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Value of Taenia Saginata Crude Antigen in Diagnosis of Bovine Cysticercosis with Reference to its Characterization

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Abstract: The surveillance system for bovine cysticercosis actually based on the traditional post-mortem inspection procedure at slaughterhouses had been hampered by poor test performance. Alternative methods should be introduced to meat inspection. Therefore, the objective of the current research was to study the reliability of indirect ELISA in detecting bovine cysticercosis, based on two types of crude antigens of different origins; *Taenia saginata* cysticerci from cattle and *T. saginata* adult worms from human patients. The detection of *Cysticercus bovis* among organs and muscle groups was done by slicing the heart, masseters, liver and muscles of 68 carcasses of cattle and buffaloes from Basateen abattoir. Three out of 68 (4.4%) examined carcasses were infected with bovine cysticercosis. While, from the 68 examined serum samples, 20 (29.4%) and 42 (61.76%) samples were positive by ELISA with crude *C. bovis* and *T. saginata* antigens, respectively. The study was extended to demonstrate shared bands between both tested antigens.Of them, the band at molecular weight 90 KDa was a major band in the electrophoretic profile of *T. saginata* crude antigen. This band might enforce the sensitivity of *T. saginata* crude antigen to be higher than that of *C. bovis* crude antigen. In conclusion, results obtained in the current study showed a considerable improvement in the detection of *C. bovis*-infected animals by ELISA based on *T. saginata* crude antigen which was superior to *C. bovis* crude antigen for detecting antibodies in sera of naturally infected animals.

Key words: *Taenia Saginata* · *Cysticercus Bovis* · Crude Antigen · Meat Inspection · ELISA

INTRODUCTION

Cysticercus bovis is the larval stage of *Taenia saginata*, the bovine tapeworm. Humans are the final host and bovines are the intermediate host to this infection. Cattle become infected after eating *T. saginata* eggs (proglottids) from infected humans. Once cattle are infected, cysticerci develop in the muscle and subsequently become infective to humans after approximately 10 weeks [1, 2]. A person infected with a single *T. saginata* tapeworm is capable of contaminating the environment with up to half a million eggs per day over the course of infection, which, if left untreated, can persist for years. Eggs contaminating the environment via defecation or spontaneous discharge of proglottids can be disseminated by water, wind, scavenging birds such as gulls feeding on raw sewage, oribatid mites, flies, earthworms, or fomites such as boots or farm machinery. Infective *Taenia* eggs can persist under a variety of environmental conditions; as with most parasite environmental stages, cool and moist conditions favour long-term survival. They can also survive in sewage and in sludge for up to several months, and are resistant to most conventional chemical and disinfecting agents [3].

In the meat industry, economic losses are closely associated with the status of infection. If a heavy infection or generalized cysticercosis is found in a carcass, it must be totally condemned. Light infection or localized cysticercosis leads to condemnation of the infected parts; furthermore, the carcass must be kept in cold storage at a temperature not exceeding -7 °C for up to 3 weeks to inactivate the parasites [4,5]. In England alone, the costs of refrigeration, handling, and transport are estimated at ∈ 100 per carcass, or ∈ 4.0 million

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annually [4]. Africa suffers great losses due to bovine cysticercosis estimated to be $1.8 billion annually [6]. Cattle with cysticercosis are unlikely to exhibit clinical signs, and detection is made during post-mortem carcass examination. In most parts of the world where regulated post-mortem screening for these parasites occurs, examination of so-called "predilection sites" is conducted during routine meat inspection. However, such procedures are insensitive, particularly for lightly infected carcasses [7, 8]. Viable cysticerci can be easily missed on meat inspection since the translucent cysts blend with the surrounding host tissue. Only upon death and degeneration of the parasite there is a sufficient host inflammatory response to create a more detectable lesion. Despite its limitations, visual inspection of carcasses remains the most common method of diagnosing bovine cysticercosis. Efforts to estimate the failure rate of detection during meat inspection show that as few as 27% [9], 28% [10] of field-infected animals are detectable. These observations were reinforced by a probabilistic model developed by Kyvsgaard *et al*. [11] which showed that over 85% of infected animals may be missed during routine meat inspection. The limitations of the current meat inspection procedures pose significant challenges for regulators and diagnostic tasked with preventing zoonotic transmission of the parasite. This could be addressed if a reliable serological test, e.g., enzyme-linked immunosorbent assay (ELISA) was available for use on live animals. The immune response against taeniid parasites is reported to be antibody-mediated. A positive antibody ELISA indicates that the animals have been exposed to the infection, but may not necessarily have a current infection. However, it is a useful method for epidemiological studies to indicate the spread of the infection in outbreaks or high-infected areas [12].

Therefore, the objective of the current research was to study the reliability of indirect ELISA, based on two types of crude antigens of different origins; *T. saginata* cysticerci from cattle and *T. saginata* adult worms from human patients, in detecting bovine cysticercosis.

MATERIALS AND METHODS

Collection of Samples:

- *T. saginata* cysticerci were obtained from carcasses following meat inspection looking for cysticerci at specific predilection sites (external and internal masseter muscles, tongue, heart and diaphragm) from Basateen abattoir in Egypt [13].
- *T. saginata* adult worms were obtained from human patients from Assuit hospital and kept in antibiotic saline at 4ºC.
- Sixty eight blood samples of cattle and buffaloes were collected in clean centrifuge tubes, allowed to clot, and then centrifuged at 3000 rpm for 10 minutes for serum separation. The clear non-hemolysed supernatant serum samples were amputated and stored at -20°C until use for serological studies. Three cattle sera proved to harbor *C. bovis* only as indicated by their post-mortem examination after slaughtering were used as positive control.

Preparation of crude antigens: Crude antigens of whole *C. bovis* from cattle origin and *T. saginata* adult worm from human origin were prepared by their homogenization and sonication in phosphate buffer saline (PBS) and centrifugation at 10,000 rpm for 30 min. Following the method described by Lightowlers *et al*. [14], a sample concentrator (speed-vac) was used for concentration of the prepared antigens. The protein content was estimated using the method of Lowery *et al*. [15].

Enzyme-linked immunosorbent assay (ELISA): ELISA was carried out according to Zimmerman *et al*. [16] and the optimal antigen, serum and conjugate concentrations were determined after preliminary checkerboard titration according to Catty and Raykundalia [17.. The wells were coated with 5 µg/ml of the tested antigens diluted in 0.1 M carbonate buffer (pH 9.6) at rate of 100 µl per well and incubated overnight at 4°C. Washed three times with PBS containing 0.01% Tween-20. . After blocking with 0.1% bovine serum albumin in 0.01 M PBS for 1.5 h at 37 °C, plates were washed three times,. a 100 µl of cattle sera (diluted 1:800 in PBS) were added, followed by incubation for 1.5 h at 37 °C. After washing, 100 µl of 1:1000 peroxidase conjugate anti-bovine IgG (Sigma) were added and the plates were incubated at 37 °C for 2 h. Finally 50 µl of orthphenylene diamine 340 µg/ml citrate /phosphate buffer, PH 5 with 0.03% hydrogen peroxide solution, was added and after incubation in the dark for 15 min at 37 °C the absorbance was read at a wavelength of 405 nm . Positive values were assigned according to Rodriguez-Peiez and Hillyer [18] as those values with absorbance readings greater than the cut off value, which calculated as mean OD of negative sera plus three standard deviations.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE): Ten mg of both *C. bovis* and *T. saginata* crude antigens were fractionated using 10% SDS-PAGE under reducing condition [19]. The fractionated antigens were stained with commassie blue dye and the molecular weight of bands was determined using Imaging Densitometer (BIO-RAD model GS-700).

RESULTS

Post-mortem examination: Three out of 68 (4.4%) examined carcasses of cattle and buffaloes were infected with bovine cysticercosis as proved by post-mortem examination.

ELISA: Checkerboard titration of both antigen preparations showed that the adult worm extract was superior to the larval cyst extract for detecting antibodies in sera from naturally infected cattle. There was greater background activity with control sera (parasite free-animals), against *C. bovis* antigen. Both antigens were used at the optimal concentration of 5 µg protein/ml. From the 68 examined serum samples, 20 (29.4%) and 42 (61.76%) samples reacted positively in the antibody ELISA with crude *C. bovis* and *T. saginata* antigens, respectively.

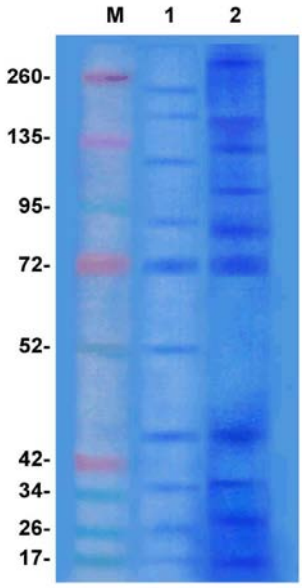

Fig. 1: SDS-PAGE of crude antigens of *C. bovis* (Lane 1) and *T. saginata* (Lane 2). M, Molecular weight marker.

SDS-PAGE: Electrophoretic profile of the tested antigens was resolved by SDS-PAGE and showed bands at both high and low molecular weight ranges. *C. bovis* and *T. saginata* crude antigens revealed at least 10 bands for each antigen. The molecular weights of the separated bands ranged from 18-276 kDa. Of them, the bands at molecular weight of about 45, 73 and 90 kDa were common (Fig.1). Two common bands (45 and 73 KDa) were major bands in *C. bovis* crude antigen, while all of them were major bands in *T. saginata* crude antigen.

DISCUSSION

The surveillance system for bovine cysticercosis actually based on the traditional post-mortem inspection procedure at slaughterhouses had been hampered by poor test performance. Alternative methods should be introduced to meat inspection. Whereas control of bovine cysticercosis could be achieved through improvements in public health, sanitation and animal management/husbandry practices [20], any effective control programme required sensitive diagnostic tools. The results of the current study showed that the antibody ELISAs were more sensitive than meat inspection for the determination of prevalence rate of bovine cysticercosis. In many countries the 'knife and eye' method was used whereby the so-called predilection sites (heart, tongue, masseter muscles, oesophagus and diaphragm) were visually examined and/or incised to detect cysticercosis. However, several studies had shown that, except for the heart, none of the other muscles should be considered as real predilection sites [11, 21]. Detailed dissection of carcasses of lightly infected animals had proven that, in 51% to 56% of them, cysticerci were not present in these presumed predilection sites [9]. Therefore, it was not surprising that routine meat inspection (if carried out properly) detected only the more heavily infected animals and underestimated the real prevalence of bovine cysticercosis by at least a factor of three to ten [22]. Another disadvantage of current meat inspection techniques was the fact that they were labor intensive and very subjective. It depended mainly on the skills and motivation of the meat inspector as to whether or not cysticerci would be detected [22]. Living cysticerci, which were usually present in small numbers, were especially difficult to detect. Once the cysticerci dead and became caseous or calcified, they had to be

differentiated from other lesion. Hence, the 4.4 percent in the current study could actually be a gross underestimation of the actual prevalence since the meat inspection methods used at the abattoirs had been found to be 2-20 times less sensitive than serologic methods [23, 24].

ELISA results showed the superior performance of crude *T. saginata* antigen than crude *C. bovis* antigen in antibody detection (61.76% and 29.30%, respectively). Indeed, the antigen played a pivotal role in the performance of an ELISA designed to detect serum antibodies. Many studies had appropriately been focused on evaluating and comparing available antigen preparations. Promising results were obtained using antigens derived from *T. saginata* [25]. Moreover, Craig and Rickard [26] reported that a crude aqueous extract of adult *T. saginata*, used as antigen in the peroxidase micro-ELISA, enabled the specific diagnosis of experimental and natural *T. saginata* cysticercosis. They added that similar results were reported by other workers using different serological tests such as the indirect haemagglutination test (IHAT) and the indirect fluorescent antibody test (IFAT). In the present study, the high percentage of positive serum samples could be attributed to the probable exposure of these animals to *T. saginata*, or could be due to cross reactions with other taeniid parasites [27], which need further investigations. However, the occurrence of other taeniid species being present in cattle is either unknown or rather unlikely, as no taeniid cestode infections with cattle as definitive hosts are known and the spectrum of metacestode stages as well is rather limited.

The present study provided evidence of shared bands between both tested antigens. Of them, the band at molecular weight 90 KDa was a major band in the electrophoretic profile of *T. saginata* crude antigen. This band might enforce the sensitivity of *T. saginata* crude antigen to be higher than that of *C. bovis* crude antigen. Indeed, Ralston and Heath [28] described an immunoblot test for antibodies to *T. ovis* based on the recognition of a 94-kDa antigen band in the sodium dodecyl sulfate-polyacrylamide gel electrophoresis profile of *T.* ovis *scolex* secretions. The test was specific for cestode infections and was 82% sensitive for the recognition of *T. ovis* infections. However, the antigenic activity of *T. saginata* band at 90 KDa in antibody detection needs further investigation.

CONCLUSION

The current study had led to a considerable improvement in the detection of *C. bovis*-infected animals by serological testing based on *T. saginata* crude antigen which could be useful for diagnosis of bovine cysticercosis.

REFERENCES

1. Flisser, A., D. Correa, G. Avilla and P. Marvilla, 2005. Biology of *Taenia solium, Taenia saginata* and Taenia saginata asiática. In: Murrel. K.D. (Ed.) WHO/FAO/OIE guidelines for the surveillance, prevention and control of taeniosis/cisticercosis. Paris, France, pp: 1-8.
2. McFadden, A.M.J., D.D. Heath, C.M. Morley and P. Dorny, 2011. Investigation of an outbreak of *Taenia saginata* cysts (*Cysticercus bovis*) in dairy cattle from two farms. Vet. Parasit., 176: 177-184.
3. Pawlowski, Z.S. and K.D. Murrel, 2001. Taeniasis and Cysticercosis. In: Hui, Y.H., Sattar, S.A., Murrell, K.D., Nip, W.K., Stanfield, P.S. (Ed.), Foodborne Disease Handbook, Volume 2, 2nd Edition, Marcel Dekker, NY, USA, pp: 217-227.
4. Gracey, J., D.S. Collins and R. Huey, 1999. Diseases caused by helminth and arthropod parasites. In: Meat hygiene, 10th Edition WB Saunders, UK, pp: 635-699.
5. Ibrahim, N. and F. Zerihum, 2012. Prevalence of *Taenia saginata* cysticercosis in cattle slaughtered I Addis Ababa municipal abattoir, Ethiopia. Global Veterinaria, 8: 467-471.
6. Harrison, L.J.S., 1996. Epidemiology and control of *Taenia saginata* cysticercosis. Centre for Tropical Veterinary Medicine, University of Edinburgh, UK, R pp: 5580.
7. Kandil, O.M., O.A. Mahdy, S.K. Abou- Dobal and W.M. Mousa, 2004. Purification and characterization of the three larval taeniid antigens by gel filtration. Vet. Med. J., 52: 449-456.
8. Oliveira, H.B., G.A. Machado, J.R. Mineo and J.M. Costa-Cruz 2010. *Taenia saginata* metacestode antigenic fractions without affinity to concanavalin A are an important source of specific antigens for the diagnosis of human neurocysticercosis. Clin. Vaccine Immunol., 17: 638-644.

9. Walther, M. and J.K. Koske, 1980. *Taenia saginata* cysticercosis: a comparison of routine meat inspection and carcase dissection results in calves. Vet. Rec., 106: 401-402.
10. Dewhirst, L.W., J.D. Cramer and J.J. Sheldon, 1967. An analysis of current inspection procedures for detecting *bovine cisticercosis*. J. Am. Vet. Med. Assoc., 150: 412-417.
11. Kyvsgaard, N.C., B. Lisose, S.A. Henriksenand, P. Nansen, 1990. Distribution of *Taenia saginata* cysts in carcasses of experimentally infected calves and significance for routine meat inspection. Res. Vet. Sci., 49: 29-33.
12. Harrison, L.J., T. Garate, D.M. Bryce, L.M. Gonzalez, M. Foster-Cuevas, L.W. Wamae, J.A. Onyango-Abuje and R.M. Parkhouse, 2005. Ag- ELISA and PCR for monitoring the vaccination of cattle against *Taenia saginata* cysticercosis using an oncospheral adhesion protein (HP6) with surface and secreted localization. Trop. Anim. Health. Prod., 37: 103-120.
13. Lopes, W.D., T.R. Santos, V.E. Soares, J.L. Nunes, R.P. Mendonça, R.C. de Lima, C.A. Sakamoto, G.H. Costa, V. Thomaz-Soccol, G.P. Oliveira and A.J. Costa, 2010. Preferential infection sites of *Cysticercus bovis* in cattle experimentally infected with Taenia saginata eggs. Res. Vet. Sci., 19: 20-26.
14. Lightowlers, M.W., R. Rolfe and C.G. Gauci, 1996. *Taenia saginata*: Vaccination against cysticercosis in cattle with recombinant oncosphere antigens. Experimental Parasitol., 84: 330-338.
15. Lowry O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
16. Zimmerman, G.L., L.W. Jen, J.E. Cerro, K.L. Farnsworth and R.B. Wescott, 1982. Diagnosis of *Fasciola hepatica* infections in sheep by an enzyme linked immunosorbent assay. Am. J. Vet. Res., 43: 2097-2100.
17. Catty, D. and C. Raykundalia, 1989. ELISA and related enzyme immunoassays. In: Catty, D. (Ed.), Antibodies, a Practical Approach, vol. II. IRL Press Oxford University Press, Oxford, pp: 97-152.
18. Rodriguez-Peiez, J. and G.V. Hillyer, 1995. Detection of excretory-secretory circulating antigens in sheep infected with *Fasciola hepatica* and with *Schistosoma mansoni* and Fasciola hepatica. Vet. Parasitol., 56: 57-66.
19. Laemmli, U.K., 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.
20. Gonzalez, L.M., N. Villalobos, E. Montero, J. Morales, R.A. Sanz, A. Muro, L.J. Harrison, R.M. Parkhouse and T. Garate, 2006. Differential molecular identification of Taeniid spp. and Sarcocystis spp. cysts isolated from infected pigs and cattle. Vet. Parasitol., 142: 95-101.
21. Maeda, G.E., N.C. Kyvsgaard, P. Nansen and H.O. Bogh, 1996. Distribution of *Taenia saginata* cysts by muscle group in naturally infected cattle in Tanzania. Prev. Vet. Med., 28: 81-89.
22. European, Commission, 2000. Opinion of the Scientific Committee on Veterinary measures related to Public Health on the control of taeniosis-cysticercosis in man and animals. Brussels, pp: 1-31.
23. Dorny, P., I. Phiri, S. Gabriel, N. Speybroeck and J. Vercuysse, 2002. A sero-epidemiological study of *Taenia saginata* cysticercosis in Zambia cattle. Vet. Parasitol., 104: 211-215.
24. Asaava, L.L., P.M. Kitala, P.B. Gathura, M.O. Nanyingi, G. Muchemi and E. Schelling, 2009. A survey of *bovine cysticercosis*/human taeniosis in Northern Turkana District, Kenya. Prev. Vet. Med., 89: 197-204.
25. Harrison, L.J.S. and M.M.H. Sewell, 1981. Antibody levels in cattle naturally infected with *Taenia saginata* metacestodes in Britain. Res. Vet. Sci., 31: 62-64.
26. Craig, P.S. and M.D. Rickard, 1980. Evaluation of "Crude" antigen prepared from Taenia saginata for the serological diagnosis of *Taenia saginata* cysticercosis in cattle using the Enzyme-Linked Immunosorbent Assay (ELISA). Z, Parasitenkd., 61: 287-297.
27. Brandt, J.R., S. Geerts, R. De Deken, V. Kumar, F. Ceulemans, L. Brijs and N. Falla, 1992. A monoclonal antibody-based ELISA for the detection of circulating excretory–secretory antigens in *Taenia saginata* cysticercosis. Int. J. Parasitol. 22: 471-477.
28. Ralston, M.J. and D.D. Heath, 1995. A defined antigen for the serodiagnosis of *T. ovis* infections in dogs. J. Parasitol., 81: 422-428.