SEROLOGICAL DIAGNOSIS OF HYDATIDOSIS IN ABATTOIRS

Nahid Arian Pour* and Rasool Kian
Faculty member-AJA
University of Medical Science- Tehran- Iran

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ABSTRACT

Echinococcosis and hydatidosis are community health problems in different parts of the world. To study the efficacy of intra-dermal test in diagnosis of hydatidosis in cattle, hydatid fluid obtained from bovine and sheep liver and lung cysts was injected intra-dermal to animals. Skin reaction was recorded compared to control. Presence of the cyst in organs in postmortem examination of carcass was considered as gold standard. After 24 hours of injection, sensitivity and specificity of ID test in cows and sheep were calculated. On the ground of its merits, intra-dermal test may be used to diagnose infection before infected carcass enters the market.

Key words: Abattoir, Echinococcosis, Hydatidosis, Hydatid cyst, Intra-dermal test, Zoonotic disease.

Hydatid disease is a food borne zoonotic parasitic disease predominantly affecting the liver, lung and other vital organs (Chharba and Singh, 2009). The most frequent causative agent is Echinococcus granulosus, the “dog tape worm” (Rodrigues et al., 2008). It is prevalent in the sheep-raising areas (Taghipour et al., 2008) and is an important health problem worldwide. The disease is relatively common in Mediterranean, the Middle East, Central Asia, East Africa and some areas of South America (Herrera et al., 2005).

Cystic echinococcosis is principally maintained in a dog–sheep–dog cycle (WHO, 2010). Vicinity of sheep with dog in places selling sheep results in contamination of sheep wool with parasite’s eggs (Rokni, 2009). Sheep and other herbivores normally acquire infection following consumption of food contaminated with dog feces containing parasite’s eggs. The infection is transmitted to dogs when they are fed infected viscera of sheep or other ruminants during the slaughter of animals (WHO, 2010).

The diagnosis of infection in human is commonly based on the results of specific serological tests and the imaging findings like myelography, computerized tomography (CT), and magnetic resonance imaging (MRI) (Taghipour et al., 2008; Chhabra and Singh, 2009). Serological assays for hydatid disease can be used both for diagnosis and post-treatment follow-up (Rodrigues et al., 2008). These tests are based on the reaction and precipitation of the test antigen and the circulating antibodies in the host and include immunoelectrophoresis, enzyme linked immunosorbent assay (ELISA) and western blot (Carmena et al., 2007).

The diagnosis of hydatid disease is confirmed after surgery by gross and histopathologic examination (Taghipour et al., 2008).

Cysts of Echinococcus spp in intermediate hosts other than human are usually detected at post-mortem abattoir examination of the viscera. Although this can provide important epidemiological data which can be used to define likely echinococcus infection pressure, the main disadvantage of the approach is that samples obtained at slaughter houses are potentially biased (Yang et al., 2009).

Intra-dermal tests are widely used to support the diagnosis of dermatological and non-dermatological diseases. They are mainly indicated for the detection of immediate (Type I) and delayed type hypersensitivity (DTH, Type IV hypersensitivity) towards organisms or their exogenous or endogenous antigens (Nagar et al., 2006).

This research has been carried out in order to find out if infected cattle in the abattoirs in...
endemic and hyper-endemic areas can be identified by performing a simple and cheap diagnostic test like intra-dermal test (ID) prior to slaughter of the animals.

Antigen preparation - This research was carried out in Azad University Research Center- Tehran-Iran. To prepare antigen required for the test, liver and lung of infected animals (cow and sheep) were obtained from one of the abattoirs (Kehenz) in Shahryar. Infected organs were carried separately to the laboratory and their hydatid fluids were aspirated under sterile conditions. Separate aliquots of hydatid fluids were prepared. Protein content of samples was estimated by biuret method (Rosenthal and Cundiff, 1956). Protein content was found out to be 185 µg/ml which was diluted by isotonic saline to 120 µg/ml and kept in water bath at 56°C for half an hour. Antigen was collected in separate vials and kept in refrigerator till use.

Animal injection - 10 cows and 15 sheep waiting to be slaughtered in Shahryar abattoir were selected. Care was taken to choose animals of almost the same age and weight. Hair and wool of the animals at the desired sites were shaved and skin was cleansed with alcohol. 3 ml of antigen prepared from cysts of bovine viscera was injected intra-dermal to cows. Similarly, same amount of antigen prepared from sheep was injected by the same route to sheep. 3 ml of normal saline was also injected at a distance of 13 cm to first injection as control to all the cases. Site of injections were marked and results were read after one and 24 hours. Any change in the skin in the form of heat, erythema and edema more than one centimeter at the site of injection, were considered positive.

Statistical analysis - Recorded skin reactions in different animals at different times were statistically analyzed and sensitivity, specificity, Positive and negative predictive values were calculated.

Skin reactions of intra-dermal test results in the form of local heat, erythema and edema more than one centimeter at the site of injection, were considered positive.

One hour after injecting antigen to cows, in 4 cases (40%) erythema was observed as skin reaction at the site of injection. Sensitivity of ID test after one hour of injection is 25% while its specificity is 50% with positive and negative predictive values of 25% and 50%.

After 24 hours, in 70% of cows skin reaction was noticed mainly in the form of edema. Measured sensitivity of ID test after 24 hours of injection is 75% while its specificity is 33.33% with positive and negative predictive values of 42.85% and 66.66% (Table 1). Out of 15 sheep tested, one hour after injecting antigen, in 4 cases no skin reaction was observed while in remainder erythema was the major skin reaction noticed (Table 1). Sensitivity of the ID test one hour after injection is 71.42 % and its specificity is 25% with positive and negative predictive values of 45.45% and 50% respectively.

Twenty four hours after injection, edema was the major skin response in 9 out of 10 responding sheep. The calculated sensitivity 24 hours after injection is 71.42 % and its specificity is 37.5% with positive and negative predictive values of 50% and 60% respectively. In these cases also the presence of the cyst in the organs in postmortem examination was considered as gold standard (Table 1).

Comparing calculated sensitivity and specificities of the ID test obtained after one hour with 24 hours readings, it may be concluded that results obtained after 24 hours in two groups of animals are closer and showed more similarity. So, 24 hours readings may be considered as the basis of reliable ID results.

Cystic echinococcosis is considered endemic in the entire Mediterranean zone including all countries from the Middle East (Sadjjadi, 2006). All assays tested like Ag-ELISA, antibody-ELISA, enzyme-linked immuno-electro transfer blot (EITB) and tongue inspection show low sensitivity in rural pigs infected naturally with low levels of T. solium (Dorny et al., 2005). A small percentage (13–22%) of cattle carrying fewer than 30–50 viable cysticerci is detected by Ag-ELISA (World Organization for Animal Health, 2008).

Immunological tests with high sensitivity and specificities are time consuming tests, requiring expensive chemicals and equipments and are to be performed by highly educated and well trained persons in highly equipped research laboratories and can not be performed in abattoirs with no or least equipment and untrained personnel. It is a fact that even many licensed abattoirs lack a well equipped
diagnostic laboratory. So, what is eagerly required is an easy-to-do diagnostic test to be done before slaughter of animals to find out the animal infection.

As we were keen to find a simple and cheap test to be performed at abattoir level for diagnosing infected animals which were being slaughtered for human consumption; after studying procedure of different serological/immunological tests performed so far for diagnosis of hydatidosis we found ID test as the easiest, cheapest and simplest test to be performed by each and every experienced person, irrespective of one’s knowledge. The procedure of ID test can be easily learnt and performed in the abattoirs, at least in endemic and hyper endemic areas.

In this study sensitivity and specificity of intra-dermal test was measured in animals which are intermediate hosts in parasite’s life cycle and consumed by human as meat. The results of showed relatively lower values than other serological tests performed using human sera which could be due to different antibody response of human and animals to the same antigen. As the sensitivity of ID test was obtained in this research was higher than its specificity if may be concluded that ID test was the target test fulfilling the aim of present study. Development of an automated sensitive diagnostic test would greatly reduce the costs of damage to the carcass and also the costs of labor (World Organization for Animal Health, 2008). As serological tests for animals have not reached the stage where commercialization for individual diagnosis or large-scale detection of infected carcasses in slaughter houses is possible (World Organization for Animal Health, 2008) a rapid test has to be found for diagnosis of infection before slaughter of infected animals at least in endemic and hyper endemic areas. Killing the infected animal and exposing the infected organs is harmful to the environment and human; especially if dogs come in contact with the infected leftovers. One way to reduce the rate of infection in the area is to minimize the slaughter of infected animals.

Employing vigilance as watching forces in monitoring performance of pre sacrifice tests may help eradicating this infection at the abattoir level and embracing the circle of scarified animal and society health (Rokni, 2009).

**CONCLUSIONS**

Many serological tests are used for diagnosis of hydatidosis with varying degrees of sensitivity and specificity, yet these are often used
for research purposes. To use a test which can be performed by staff of abattoir, not consuming much time and money and be easy-to-perform may be used as a routine diagnostic test in the abattoirs. Intra-dermal test, compared to other serological tests, is an easy-to-do, quick and simple test which can be performed in abattoirs and its result can be read easily.

REFERENCES


