivities and specificities of both ELISA tests from previously reported data (Benito et al., 2001; Benito and Carmena, 2005) comparing these tests to necropsy (Tables 1 and 2). Parasitological examination of the small intestine at necropsy is considered the gold standard method for the diagnosis of *E. granulosus* in the definitive host, with sensitivity and specificity each just below 100% (Eckert and Deplazes, 2004; WHO/OIE, 2001). We carried out two first stage analyses, one assuming 100% sensitivity and specificity for necropsy, and the other assuming 95% prior ranges for the sensitivity and specificity of 95–100% and 98–100%, respectively. This allowed us to determine robustness of our final estimates to these inputs.

Table 1
Summary of the diagnostic characteristics of the serum antibody ELISA assay (Ab ELISA) used for the development of prior distribution of this test

	Necropsy results		Total (n = 289)
	Positive (n = 36)	Negative (n = 253)	
Ab ELISA (+)	29 (80.5%)	20 (7.9%)	49 (17.0%)
Ab ELISA (−)	7 (19.4%)	233 (92.1%)	240 (83.0%)

Necropsy was considered the gold standard method (Benito et al., 2001).

Our Bayesian analysis was performed using the BayesDiagnosticTests Version 2.1 Software Package (http://www.medicine.mcgill.ca/epidemiology/Joseph/Bayesian-Software-Diagnostic-Testing.html), which implements the methods of Joseph et al. (1995). Briefly, the method operates as follows: the probability of landing in each cell in a two by two table of data such as those given in Tables 1, 2 and 4 depends on the prevalence (π), and the sensitivities (S_1, S_2) and specificities (C_1, C_2) of the two tests. For example, in order to land in the first cell, the subject must either be truly positive, and have both tests correctly identify this true positive (which occurs with probability $\pi S_1 S_2$), or be truly negative, and have both tests incorrectly identify the subject as positive (which occurs with probability $(1-\pi)(1-C_1)(1-C_2)$). Calculating these probabilities across all four cells in each table, and raising each term to the number of subjects falling into that cell provides the likelihood function for that table of data. As in all Bayesian approaches, this likelihood function must be multiplied by the joint prior distribution over all unknown parameters, which by Bayes Theorem gives the posterior distribution from which all statistical inferences including 95% credible intervals (Bayesian analogue of standard confidence intervals) follow. At the first stage, we used uniform

Table 2
Summary of the diagnostic characteristics of the coproantigen ELISA assay (CpAg ELISA) used for the development of prior distribution of this test

	Necropsy results		Total (n = 200)
	Positive (n = 37)	Negative (n = 163)	
CpAg ELISA (+)	29 (78.4%)	11 (6.7%)	40 (20%)
CpAg ELISA (−)	8 (21.6%)	152 (93.3%)	160 (80%)

Necropsy was considered the gold standard method (Benito and Carmena, 2005).

Table 3
Results of the diagnostic tests for the detection of *Echinococcus granulosus*, according to the sheep-dog origin

	Coproparasitological exam[a] ($n = 726$)		Serum antibody ELISA ($n = 754$)		Coproantigen ELISA ($n = 721$)	
	Positive	Negative	Positive	Negative	Positive	Negative
Northern region	21 (7.1%)	273 (92.9%)	29 (9.0%)	294 (91.0%)	47 (13.7%)	295 (86.3)
Central region	49 (12.7%)	336 (87.3%)	29 (7.9%)	339 (92.1%)	44 (13.6%)	280 (86.4%)
Southern region	5 (10.6%)	42 (89.4%)	11 (17.5%)	52 (82.5%)	10 (18.2%)	45 (81.8%)
Total	75	651	69	685	101	620

[a] Family Taeniidae only.

prior distributions over the properties of the ELISA tests, but informative priors (as described above) for the necropsy properties, in order to estimate the ELISA test parameters. At the second stage, we took the results (posterior distributions for the sensitivities and specificities of both ELISA tests) from the first stage to form prior distributions as inputs to the analysis of the data in Table 3. Throughout all analyses, uniform prior distributions were used for the prevalences.

The Normal approximation to the difference between two binomial proportions was used to estimate the differences between the results of the diagnostic tests and the sex and origin of the sheep-dogs. Results derived from the dog's origin were dichotomized into South versus non-South regions, as sheep-dogs from the Southern region were expected to bear higher *E. granulosus* infection rates (see Section 4). Nonparametric Spearman's ρ was calculated to measure the degree of association between the Ab and CpAg ELISA results, and to evaluate whether *Echinococcus* coproantigen OD values are related to dog age.

3. Results

3.1. Coproparasitological examination

A total of 726 dog stool samples were obtained for coproparasitological exam. The presence of intestinal helminths were recorded in 416/726 (57.3%) of faecal samples after laboratory examination by at least one of the concentration methods used. Taeniid eggs were found in 75/726 (10.3%) of the examined samples (Table 1). Neither dog sex (difference (D) = 0.01, 95% CI: −0.03 to 0.06) nor dog region of origin ($D = -0.003$, 95% CI: −0.10 to 0.09) seem to have an effect on the copro-prevalence of members of the family Taeniidae. Other helminth species found were *Trichuris vulpis* (38.3%), *Uncinaria stenocephala* (26.3%), *Toxocara canis* (5.2%), *Toxascaris leonina* (1.4%), *Capillaria* spp. (0.5%), and *Ancylostoma caninum* (0.3%). No important differences were found between the detection of enteroparasite eggs and the kind of concentration method used, except in the cases of *T. vulpis* ($D = 0.55$, 95% CI: 0.47–0.62) and *T. canis* ($D = 0.75$, 95% CI: 0.57–0.93), which were better detected by sedimentation.

3.2. Immunodiagnostic assays

The Ab ELISA was assessed against 754 dog serum samples of which 69 (9.1%) tested positive for *E. granulosus* specific antibodies. Of 721 dog faecal supernatants assayed for *E. granulosus* coproantigens, 101 (14.0%) were positive (Table 1). No important differences were found between the *Echinococcus* prevalence rates obtained by Ab ELISA ($D = -0.002$, 95% CI: −0.05 to 0.04) or CpAg ELISA ($D = 0.005$, 95% CI: −0.05 to 0.06) and dog sex. When tested by both Ab or CpAg ELISA assays, sheep-dogs from cattle farms of the Southern region showed higher *E. granulosus* infection rates than those from the rest of the province, although these results are accompanied by wide confidence intervals (Ab ELISA – $D = -0.09$, 95% CI: −0.19 to 0.01; CpAg ELISA – $D = -0.05$, 95% CI: −0.16 to 0.07). Taking together, these results show that CpAg ELISA detects a 4–42% more positive samples than Ab ELISA, depending on the dog region of origin.

Matched samples (coproparasitological examination with respective blood and stool specimens) were obtained from a total of 666 dogs. A weak statistically significant correlation (Spearman's $\rho = 0.10$, 95% CI: 0.02–0.18) between the Ab and CpAg ELISA tests was found (Fig. 2). Forty-one dogs (6.1%) tested positive for *E. granulosus* by both immunoassays. Eighteen dogs (2.7%) were Ab ELISA positive but CpAg ELISA negative, and 56 (8.4%) animals were CpAg ELISA positive but failed to show specific antibody levels (Table 4). Taking together, these data indicate that 115 (18.2%) sheep-dogs tested positive for *E. granulosus* on

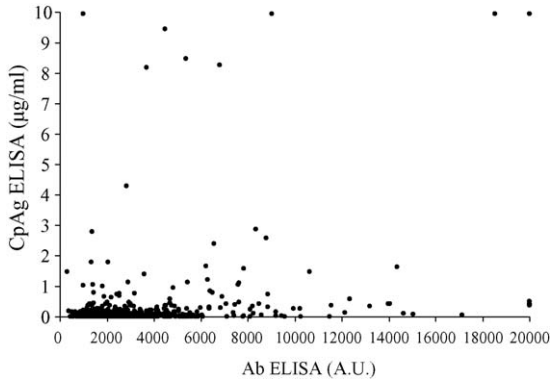

Fig. 2. Correlation between *Echinococcus granulosus* antibody and coproantigen OD values obtained by ELISA ($n = 666$). Nonparametric Spearman's $\rho = 0.103$ (95% CI: 0.023–0.178).

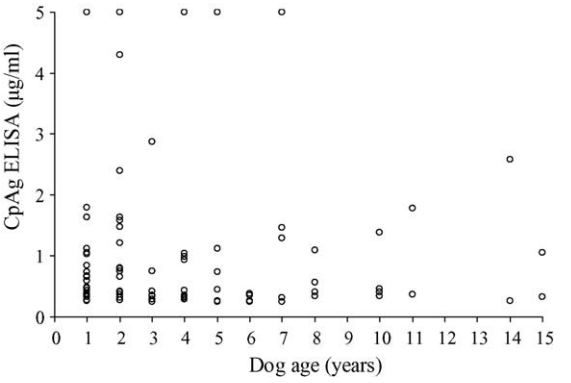

Fig. 3. Correlation between positive *E. granulosus* coproantigen OD values and sheep-dog age from cattle farms of the province of Álava ($n = 101$). Nonparametric Spearman's $\rho = -0.049$ (95% CI: −0.234 to 0.135).

Table 4
Comparison of antibody (Ab) and coproantigen (CpAg) ELISA assays for the detection of *E. granulosus* in the studied sheep-dog population ($n = 666$)

	Ab ELISA (+)	Ab ELISA (−)	Total
CpAg ELISA (+)	41 (6.1%)	56 (8.4%)	97 (14.6%)
CpAg ELISA (−)	18 (2.7%)	551 (82.7%)	569 (85.4%)

at least one of the immunoassays. Using a Bayesian statistical approach that adjusts for the imperfect sensitivities and specificities of both ELISA tests, the prevalence of *E. granulosus* infection in this sheep-dog population was estimated to be 8.0% (95% credible interval: 5.4–11.4%). Diagnostic performance characteristics of both Ab and CpAg ELISA assays in the studied dog population are shown in Table 5.

Table 5
Bayesian estimation of the diagnostic characteristics of antibody (Ab) and coproantigen (CpAg) ELISA assays in the studied sheep-dog population, assuming necropsy is an imperfect test with 95% sensitivity and 98% specificity

	Mean	S.D.	95% Credibility interval
Ab ELISA			
Sensitivity	0.822	0.078	0.648–0.942
Specificity	0.951	0.007	0.942–0.971
Positive predictive value	0.627	0.062	0.500–0.745
Negative predictive value	0.983	0.009	0.958–0.996
Positive likelihood ratio	20.28	4.291	13.26–30.05
Negative likelihood ratio	0.184	0.082	0.052–0.367
CpAg ELISA			
Sensitivity	0.870	0.046	0.769–0.948
Specificity	0.922	0.012	0.898–0.946
Positive predictive value	0.492	0.076	0.354–0.657
Negative predictive value	0.987	0.005	0.975–0.995
Positive likelihood ratio	11.5	2.114	8.308–16.55
Negative likelihood ratio	0.140	0.050	0.055–0.250

When the relationship between the CpAg ELISA OD values and the dog age was analyzed, no significant linear correlation (Spearman's $\rho = -0.05$, 95% CI: −0.23 to 0.13) could be demonstrated, and high *E. granulosus* coproantigen levels were detected even in dogs aged 10 years or more (Fig. 3). This seems to indicate that there were not differences in the prevalence of *E. granulosus* between old and young dogs.

4. Discussion

Determining the rate of infection and mean abundance in dogs is probably the best index of the degree of transmission of *E. granulosus* in a local region (Craig et al., 2003; Jenkins et al., 2000), a fact that is essential for the establishment of baseline data on prevalence, and in surveillance of hydatid control programmes in endemic areas (WHO/OIE, 2001). In the province of Álava (northern Spain), we previously found a low prevalence (0.5%, 5/1040) of *E. granulosus* infection in dogs at necropsy, although the majority of the dogs studied in that survey came from urban environments (Benito et al., 2003). Prevalences ranging from 0.2 to 1.3% have also been reported in dogs from diverse origins in the adjacent Autonomous Communities of Navarra (Gobierno de Navarra, 1987) and La Rioja (Jiménez et al., 2002).

Bayesian methods have become a powerful statistical tool for the analysis of parasitological data and accurate estimation of levels of infection endemicity (Basáñez et al., 2004). In the present study, a large sheep-dog population from the province of Álava has

been investigated by using coproparasitological and immunoenzymatic assays in order to determine the *E. granulosus* infection rate. Following a Bayesian approach which assumes necropsy is an imperfect test with sensitivity assumed to be in the range from 95 to 100% and specificity assumed to be in the range from 98 to 100%, the estimated parasite prevalence in the studied sheep-dog population was 8.0%. A very similar prevalence of 8.4% (data not shown) was also calculated assuming necropsy is a perfect test with 100% sensitivity and specificity, a feature that demonstrates the robustness of our estimates to the choice of prior distribution. This prevalence value is 6–40-fold higher than previously reported in this province (Benito et al., 2003), and in the bordering Autonomous Communities of Navarra (Gobierno de Navarra, 1987) and La Rioja; (Jiménez et al., 2002). As expected, these data are in agreement with those obtained in other previous surveys (Buishi et al., 2005; Shaikenov et al., 2003), demonstrating that shepherd dogs are the main dog class involved in the (domestic) transmission dynamic of *E. granulosus*.

In our work, the coproparasitological survey revealed the presence of taeniid eggs in 10.4% (75/726) of the stool samples analyzed, an elevated rate taking into account that this methodology considerably underestimates the true prevalence of the infection (Jenkins et al., 2000). When matched samples were assayed using both Ab and CpAg ELISA assays, a weak but significant correlation between the test results could be demonstrated (Spearman's $\rho = 0.10$, 95% CI: 0.02–0.18). However, 56 (8.4%) sheep-dogs which were CpAg ELISA positive have undetectable *E. granulosus* specific antibody levels. Very likely many of these results correspond to CpAg ELISA false positive reactions. It is well-known that members of the family Taeniidae are a common source of cross-reactivity in the immunodiagnosis of intestinal dog echinococcosis (see Carmena et al., 2006). This could be the case in the present survey, where an elevated rate (10.4%) of taeniid eggs was detected by coprological examination. On the other hand, taking into account that a true positive CpAg ELISA result is indicative of current infection (Fraser and Craig, 1997), the seronegativity of those dogs may suggest a recent infection which still has not elicited a specific antibody response. This phenomenon may also be due to the sequestration of antibodies and the formation of circulating immunocomplexes (Gasser et al., 1993; Spinelli et al., 1996), or to immune evasion mechanisms of the parasite (Gasser et al., 1994), or to a low immune response of the host (Gasser et al., 1993, 1994). Host nutritional status has also been suggested to have an impact on the antibody levels (Jenkins et al., 1991). Eighteen (2.7%) sheep-dogs were Ab ELISA positive but CpAg ELISA negative. This finding may indicate the possibility of recent exposure, with previous infection eliminated spontaneously or by owners with anticestodal drug treatment. Because specific serum antibody levels can persist for several months after the worms have been removed (Gasser et al., 1990), this fact may be an important cause of false-positive reaction in the serodiagnosis of the disease.

CpAg ELISA showed a higher diagnostic sensitivity than Ab ELISA (87.0 and 82.2%, respectively). A similar result has been previously reported by Craig et al. (1995). Both Ab and CpAg ELISA assays showed a very high negative predictive value (98.3 and 98.8%, respectively), a characteristic that make them specially suited for the mass-screening of dog populations with low prevalence of *E. granulosus*. However, the relatively low positive predictive values of these techniques (62.7% for the Ab ELISA, and 49.2% for the CpAg ELISA) strongly recommend their simultaneous use in epidemiological surveys, in order to reduce the risk of false positive results. In an scenario of low-medium parasite prevalence like the one described in this study, the superior sensitivity of the CpAg ELISA, together with its ability to estimate the current status of the infection and the parasite burden, make this assay the immunodiagnostic test of choice for the detection of *E. granulosus* in the definitive host. However, confirmation of Ab and/or CpAg ELISA positive results by detection of *E. granulous* DNA in faeces using PCR is highly desirable (Deplazes et al., 2003).

Dog sex did not appear to influence the prevalence of *E. granulosus*, independently of the diagnostic test used or the dog region of origin. Concerning dog origin, the Southern region was found to bear the highest parasite rates by the three techniques used. Although statistically inconclusive, this result was predictable taking into account that the geoclimatic characteristics of this area present favorable conditions for the dynamic transmission of the infection. In addition, this region borders the Autonomous Communities of Navarra and La Rioja, where the presence of the parasite infection in dogs has been previously reported (Gobierno de Navarra, 1987; Jiménez et al., 2002).

In recent years a number of field surveys based on arecoline purgation or necropsy have been conducted in Eastern Tibetan China (Budke et al., 2005), Kazakhstan (Torgerson et al., 2003), and Tunisia (Lahmar et al., 2001) in order to determine the abundance and prevalence of *E. granulosus* in dogs and to investigate

the transmission dynamic of the parasite. These studies showed a pattern of age related infection, with young dogs bearing the highest abundance and prevalence rates, whereas older animals had lower parasite burdens and prevalence. Similar results have also been reported in naturally *E. multilocularis* infected foxes in high endemic areas of Germany (Tackmann et al., 2001) and Switzerland (Hofer et al., 2000). However, other studies have failed to demonstrate a difference in the *E. multilocularis* prevalence rates in dogs or foxes of different ages (Budke et al., 2005; Tackmann et al., 2001). It has been suggested that the pattern of age related infection may be due to the development of a host protective immune response against the *Echinococcus* reinfection with dog age. The use of mathematical models can provide insight into our understanding of this phenomenon. Thus, in a high endemic region of the Tibetan plateau (People's Republic of China) a model assuming the presence of host's immunity was the best fit for the *E. granulosus* natural infection in dogs (Budke et al., 2005). However, the same model was not valid for the *E. multilocularis* infection in the same area. In Kazakhstan Torgerson et al. (2003) reported that farm dogs with an *E. granulosus* prevalence rate of 23% showed a clear age related infection pattern, whereas a village dog population with lower prevalence rate of 5.8% (similar to the estimated *E. granulosus* prevalence of 8.0% in our study) failed to demonstrate any decrease in the mean prevalence in older dogs. Using the same abundance model of Budke et al., the authors found that assuming the presence of immunity was the best fit for farm dogs, but not for village dogs. Taken together these data provide evidence that in conditions of high infection pressure the acquired host protective immune response is the most likely explanation of the observed pattern of age related infection (Torgerson, 2006).

In a recent study carried out in a high endemic region of northwest Libya, Buishi et al. (2005) reported a prevalence of *E. granulosus* of 25.8% in stray dogs by necropsy and of 21.6% in owned dogs by CpAg ELISA. A significant inverse correlation between the *E. granulosus* CpAg ELISA OD values and the dog age was found, with dogs aged 3 years or less seeming to bear the heaviest adult parasite burdens, whereas intensity of the infection seemed to decrease with the dog age. In our study no significant linear correlation (Spearman's $\rho = -0.05$, 95% CI: -0.23 to 0.13) could be found between CpAg ELISA OD values and dog age, suggesting that there were no differences in the prevalence of *E. granulosus* between old and young dogs. As discussed before, this situation may be a consequence of a low parasite infection pressure, where an insufficient number of worms are not able to stimulate the host specific immune response (Torgerson, 2006). Other possible explanations may be the inability of some dogs to develop a host protective immune response against the challenging infection, or the fact that some animals were primary infected at an advanced age.

In conclusion, we have shown that the overall prevalence of *E. granulosus* in sheep-dogs in the province of Álava is 8.0%, a rate that represents a public health threat. This finding is in line with previous investigations, corroborating that sheep-dog is the dog class most vulnerable to be infected by *E. granulosus*. The results indicate that the studied sheep-dog population has access to infected viscera, being home slaughtering the most probable cause of maintaining the infection. Under this situation, a control program based on regular treatment of the dogs with praziquantel should be initiated.

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