

ECHINOCOCCUS MULTILOCULARIS COPROANTIGEN DETECTION BY ENZYME-LINKED IMMUNOSORBENT ASSAY IN FOX, DOG, AND CAT POPULATIONS

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ABSTRACT: A sandwich enzyme-linked immunosorbent assay (ELISA) for the detection of *Echinococcus multilocularis* coproantigens (EM-ELISA) was developed with polyclonal rabbit (solid phase) and chicken egg (catching) antibodies that were directed against *E. multilocularis* coproantigens and somatic worm antigens, respectively. In experimentally infected dogs and cats, coproantigens were first detectable 6–17 days postinfection (PI) in samples of 8 dogs (worm burdens at necropsy: 6,330–43,200) and from 11 days PI onward in samples of 5 cats infected with 20–6,833 worms. After anthelmintic treatment of 4 dogs and 5 cats at day 20 PI, coproantigen excretion disappeared within 3–5 days. The sensitivity of the ELISA was 83.6% in 55 foxes infected with 4–60,000 *E. multilocularis*, but reached 93.3% in the 45 foxes harboring more than 20 worms. The EM-ELISA was used in surveys of “normal” dog and cat populations in Switzerland. Among 660 dogs and 263 cats, 5 dogs and 2 cats exhibited a positive reaction. In 2 of these dogs (0.30%) and 1 cat (0.38%), intestinal *E. multilocularis* infections were confirmed by necropsy, polymerase chain reaction PCR, or both. The specificities of the ELISA in these groups were found to be 99.5% and 99.6%, respectively, if positive ELISA results that could not be confirmed by other methods were classified as “false positive” reactions.

Echinococcus multilocularis, a tapeworm inhabiting the small intestine of carnivorous mammals, is the causative agent of alveolar echinococcosis, one of the most lethal helminthic infections of humans. The parasite is endemic in a large belt of the northern hemisphere stretching from North America through northern Europe, Russia, and southern Asia to China and Japan (Rausch, 1995; Schantz et al., 1995; Eckert, 1998). The sylvatic cycle of *E. multilocularis* predominantly involves foxes (genera *Vulpes* and *Alopex*) as definitive hosts and many species of rodents as intermediate hosts (Schantz et al., 1995). Domestic dogs and cats can also act as definitive hosts, but foxes are thought to be the main sources of environmental contamination with eggs of *E. multilocularis* in most of the endemic areas (Schantz et al., 1995; Eckert and Deplazes, 1997).

An accurate determination of the prevalence of *E. multilocularis* in populations of final hosts is an essential requirement for establishing epidemiological baseline data and for estimating the potential infection risk for humans (Eckert, 1998). Currently, the most reliable technique for the diagnosis of *E. multilocularis* infection in foxes and other definitive hosts is parasitological examination of the small intestine at necropsy. Until recently, methods for an accurate and sensitive identification or exclusion of the infection in living animals were not available. The standard purgation technique with arecoline hydrobromide routinely used for screening dog populations for *Echinococcus granulosus* is not applicable to foxes and cats. In dogs, this technique is hampered by its relatively low sensitivity (65.2% after 1 dose, 78.3% after 2 doses), and it is inefficient in up to 32% of the dogs that do not purge (Schantz, 1997). Moreover, the technique is biohazardous, labor intensive, and costly.

Recent developments in serum antibody, fecal antigen, and DNA detection for the diagnosis of intestinal infections with *E. granulosus* or *E. multilocularis* have provided alternatives to current techniques (reviewed by Craig et al., 1996; Deplazes and Eckert, 1996). In particular, the detection of parasite coproantigens by sandwich enzyme-linked immunosorbent assay (ELISA) has become a general focus of interest in the diagnosis

of intestinal *Echinococcus* infections in carnivores (Allan et al., 1992; Deplazes et al., 1992; Craig et al., 1995; Kohno et al., 1995).

In a previous publication, Deplazes et al. (1992) reported for the first time the detection of *E. multilocularis* coproantigens in fecal samples of foxes and dogs by a sandwich ELISA. However, the sensitivity of this test system designed for the detection of *E. granulosus* was not satisfactory. In the present paper, we describe the development and evaluation of a sandwich ELISA for the sensitive and specific detection of *E. multilocularis* coproantigens (EM-ELISA) and its use for parasite diagnosis in experimentally infected dogs and cats as well as in populations of domestic dogs, cats, and wild red foxes.

MATERIALS AND METHODS

Animals and samples

The numbers and sources of the fecal samples (FS) or intestinal contents (IC) and some characteristics of the materials are listed in Table I. For the experimental infections, 11 dogs (Niederlaufhund–Beagle crossbreeds, 9 females and 2 males, 1-yr-old) and 10 cats (European shorthair, female and males, 14–16 wk old) were raised under helminth-free conditions. Procedures for experimental infection, necropsy of infected dogs and cats, and worm recovery after necropsy were as described previously (Thompson and Eckert, 1983).

All FS or IC samples were mixed at a ratio of 1:4 (v/v) or 1:8 (v/v), respectively, with buffer solution (phosphate-buffered saline [PBS] containing 0.04% Na₂S₂O₃, 0.05% bovine hemoglobin [Fluka, Buchs, Switzerland], and 0.3% Tween-20 [PBS-Tween]). In order to kill *Echinococcus* eggs possibly present in the samples, FS and IC were frozen for at least 5 days at –80 C and then maintained at –20 C until examination. After centrifugation (3,000 g, 10 min, room temperature), the supernatants of the samples were used for the ELISA.

Parasite antigens and antigen-IgG complexes

For the production of polyclonal antibodies, the following antigens were used. Excretory/secretory (E/S) antigens of preadult intestinal *E. multilocularis* stages were prepared according to Deplazes et al. (1992). Antigen of nongravid adult *E. multilocularis* worms (isolated from dogs 21 days PI) was prepared by freezing at –20 C in PBS and shaking the suspension after thawing (this procedure leads to the dissociation of the worm tegument). After the worms and larger particles were sedimented (1 g, 2 min, room temperature), the supernatant was ultrasonicated (60 sec, 40 W, 80% pulse), centrifuged (10,000 g, 30 min, 4 C), and used as antigen (=“worm antigen”).

Echinococcus multilocularis coproantigen-IgG complexes (cAg-IgG-

Received 15 April 1998; revised 21 July 1998; accepted 21 July 1998.

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TABLE I. Origin and characteristics of fecal samples (FS) or intestinal content (IC) used for the evaluation of the EM-ELISA.

Group	Species (no.) and origin of animals	No. and type of samples	Sample characteristics
1a	Dogs (100), Zurich area, Switzerland	100, FS	50 without helminthic infection, 50 with natural nematode infection and egg excretion: 36% <i>Toxocara</i> , 36% <i>Trichuris</i> , 24% hookworms, 2% <i>Toxascaris</i> , 2% <i>Capillaria</i>
1b	Stray dogs (43), Spain	43, IC	Single or multiple <i>Taenia</i> infection: 56% <i>T. pisiformis</i> , 44%, <i>T. hydatigena</i> , 2% <i>T. multiceps</i> (Deplazes et al., 1994)
1c	Stray dogs (23), Spain	23, IC	Natural <i>Echinococcus granulosus</i> infection: with 2 to >500 worms/animal: 13 positive for <i>E. granulosus</i> coproantigen (Deplazes et al., 1994)
1d	Dogs (9)	9, FS	Experimental infection with <i>E. granulosus</i> (Deplazes et al., 1992): all positive for <i>E. granulosus</i> coproantigen
1e	Dogs (6), Switzerland	6, FS	Natural infection with <i>Echinococcus multilocularis</i> (confirmed by necropsy or by PCR egg identification; Mathis et al., 1996)
2a	Dogs (11)	116, FS	Experimental infection with <i>E. multilocularis</i> (for worm burden found at necropsy, see Fig. 3)
2b	Cats (10)	10, IC; 125, FS	Experimental infection with <i>E. multilocularis</i> . FS without individual animal identification (for worm burden found at necropsy, see Fig. 4)
3a	Red foxes, (32), Switzerland	32, IC*	Free of <i>E. multilocularis</i> but infected with <i>Taenia</i> spp. (41%), <i>Mesocostoides</i> (12%), <i>Toxocara</i> (50%), hookworms (66%), <i>Trichuris</i> (13%), <i>Capillaria</i> (47%)
3b	Red foxes, (55), northeastern Switzerland	55, IC*	Infected with <i>E. multilocularis</i> (between 4 and approximately 60,000 worms per fox, 3 foxes harbored only immature stages (prepatent infection))
4	Dogs (661), northeastern Switzerland	661, FS†	Of different ages and breeds (151 farm dogs, 77 kept in kennel, 416 kept in households, 17 without further data, 80 of the totally examined dogs were hunting dogs). Coproscopic egg examination revealed 5.7% <i>Toxocara</i> , 3.6% <i>Trichuris</i> , 1.8% taeniid, 1.8% hookworms, 0.2% <i>Toxascaris</i> , 0.2% <i>Capillaria</i> infections
5	Cats (265), northeastern Switzerland	265, FS†	130 free-roaming animals, 135 kept in households. Coproscopic egg examination revealed 15% <i>Toxocara</i> , 4.1% taeniids, 1.9% <i>Capillaria</i> , 0.4% <i>Toxascaris</i> infections

* Samples were taken from the rectum 18 hr after being shot. Parasitological examination of the whole small intestine was performed by the sedimentation technique according to Mathis et al. (1996).

† All FS of dogs and cats were sent by the owners to the laboratory at room temperature within 4 days in buffer solution.

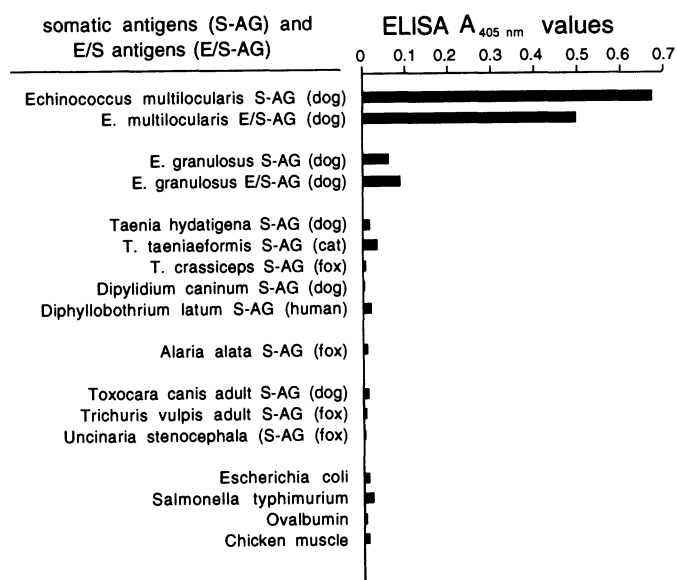


FIGURE 1. Specificity evaluation of the ELISA with 1 µg/well *Echinococcus* or other antigens in control fecal suspension (CFS). Ovalbumin and chicken muscle protein were tested at a concentration of 50 µg/well in CFS. The host origin of the parasites is given in parentheses.

c) included serum of a rabbit hyperimmunized with *E. multilocularis* E/S antigen that was diluted 1:4 with supernatant of a fecal sample collected at the end of the prepatent period (day 21 PI) from a dog experimentally infected with more than 10,000 *E. multilocularis* worms. The cAg-IgG-c suspension was affinity purified on a protein A-sepharose CL-4B column according to the manufacturer's instructions (Pharmacia Fine Chemicals, Uppsala, Sweden), and the eluted fraction was used as antigen.

All antigens used for the evaluation of the specificity of the sandwich ELISA (Fig. 1) were prepared according to Deplazes et al. (1990, 1991) and diluted in a control fecal suspension (CFS) from a helminth-free dog. The protein concentration of antigens and antibody fractions was assessed by a protein assay (Bio-Rad, Glattdbrugg, Switzerland) with bovine plasma gammaglobulin as standard.

Anti-*E. multilocularis* hyperimmunoglobulins

Rabbits were immunized by subcutaneous injection of 0.3 mg E/S antigen or 1 mg cAg-IgG-c of *E. multilocularis* in complete Freund's adjuvant (FA) and boosted with the same antigens 2 wk later with incomplete FA. The rabbit immunized with the cAg-IgG-c was boosted again 2 mo later with *E. multilocularis* adult antigen in incomplete FA. The rabbits were killed and bled 7 days after the last booster.

A 1-yr-old hen was immunized intramuscularly in the wing with 1 mg *E. multilocularis* worm antigen in complete FA and boosted with the same antigen 3 wk later with incomplete FA. Eggs laid before ("preimmune" eggs) and 1–2 mo after the immunization procedure were used as sources of secondary antibodies in the sandwich ELISA. The egg yolk was diluted 1:10 in distilled water, frozen for 24 hr at –20 C, thawed at 4 C, and centrifuged (5,000 g, 20 min, 4 C). An

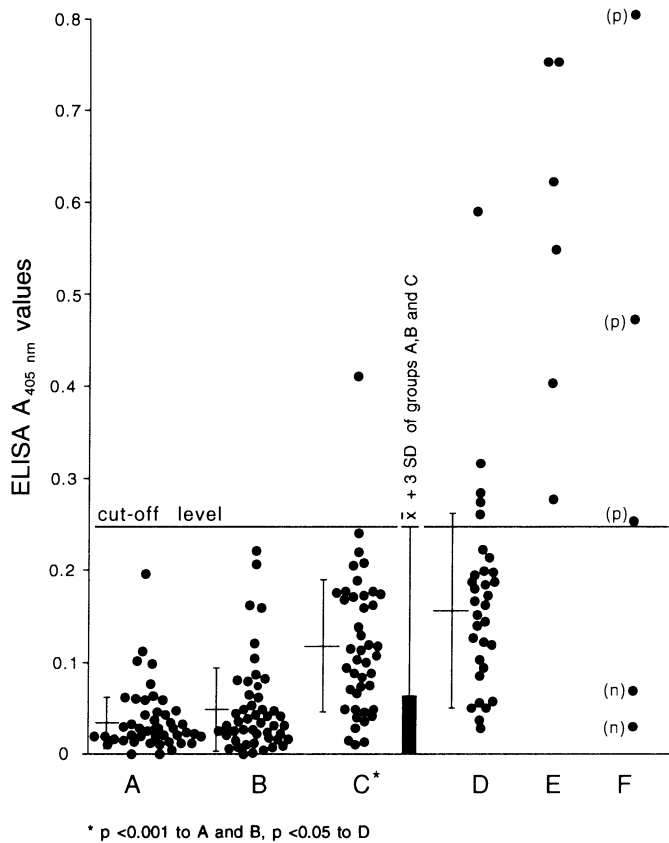


FIGURE 2. Determination of a cut-off value with fecal samples (FS) of groups A, B, and C. **A.** Fifty helminth-free dogs. **B.** Fifty dogs infected with nematodes. **C.** Forty-three dogs infected with *Taenia* spp. (1 strong false-positive reaction was excluded from the calculation). **D.** Cross-reactivity to *Echinococcus granulosus* coproantigens was tested with 23 intestinal contents of dogs with natural *E. granulosus* infection and 9 FS of experimentally infected dogs. **E.** FS of 6 dogs naturally infected with *Echinococcus multilocularis*. **F.** Three dogs experimentally infected with *E. multilocularis* and 2 samples of helminth-free dogs served as positive (p) and negative (n) controls, respectively. Means \pm 1 SD are given for the groups A–D.

antibody suspension in the supernatant (egg-IgG suspension) was used in the ELISA. Chicken antibodies were used because of the convenience of their production and their suitability in our test system as revealed by previous studies (data not shown).

Sandwich ELISA

The development of the sandwich ELISA for detecting *E. multilocularis* coproantigens was based on principles previously described for similar tests to diagnose *Taenia* or *E. granulosus* (Deplazes et al., 1990, 1991, 1992), but the following modifications were made. Protein-A-purified rabbit antibodies directed to *E. multilocularis* cAg-IgG-c were used as catching antibody. In parallel, purified IgG from a nonimmunized rabbit was used as a control antibody. ELISA plates (96-well, MaxiSorp, Nunc, Roskilde, Denmark) were coated with both antibodies (20 μ g protein/ml coating buffer, 100 μ l per well) overnight at 4 C. The plates were saturated (30 min, 37 C) with PBS-Tween containing 0.5% normal rabbit serum before use. Fecal supernatants of 100 μ l per well were added and incubated for 90 min at 37 C. The plate was washed and chicken IgG directed against *E. multilocularis* adult antigen (for the antigen-specific reaction) and IgG of “preimmune” eggs (for the control reaction) was added. The egg-IgG suspensions (diluted 1:200 in PBS-Tween) were incubated for 90 min. Visualization of the immune reaction was obtained by a further incubation (60 min) with goat anti-chicken IgG labeled with alkaline phosphatase (Fc Fragment,

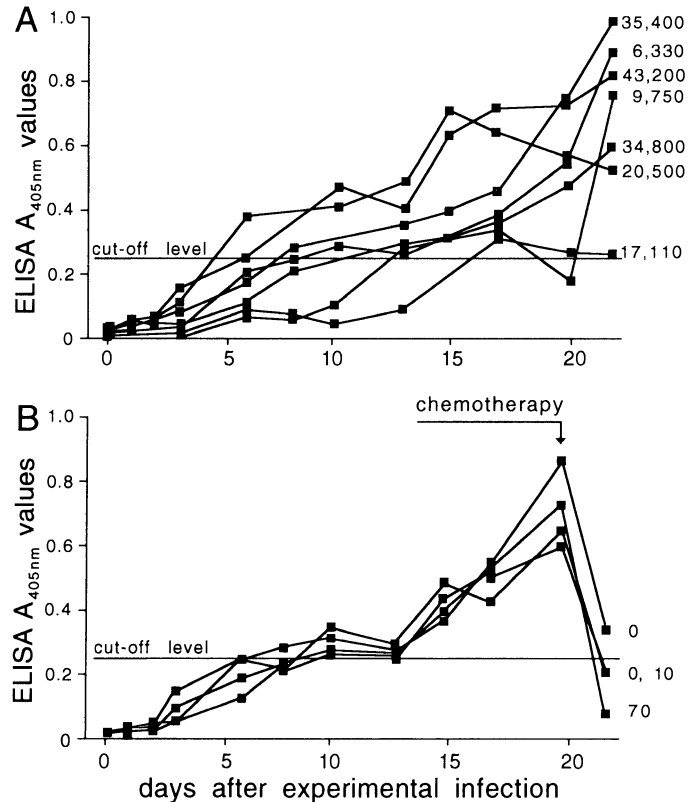


FIGURE 3. Follow-up coproantigen examination in 11 dogs during experimentally induced infection with *Echinococcus multilocularis*. Right-hand numbers indicate worm burdens found at necropsy on days 22 or 25, respectively. **A.** Seven untreated dogs. **B.** Four dogs treated on day 20 with epsiprantel (5.4 mg/kg body weight). The cut-off value was determined by calculating the mean A_{405nm} value + 3 SD of fecal samples of 142 *Echinococcus*-free dogs.

Bethyl Laboratories Inc., Montgomery, Texas, diluted 1:2,000 in PBS-Tween) and the corresponding chromogenic substrate solution (4-nitrophenyl phosphatase). Results were expressed as corrected A_{405nm} values (A_{405nm} value of the specific reaction minus A_{405nm} value of the control reaction) as described by Deplazes et al. (1992).

Cut-off determination and diagnostic parameters of the ELISA

Determination of cut-off values by calculating the mean A_{405nm} value + 3 SD of fecal samples or intestinal contents of *Echinococcus*-free dogs and foxes is shown in Figures 2 and 5. Mean A_{405nm} values of the different dog groups (Fig. 2) were compared by 1-way analysis of variance. Predictive values of the ELISA were calculated according to Lutz and Winkler (1995).

RESULTS

The specificity of the ELISA was first tested with 1 μ g/well parasite or bacterial antigens in CFS. Compared with the *E. multilocularis* antigens tested, the *E. granulosus* antigen showed only moderate cross-reactivity in the ELISA. The reactions with antigens from several other cestode species, a trematode species (*Alaria alata*), and various nematode species, as well as from bacteria and from chickens, were negligible (Fig. 1).

The diagnostic cut-off value for samples from dogs was determined by calculating the mean A_{405nm} value + 3 SD of fecal samples from 50 helminth-free dogs, 50 dogs with nematode

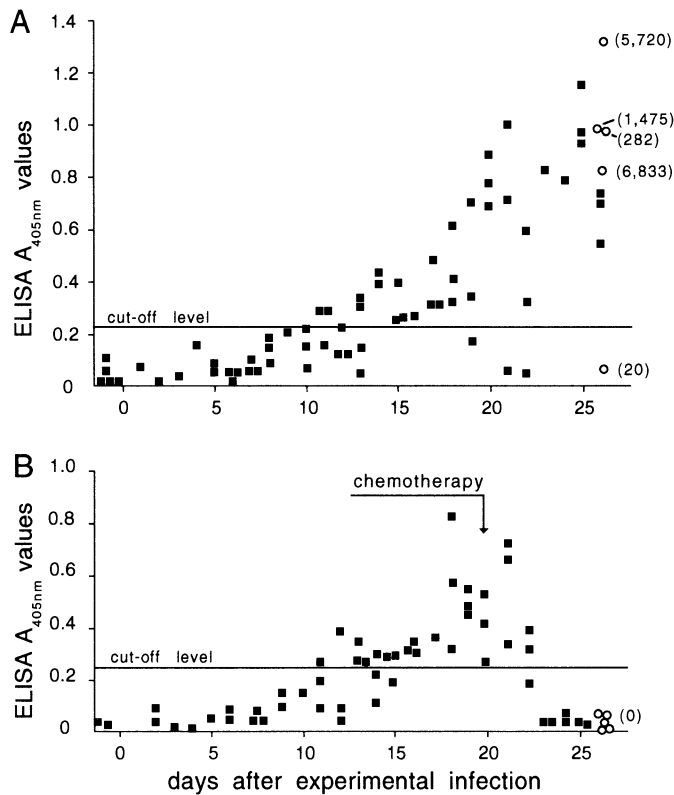


FIGURE 4. Follow-up coproantigen examination in 10 cats during experimentally induced infection with *Echinococcus multilocularis*. Solid symbols represent individual fecal samples of the cats without animal identification. Open symbols represent intestinal contents of the individual cats (number of *E. multilocularis* worms determined at necropsy is given in parentheses on the right-hand side). The cut-off value was determined by calculating the mean A_{405nm} value + 3 SD of fecal samples of 142 *Echinococcus*-free dogs.

infections, and 43 dogs with naturally acquired *Taenia* infection (Table I, groups 1a and 1b). The results are presented in Figure 2. One strongly cross-reacting sample of the *Taenia*-infected group was regarded as false positive and excluded from the cut-off calculation. On the other hand, some cross-reactivity (16%) was observed with samples of 32 dogs naturally or experimentally infected with *E. granulosus*. In comparison, fecal samples of 6 dogs naturally infected with *E. multilocularis*, as diagnosed by necropsy or egg identification by PCR (Mathis et al., 1996), were positive in the ELISA.

Coproantigen detection in dogs with prepatent *E. multilocularis* infection

The results obtained from 116 fecal samples of 11 dogs experimentally infected with *E. multilocularis* (Table I, group 2a) are shown in Figure 3. In all cases, the absorbance values of fecal samples collected prior to experimental infection (day 0) were low. Follow-up studies with the coproantigen ELISA revealed elevated absorbance values in fecal samples from day 3 after infection. With the use of the diagnostic cut-off value as determined in Figure 2, these reactions could be interpreted as specific from day 6 postinfection (PI) in 4 dogs and from day 10 in 9 of the 11 dogs. Two dogs were positive for the first time at days 13 and 17 PI, respectively. Only 1 dog had a

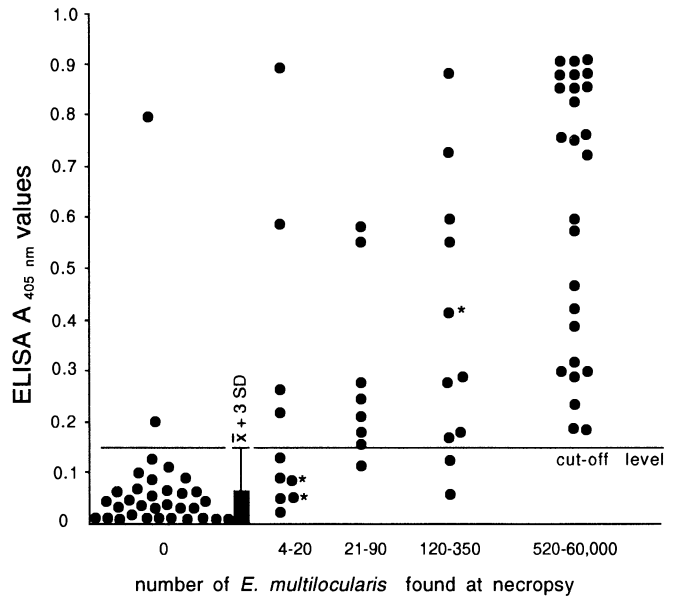


FIGURE 5. Detection of *Echinococcus multilocularis* coproantigens by ELISA in intestinal contents (IC) of 87 wild foxes of the Zürich area, 52 infected with variable numbers of gravid stages and 3 (*) with nongravid stages of *E. multilocularis*. The cut-off value for fox samples was calculated with 31 IC of *Echinococcus*-free foxes of the same population (1 strong false-positive reaction was excluded from the calculation).

subsequent negative coproantigen result at day 22 PI. Four dogs of the group were treated with epsiprantel (5.4 mg/kg body weight) at day 20 PI. Coproantigen values dropped below or close to the cut-off value within 2 days. Examination of the small intestine at necropsy on days 22–24 PI revealed burdens of 6,330–43,200 nonfertile *E. multilocularis* in 7 untreated dogs (Fig. 3A), whereas worm numbers in the 4 treated dogs ranged between 0 and 70 worms (Fig. 3B). In all cases, an accurate interpretation of the results was possible because of low A_{405nm} absorbance values in the control reaction. Overall, the sensitivity for the detection of coproantigens within the first 13 days PI in the 11 dogs was 32% (13 of 41 samples) but increased to 96% (24 of 25 samples) from day 15 until day 22.

Coproantigen detection in cats with prepatent *E. multilocularis* infection

The results obtained from 125 FS and 10 IC of 10 cats experimentally infected with *E. multilocularis* (Table I, group 2b) are shown in Figure 4. Because the cats were kept in groups of 5 animals, individual follow-up examinations were not possible. In both groups, specific reactions could be found from day 11 PI onwards. One group of 5 cats was treated on day 20 PI with 5.5 mg/kg epsiprantel (group 2b) and coproantigen values dropped below the cut-off value within 3 days after treatment (Fig. 4B). Examination of the small intestine at necropsy on day 26 PI revealed worm burdens between 20 and 6,833 in 5 untreated cats (Fig. 4A), whereas no worm was found in the treated cats (Fig. 4B). From 8 infected cats, coproantigens could be detected in 18 of 62 samples (29%) within the first 15 days PI and in 42 of 45 samples (93%) collected from day 16 PI until the end of the experiment or until the beginning of the chemotherapy (Fig. 4A, B). In 4 of the totally examined 135

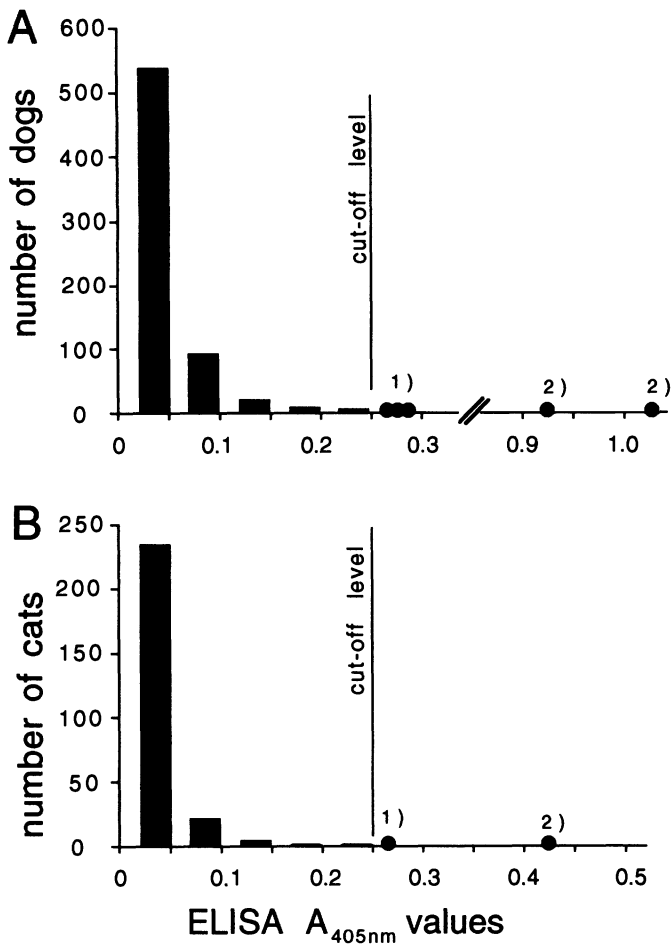


FIGURE 6. Frequency distribution of the *Echinococcus multilocularis* coproantigen ELISA A_{405nm} values. **A.** ELISA results of 660 fecal samples (FS) from dogs. **B.** ELISA results of 263 FS from cats. Dots represent single cases: 1) = *E. multilocularis* infection not confirmed, 2) = *E. multilocularis* infection subsequently confirmed by necropsy examination or egg characterization by PCR. The cut-off value was determined by calculating the mean A_{405nm} value + 3 SD of fecal samples of 142 *Echinococcus*-free dogs.

samples, an accurate interpretation of the results was not possible because of high A_{405nm} values in the control reaction (higher than 50% of the specific reaction).

Detection of *E. multilocularis* in populations of wild foxes and domestic dogs and cats

Fox population: Figure 5 shows the ELISA results of 87 foxes. The corresponding cut-off value calculated for this group (Table I, group 3a) was markedly lower than the value determined for samples of dogs (A_{405nm} value of 0.15 compared with 0.25 for samples from dogs in Fig. 2). Overall, the coproantigen prevalence was 55% as compared with the prevalence of 63% determined by the parasitological sedimentation technique. The overall diagnostic sensitivity was 83.6% in the 55 foxes infected with *E. multilocularis* but reached 93.3% in 45 foxes harboring more than 20 worms and dropped to 40% in 10 animals infected with less than 21 worms (Fig. 5). From a total of 208,300 *E. multilocularis* worms detected in the 55 infected

TABLE II. Predictive values of the *Echinococcus multilocularis* coproantigen ELISA for dog populations. Test parameters: specificity 99.5% as determined with a dog population of 658 samples, sensitivity 83.6% as determined with a fox population.

Predictive values	Prevalence of <i>E. multilocularis</i> in dogs			
	10%	1%	0.5%	0.1%
Negative predictive value	98.2	99.8	99.9	>99.9
Positive predictive value	94.9	62.8	45.7	14.3

foxes, 99.7% were found in foxes excreting *E. multilocularis* coproantigens.

Dog population: One of the fecal samples of 661 dogs (Table I, group 4) tested by ELISA for *E. multilocularis* coproantigens could not be interpreted accurately because of strong reactions with both specific and control IgG and was, therefore, excluded from the study. The frequency distribution of the ELISA values for the dog population is depicted in Figure 6A. Specific coproantigen reactions were found in 5 (0.76%) of the remaining 660 samples. In 2 of 3 samples with relatively low positive absorbance values, helminth eggs were not detected in a coproscopic examination. However, the third dog excreted *Toxocara* and taeniid eggs. The status of infection of these 3 dogs could not be investigated further. The fecal samples of the 2 remaining dogs with strong ELISA reactions contained taeniid eggs, and, in both cases, *E. multilocularis* infection could be confirmed by polymerase chain reaction (PCR) (Mathis et al., 1996). One of these dogs, a hunting dog, was examined after necropsy, and 250 gravid *E. multilocularis* were detected; the other dog was treated with praziquantel, and a coproantigen examination 4 days after therapy revealed a negative result. These 2 proven cases of *E. multilocularis* infection correspond to a prevalence of 0.30% in this population of 660 dogs.

The specificity of the ELISA in this group was found to be 99.5% if 3 positive ELISA results that could not be confirmed by other methods were classified as “false positive” reactions. The predictive values of the EM-ELISA for dog populations with *E. multilocularis* prevalence rates between 0.1% and 10% are presented in Table II.

Cat population: Examination of the first 50 fecal samples with the coproantigen ELISA revealed a comparable cut-off value as determined for the dog population (data not shown). Two of the totally examined 265 samples (Table I, group 5) from cats had to be excluded from the study because of a strong reaction with the control IgG. Frequency distributions of coproantigen levels determined with this cat population in the ELISA are depicted in Figure 6B. In 2 cases (0.76%), positive reactions were detected in the ELISA. One sample with a low positive reaction contained *Toxocara* eggs, but further investigations on the infection status of this cat could not be performed. The other sample with an elevated ELISA value was from a stray cat and contained few taeniid eggs. Five *E. multilocularis* worms, 1 gravid with fully developed, thick-shelled eggs, were detected at necropsy. These data indicate a prevalence of proven *E. multilocularis* infection of the total cat population of 0.38% or of 0.77% for the free-roaming cat population.

DISCUSSION

The high specificity of the described EM-ELISA is demonstrated primarily by the fact that a wide spectrum of antigens derived from nonechinococcal cestodes or other helminths did not induce significant levels of cross-reactivity. The low cross-reactivity to *E. granulosus* somatic or E/S antigens (Fig. 1) and the low rate of cross-reactivity of 16% with samples of 32 dogs infected with *E. granulosus* (Fig. 2) were surprising. Compared with other coproantigen tests, which were shown to be specific at the *Echinococcus* genus level (Deplazes et al., 1992; Craig et al., 1995), this EM-ELISA is a highly species-specific test system. The specificity of the EM-ELISA was determined in 2 surveys of "normal" dog and cat populations (Fig. 6) and was found to be very high (99.5% and 99.6%, respectively) if positive ELISA results that could not be confirmed by other methods were classified as "false positive" reactions.

Time courses of coproantigen excretion were investigated with fecal samples and intestinal contents of 11 dogs and 10 cats experimentally infected with *E. multilocularis*. Worm burdens at necropsy were high in dogs (6,330–43,200) and lower in cats (20–6,833). ELISA values were elevated from day 3 in dog samples and from day 8 in cat samples. However, specific reactions related to the diagnostic cut-off value could be detected in the dogs from day 6 PI and in cats from 11 days onward. With few exceptions, the ELISA values rose consistently during the experimental infection until the end of the experiment. The courses of coproantigen excretion in our experimental infections in dogs differed to some extent from experimental infections of foxes with *E. multilocularis*, where the ELISA values peaked before day 20 PI and subsequently tended to decrease (Nonaka et al., 1996). After chemotherapy, coproantigen excretion dropped below, or close to, the cut-off level within 2–3 days in 4 dogs and 5 cats. Coproantigen disappearance within 3–5 days after chemotherapy also was demonstrated recently in *Taenia hydatigena* and *E. multilocularis* infections (Deplazes et al., 1990; Sakashita et al., 1995; Nonaka et al., 1996).

The sensitivity of the EM-ELISA was relatively low within the first 15 days after experimental infection in dogs and cats but reached 96% in dogs and 91% in cats from day 15 onward. In a population of 87 randomly collected foxes, with the use of careful parasitological examination by the sedimentation technique (Mathis et al., 1996), the prevalence of *E. multilocularis* was found to be 63%. The mean diagnostic sensitivity of the EM-ELISA in these 55 infected foxes was 83.6% and is comparable with that achieved with the current standard technique (stereomicroscopic examination of intestinal smears at necropsy), which was 80% if compared with the sedimentation technique (Deplazes et al., 1997).

The calculation of the predictive values of the EM-ELISA (Table II) was based on *E. multilocularis* prevalences between 0.1% and 10%, which are realistic assumptions for dog populations in the endemic area of central Europe. Because the sensitivity could be determined only with a fox population, the predictive values for the test with dog samples must be regarded as preliminary. However, the very high negative predictive values indicate that the use of the EM-ELISA is especially suited for mass screening of definitive host populations with a low prevalence of *E. multilocularis*. In contrast, in such populations,

the positive predictive values of the EM-ELISA are relatively low. A correct diagnosis of an *E. multilocularis* infection is of special importance in individual domestic dogs and cats because the animals' owners are at particular risk of being exposed to infection. As a result, infected animals may have to be killed or treated under biohazard safety precautions (Eckert and Deplazes, 1997). Therefore, Deplazes et al. (1997) proposed that positive EM-ELISA results from dog samples should be confirmed further with the more laborious PCR assay (Mathis et al., 1996) for the detection of species-specific DNA derived from *E. multilocularis* eggs.

In certain epidemiological situations, relatively high prevalences of *E. multilocularis* have been found in domestic dogs (1–12%), e.g., in endemic foci of Alaska, China, France, and Japan (Schantz et al., 1995; Eckert, 1998). Under these conditions, domestic dogs may play a role in the transmission of the infection to humans. The EM-ELISA described in the present paper was used for a survey in eastern Switzerland with the aim of determining the prevalence of *E. multilocularis* in "normal" populations of dogs and cats. In the past, such studies could be based only on necropsy results, with the disadvantage that the animals examined represented a selected population. In our study, 5 of 660 dogs (0.76%) exhibited a positive reaction in the EM-ELISA, of which, 2 strong positive results were confirmed by necropsy, PCR, or both. Both dogs were dachshunds. One of these animals was a hunting dog known to be a successful mouse and vole catcher, and the other dog regularly ingested rodents that had been caught but not eaten by the cat of the pet owners. Thus, in the future, regular treatment should be considered for all dogs and cats with free access to rodents in an endemic area.

The role of dogs and cats as a potential source of human infection with *E. multilocularis* is poorly understood. Results of experimental studies revealed that cats appear to be less susceptible to *E. multilocularis* than dogs, with retarded parasite development and lower worm burdens (Crellin et al., 1981; Thompson and Eckert, 1983; Kamiya et al., 1985, 1986). However, in the present study, 3 of the 5 infected young cats harbored more than 1,000 worms, with only small differences in growth, development, and maturation of worms as compared with worms in dogs (data not shown). No information is available on the susceptibility of cats with immunosuppression, e.g., caused by viral infections. In recent years, prevalences of *E. multilocularis* in cat populations, as determined at necropsy, ranged between 0 and 5.5% in various endemic areas (Eckert, 1998). Considering the high number of domestic cats in countries where *E. multilocularis* is endemic, even a relatively low prevalence of 0.38%, as determined in the present study, may pose a certain infection risk for the human population.

Undoubtedly, the EM-ELISA, alone or in combination with PCR, represents a significant advance in the diagnosis of *E. multilocularis* infections in both living and dead animals. Furthermore, the EM-ELISA can also be used for testing fecal samples of carnivores collected in the field (P. Deplazes, unpubl. obs.). However, several factors, including stability of coproantigen under various environmental conditions, that might influence the test reaction have yet to be carefully evaluated. For further improvement and standardization of the coproantigen ELISA, there is a need to determine the nature of coproantigens of *E. multilocularis*. Such antigens or components of

them have been shown to be heat-resistant (Nonaka et al. 1996), and they may be predominant protein-, carbohydrate-, or lipid-rich molecules (Craig, 1997).

ACKNOWLEDGMENTS

Funding for this study was partially supplied by the Swiss Federal Health Office, Liebefeld-Berne, and by the Swiss National Science Foundation (grant 31 47031.96). Thanks are due to J. Skaggs for technical assistance and S. Hofer for supplying us with fecal samples of foxes.

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