

Absence of link between abortion and seropositivity of cystic hydatid disease in ewes and female goats in Turkey

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Summary

This study was conducted to test whether there is a statistically significant association between seropositivity to cystic echinococcosis and abortion in ewes and female goats from the Turkish provinces of Elazig (east Anatolia), Sanliurfa (south-east Anatolia) and Kayseri (Inner Anatolia) using the enzyme-linked immunosorbent assay (ELISA). A total of 20 of 133 sera (15.1%) from ewes and 5 of 101 sera (4.9%) from goats with a history of abortion gave seropositive results that were not significantly different ($p>0.05$) from these, 9.9% (10/101) were reported for ewes and 1.6% (2/122) for female goats without a history of abortion. Serological prevalence rates among those animals with a history of abortion were not significantly different from the control group. No positive association was established between seropositivity for cystic echinococcosis and abortion in ewes and female goats.

Keywords

Abortion, Anatolia, ELISA, Enzyme-linked immunosorbent assay, Ewe, Hydatid disease, Goat, Turkey.

Assenza di associazione tra aborto e sieropositività da idatidosi in pecore e capre in Turchia

Riassunto

Questo studio è stato condotto per verificare se vi è una associazione statisticamente significativa tra sieropositività per echinococcosi cistica e aborto in pecore e capre delle province turche di Elazig (est Anatolia), Sanliurfa (sud-est Anatolia) e Kayseri (Anatolia interna) utilizzando il enzyme-linked immunosorbent assay (ELISA). Su un totale di 133 sieri di pecora e 101 sieri di capra con una storia di aborto, rispettivamente 20 (pari al 15,1%) e 5 (pari al 4,9%) ha dato risultati sieropositivi che non erano significativamente differenti ($p>0,05$) rispetto a sieri di pecora e capra senza una precedente storia di aborto: 9,9% (10/101) nelle pecore e 1,6% (2/122) nelle capre. I tassi di prevalenza sierologica tra gli animali con una storia di aborto non erano significativamente differenti dal gruppo di controllo. Nessuna associazione positiva è stata stabilita tra sieropositività per l'echinococcosi cistica e l'aborto in pecore e capre.

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Parole chiave

Aborto, Anatolia, Capra, ELISA, Enzyme-linked immunosorbent assay, Idatidosi, Pecora, Turchia.

Introduction

Cystic echinococcosis or unilocular hydatidosis is a zoonotic parasitic disease that is caused by *Echinococcus granulosus*. The disease occurs throughout the world and causes considerable economic losses and public health problems in many, especially developing countries (principally in Africa and Asia). Domestic intermediate hosts (cattle, sheep and goats) are the principal reservoirs for the disease in humans in Turkey. In addition, the disease in sheep and cattle incurs significant economic losses to the meat industry through condemnation of the infected organs (11, 12). Many studies have been published on the distribution and prevalence of hydatidosis in livestock in Turkey and the high prevalence of hydatidosis has been reported in animals in Turkey. In sheep, seroprevalence rates ranged between 51.9% and 63.3%, while the prevalence rate was found to be 51.9% at autopsy. The prevalence in goats and cattle at autopsy were recorded as 22.1% and 39.7%, respectively (10, 11, 12).

Abortion is one of the major causes of economic losses in livestock. Although the risk of abortion depends on many factors, it is likely that infectious agents are one of the most important risk factors associated with abortions. A variety of parasitic agents, such as *Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis* spp. have been reported to cause abortion in livestock worldwide (4, 6). However, there are no published reports on the association between abortion and hydatid cyst infections in livestock.

Turkey is a major sheep-producing country with a breeding population of approximately 40 million head and ovine abortions is a major concern for farmers. The overall abortion rate amongst sheep flocks was reported as 15.6% and this rate was recorded as 10%, 18% and 7.3% in the east, south-east and Inner Anatolian regions, respectively (13). There are many infectious agents that cause abortion in

sheep, including *Chlamydia*, *Toxoplasma*, *Listeria*, *Brucella*, *Coxiella*, *Salmonella* and *Campylobacter* (1). However, we believe that certain disorders, such as hydatid cysts, that affect the liver can cause some idiopathic aborting in ewes.

In our study, we demonstrate that there is no statistically significant association between seropositivity to cystic echinococcosis and abortion in sheep and goats.

Materials and methods

Sample collection

Blood samples were collected from the jugular vein of animals and sera were stored at -20°C until use. A total of 133 sera from ewes that had aborted (aged between 2 and 8 years with 100 from the Akkaraman breed and 33 the Awassi breed) and 101 sera from aborted goats (aged between 2 and 6 years, of which 90 were cross-breeds and 11 of the Damascus breed) were collected. The sheep and goats had a recorded history of abortion. Blood samples were taken from animals in the laboratory after complaints of abortion. We also collected sera from ewes and female goats without a history of abortion; these included 101 ewes (aged between 2 and 8 years, with 77 from the Akkaraman breed and 24 from the Awassi breed) and 122 female goats (aged between 2 and 7 years, all of which were cross-breeds) as control groups. All samples were obtained from Elazig (east Anatolia), Sanliurfa (south-east Anatolia) and Kayseri (Inner Anatolia) in 2007. The animals were selected randomly from flocks in these regions. The average herd size was 250-300 animals. Samples were taken from animals that had aborted flocks. Blood samples were taken from animals to determine the cause of abortion (viral, bacterial and parasitological).

Antigen preparation for the enzyme-linked immunosorbent assay

We collected sheep hydatid cysts and used a method described by Williams *et al.* (14) to enrich antigen B (AgB) from hydatid cyst fluid (HCF) for the enzyme-linked immunosorbent assay (ELISA). Briefly, HCF was aspirated in

sterile conditions and examined microscopically for the presence of protoscoleces. HCF was then centrifuged at 2 000 g for 15 min to remove the protoscoleces and any other solid materials. The supernatant was dialysed overnight at +4°C against 0.005 M acetate buffer, pH 5.0 and then centrifuged at 15 000 g for 30 min at 4°C. The pellet was dissolved in 10 ml of 0.2 M phosphate buffer saline (PBS), pH 8.0, and boiled in a water bath for 15 min. After centrifugation at 20 000 g for 1 h at +4°C, the precipitate pellet was discarded and the supernatant containing AgB was measured for protein concentration using a method described by Lowry *et al.* (7). The sample was aliquot and stored at -20°C until use.

Control sera for the enzyme-linked immunosorbent assay

Positive control sera obtained from a sheep and a goat that had been diagnosed with hydatidosis upon post-mortem examination was positive in the immunofluorescent assay (IFA) (1/512) and IgG-ELISA (1/2 560). Negative control sera were obtained from uninfected animals which were negative in the IFA (1/4) and IgG-ELISA (1/20) and also upon post-mortem inspection.

Optimisation of the enzyme-linked immunosorbent assay procedure

The test was optimised by performing checkerboard titration for partially purified AgB antigen in combination with a positive and a negative control serum for each sheep and goat. Various coating buffers (50 mM PBS, pH 7.5, 50 mM carbonate buffer, pH 9.6 and 50 mM carbonate/bicarbonate buffer, pH 9.6) and a number of blocking reagents (the PBS contained Tween-20, bovine serum albumin and non-fatty milk powder) were also tested. All washing steps were performed with distilled water containing 0.05% Tween-20. The optimised ELISA procedures were as described below.

Enzyme-linked immunosorbent assay

The test was performed essentially as described by Simsek *et al.* (11) with minor modifications. Briefly, 10 µg/ml of antigen was coated onto the wells of ELISA plates

(Dynatech Laboratories, El Paso, Texas) in a carbonate/bicarbonate buffer, pH 9.6, and remained overnight at +4°C. After thorough washing (PBS containing %0.1 Tween-20 [PBST]), the wells were blocked with 5% (w/v) of skimmed milk in PBS (pH 7.2) for 2 h. Sera diluted 1:200 were added after two washes with PBST. The plates were incubated for 2 h at 37°C. Peroxidase conjugated antibodies against sheep IgG (Sigma A3415, St Louis, Missouri) and against goat IgG (Sigma A5420, St Louis, Missouri) were diluted 1:1 000 and added to each of the wells to test the sheep and goat IgG, respectively, after 5 washes with PBST. After 2 h incubation at 37°C and further washing to remove unbound conjugate, the amount of enzyme bound was assayed using an o-phenylene diamine and hydrogen peroxide in citrate/PBS as substrate. Absorbance values were read at 450 nm using an ELISA reader (Medispec ESR 200) after 15 min incubation at room temperature. The cut-off value was calculated as the mean of the negative control sera absorbance values plus 2 standard deviation.

Sensitivity and specificity of the test

To determine the sensitivity of the assay, 48 serum samples were obtained from sheep that had been naturally infected with cystic echinococcosis at an abattoir in Elazig which is an endemic region in Turkey. Blood samples were collected just before slaughter and transferred at +4°C to the laboratory where they were centrifuged, aliquoted, and stored at -20°C until use. These sheep were examined for the presence of hydatid cysts in internal organs (liver, lung, heart and spleen). The type and location of cysts, as well as the presence of other parasites, were recorded post mortem. Organs in which the presence of cysts was recorded during post-mortem inspection and stool samples from each animal were collected for parasitological examination.

To determine specificity, 48 sera samples were obtained from sheep that did not present hydatid cysts at slaughter. These sheep were also monitored during the dressing and evisceration procedures for offal and carcass inspection. When detected, other parasites

were recorded post mortem and stool samples from each animal were collected for parasitological examination (11).

Statistical analysis

The data were analysed with Statistical Package for Social Sciences software (SPSS, Version 11.0). The Chi-square test was performed to confirm the differences observed between groups. For all statistical analyses, a significance level (*p*) of <0.05 was used to reject the null hypothesis.

Results

A total of 234 sheep and 223 goats were examined by indirect-ELISA and 30 (12.8%) sheep and 7 (5.7%) goats were revealed as being seropositive.

Hydatidosis prevalence was 15.1% in aborted sheep while this rate was 9.9% in control sheep. There was no significant difference between sheep that had aborted and those that had not (*p*>0.05). A similar situation was observed in goats where seropositivity was 4.9% in aborted goats, whereas the rate was 1.6% in goats that had not aborted (*p*>0.05). All results are summarised in Table I.

There was no statistically significant relationship between seroprevalence, breed and age groups of the animals (*p*>0.05).

Discussion

Hydatidosis is one of the most invasive zoonotic helminth infections but it has been difficult to establish an accurate prevalence status in intermediate hosts. This is partly due to optimisation difficulties of the available

diagnostic tests and the high costs of performing these tests in field conditions. For this reason, most prevalence studies have relied on slaughter data (12). Currently, ELISA and immunoblotting are the most reliable tests for serological and diagnostic purposes, although their accuracy is largely dependent on the quality of the antigenic source used. Hydatid cyst fluid has been the antigenic extract of choice for primary immunodiagnosis of the disease; this is mainly based on the detection of antigen B (5, 10, 11). The use of purified antigenic fractions in highly sensitive techniques has obviated cross-reactions with most of the other infections (8). Partially purified HCF of sheep was used as a source of antigen in the ELISA in our study.

The most common organ in which hydatid cysts are observed is the liver (75%) followed by the lung (15%) and the remainder of the body (10%). The involvement of the genital tract is rare and the occurrence in the uterus is very rare (3). Despite the fact that there are many reports on uterine hydatid cyst in women, there are no detailed studies on uterine hydatid cysts in livestock (2). This is the first report of hydatid serology in aborted ewes and female goats. However, higher seropositivity rates were detected in aborted ewes and goats than in the control group (15.1% in aborted ewes and 9.9% in control ewes; 4.9% in aborted female goats and 1.6% in control goats) but no statistical difference was detected among groups (*p*>0.05). Aborted ewes and goats may not have uterine hydatid cysts but we believe that chronic disease can cause sequels that can include low mineral and protein supply to the bone marrow as a result of extensive liver damage, a characteristic of

Table I
Percent seropositivity to cystic echinococcosis in aborting and non-aborting sheep and goats in Turkey

Species	Aborted			Healthy parturient (controls)		
	No. examined	No. infected	Percent seropositive	No. examined	No. infected	Percent seropositive
Sheep	133	20	15.1 ^(a)	101	10	9.9 ^(a)
Goat	101	5	4.9 ^(b)	122	2	1.6 ^(b)

a) values with the same letters on the same line are not significantly different (*p*>0.05, χ^2 :1.35)

b) values with the same letters on the same line are not significantly different (*p*>0.05, χ^2 :1.99)

chronic hydatidosis Furthermore, this effect can cause hormonal change and abortion in livestock. Furthermore, it should be kept in mind that during pregnancy, the cellular immune status of the host is weakened and there is some evidence that this may favour the proliferation of hydatid cysts in infected hosts (9).

Conclusions

Further studies on the effects of hydatidosis on abortions in livestock need to be conducted. In particular, experimental studies will assist in

the understanding of hydatidosis and its relation to abortion in livestock.

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