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Research Article

A comparative study of sheep infected with *Echinococcus granulosus* and *Taenia hydatigena* using antigenic based ELISA and postmortem analyses

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Abstract

The present work was performed to examine the presence of cystic *Echinococcus granulosus* and *Taenia hydatigena* in sheep using antigenic based indirect enzyme-linked immunosorbent assay (iELISA) and postmortem analyses. For this purpose, the blood samples of 120 sheep from Afak, Dagharah and Diwaniyah cities were taken. The results showed that, for Afak, 20 and 23 samples were respectively positive for the parasitic antigens and for Dagarah and Diwanyiah, 18 and 22, and 22 and 22 samples were respectively positive for both parasitic antigens. The specificity values recorded were 50 and 63.3%, respectively. In the postmortem examination (PM), the results showed a lower presence of the parasites. Based on the results, ELISA provides a better method for the clinical detection of sheep infected with *E. granulosus* or *C. tenuicollis*.

Keywords: Cestode, Echinococcosis, Taenia, ELISA.

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Introduction

The larval stage of *Echinococcus* cestodes causes echinococcosis, a zoonotic disease. While *E. granulosus* was thought to be the sole cause of cystic echinococcosis (CE), it was soon discovered that there are other taxa with distinct adult phenotypes, host specialization, and pathogenic properties causing this disease (Nakao et al. 2013). Various strains of *E. granulosus* were described based on their intermediate host specialization, such as sheep, buffalo, cattle, horses, camels, etc.

Nine *Echinococcus* species were identified viz. *E. granulosus sensu stricto*, *E. ortleppi*, *E. equinus*, *E. canadensis*, *E. vogeli*, *E. multilocularis*, *E. felidis*, and *E. oligarthrus* (Hüttner et al. 2008). The classification of CE is still being debated and needs a comprehensive study (Lymbery et al. 2015; Romig et al. 2015; Al-Mahmoudi et al. 2019).

Carnivores serve as final hosts (canines, felines, etc), and intermediate animals, which are normally grazers (e.g. sheep and goats), shelter the parasitic larval phase (metacestode), providing good maintenance for the parasites. The cestode adult egg-laying stage lives in the small intestine of the final hosts. Hundreds of egg-generating worms could invade the definitive host, with each parasite releasing thousands of eggs per day. When the eggs are released from the host's stool, they become

Table 1. Serological data of larval *Echinococcus granulosus* and *Tenuicolic hydatigena* by iELISA.

Sheep sera studied	Number of samples studied	Results of ELISA with antigen							
		E. granulosus				*C. tenuicollis*			
		positive	negative	Sensitivity (%)	Specificity (%)	positive	negative	Sensitivity (%)	Specificity (%)
Afak	30	20	10	66.7	50	23	7	76.7	63.3
Dagharah	30	18	12	60	-	22	8	73.3	-
Diwanyiah	30	22	8	73.3	-	22	8	73.3	-
Clinically healthy	30	15	15	-	-	11	19	-	-
Average	-	-	-	66.7	-	-	-	74.4	-

infective. Depending on the climate, the eggs will stay viable for months or even a year. Desiccation and heat harm the eggs, but they may withstand freezing temperatures (Higuita et al. 2016).

Taenia hydatigena is a dog worm with a metacestode phase (*Cysticercus tenuicollis*) living in ruminants and pigs. The *C. tenuicollis* metacestode infection is crucial since it results in significant economic damages owing to the disapproval of contaminated offal or meat (Valieva et al. 2014; Singh et al. 2015; Abdulhameed et al. 2018). *Taenia hydatigena* cysticerci are led to animal yield declines and fatalities. Based on the organ affected, worm infestation, and other overlapping infections, cysticerci migration may result in hemorrhages and fibrotic passages, liver peritonitis, and heavy infections contributing to serious hepatitis and mortality in young animals (Valieva et al. 2014). If a metacestode is discovered during a meat examination or necropsy, the host and site of the metacestode are used to make a diagnostic decision in sheep. The cysts (*C. tenuicollis*) range from 1-6cm with a long-neck scolex. They are commonly connected to the omentum, mesenteric layers, and the liver surface (Valieva et al. 2014, Ali et al. 2018). Based on the above-mentioned background, this work aimed to examine the presence of *E. granulosus* and *C. tenuicollis* in sheep using antigenic based indirect enzyme-linked immunosorbent assay (iELISA) and postmortem analyses in Afak, Dagharah, and Diwaniyah cities of Iraq.

Materials and methods

The blood samples were taken from 120 sheep from Afak, Dagharah and Diwaniyah cities (30 samples of the infected animals per city) and 30 samples from serologically healthy sheep as control). The samples were collected from October 1, 2020, to February 25, 2021. The sera of the collected samples were prepared. IELISA method was used to determine circulating excreta and secretion antigens of *E. granulosus* and *C. tenuicollis* protoscolices in the blood samples (Craig et al. 2015). The optimal concentration of the antigens, dilutions of the antispecies conjugate, and serum titer were determined ahead of starting the ELISA. Peroxidase-labeled and affinity-purified rabbit antibodies specialized against sheep immunoglobulins (IgG, IgA, and IgM) functioned as conjugates in the ELISA (Craig et al. 2015).

Results

The results showed 20 (*E. granulosus*) and 23 (*C. tenuicollis*) infected samples for Afak, respectively positive for the parasitic antigens (66.7 and 76.7%, respectively). For Dagarah, 18 (*E. granulosus*) and 22 (*C. tenuicollis*) infected samples were respectively positive for the parasitic

Table 2. Comparison between iELISA and postmortem results on the presence of *Echinococcus granulosus* cysts.

Area	Total sheep examined	Number of sheep			
		ELISA positive with *E. granulosus* antigen	Infested with *E. granulosus*	With a mixed infestation (*E. granulosus* + *C. tenuicollis*)	No infestation found
Afak	30	20	16	9	5
Dagharah	30	18	17	10	3
Diwanyiah	30	22	15	9	6

Table 3. Comparison between iELISA and postmortem examination results based on the presence of *Tenuicolic cysticercosis*.

Area	Total sheep examined	Number of sheep			
		ELISA positive with *C. tenuicollis* antigen	Infested with *C. tenuicollis*	With mixed infestation (*E. granulosus* + *C. tenuicollis*)	No infestation found
Afak	30	23	19	9	2
Dagharah	30	22	18	10	2
Diwaniyah	30	22	18	9	3

antigens (60 and 73.3%, respectively, and for Diwanyiah for both parasites, 22 infected samples were found (73.3%). The specificity values recorded were 50 and 63.3%, respectively (Table 1).

In the postmortem examination (PM), differences showed a lower presence of the parasites in the tested sheep. In Afak, *E. granulosus* or mixed infections were 16 and 5 sheep, respectively. In Dagharah, *E. granulosus* or mixed infections were found in 17 and 3 sheep. In Al-Diwaniyah, *E. granulosus* or mixed infections were reported in 15 and 6 sheep, respectively (Table 2). In Afak, *C. tenuicollis* or mixed infections were detected in 19 and 2 sheep, respectively. In Dagharah, *C. tenuicollis* or mixed infections were found in 18 and 2 sheep, respectively. As for Al-Diwaniyah, 18 and 3 sheep were either with *C. tenuicollis* or mixed infections (Table 3).

Discussion

In Korea, ELISA was used to diagnose sheep infected with echinococcosis on the samples from Uzbekistan and they reported 59 infected positive and 39 healthy animals with a cut-off of 0.27 (Jin et al. 2013). In this study, the ELISA sensitivity and specificity toward the antigens of the hydatid fluids were 91.5 and 96%, respectively. Further detection using immunoblot analysis of the antigen proteins, by bands of 7-, 16-, and 24-kDa were detected (Jin et al. 2013). This study agrees with our finding that ELISA could provide higher intermediate levels of confidence in detecting Echinococcosis antigens. This analysis aimed to provide seroepidemiological systematic information on CE in infected sheep using ELISA. In Pakistan, 728 sheep sera were tested for IgG antibodies for *E. granulosus* using an EgAgB-based ELISA package. The average seroprevalence in the studied sheep was 21.98%, nearly identical to the measured prevalence rate (21.77%) (Alvi et al. 2020).

For the *C. tenuicollis*, the results showed the ability of ELISA and antibodies against the worm antigens. In Italy, 7781 lambs were studied at slaughterhouses to identify *C. tenuicollis* or larval migration based on necrotic-hemorrhagic canals. The prevalence of the parasite in the tested sheep was determined as 14.6%. About 10,807 larval migration-based necrotic-hemorrhagic canals were discovered (Scala et al. 2015). Tianli et al. (2019) usingte ELISA showed 93.41 and 99.31% of high sensitivity and specificity, respectively, with a coincidence rat at 97.02%.

The ELISA, in the current work, was confirmed the results of autopsy with a high accuracy rate. This agrees with Brik et al. (2018), who investigated 1600 sheep using PM in Morocco, that CE was 7.63%.

Protoscoleces are only discovered in the cystic fluids responsible for their fertility, at 66.66% in the liver and 57.74% in the lungs. Furthermore, calcified cysts were at 12.24% in the liver and 21.11% in the lungs (Brik et al. 2018). In conclusion, ELISA showed a better mothed for the clinical detection of sheep infected with *E. granulosus* or *C. tenuicollis.* PM analysis can confirm the ELISA results with slightly higher sensitivity and specificity.

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