Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(1s), 22-29

Determination of Immune Reactive Proteins of *Cysticercus Tenuicollis* from Naturally Infected Sheep and Goats in AL-Muthanna Province /Iraq

[1]Khaldoun M. Al-Rishawi, [2]Hadi M. Al-Mayali

sci.bio.ph.20.3@qu.edu.iq, Hadi.Hamza@qu.edu.iq

[1, 2]University of Al-Qadisiyah / College of Education / Biology Department

(Received: 04 August 2023 Revised: 12 September Accepted: 06 October)

KEYWORDS

Cysticercus,

Tenuicollis,

Proteins

ABSTRACT:

In the current study, 8 samples of *Cysticercus tenuicollis* cysts were collected from sheep and goats (four cysts for each) from slaughterhouses of AL-Muthanna province during the period from January - February 2023. The study aimed to identify the immune reactive proteins present in the cysts fluid, using SDS-PAGE technique (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis) and Western blot technique, by using the Protein Ladder with molecular weights of 10.5 - 14 - 22 - 29 - 42 - 51 - 62 – 70 – 95 – 130 – 175 KDa.

The results of the SDS-PAGE showed the presence of 7 bands of proteins in the cyst fluid, molecular weight in the range of 120 - 27 kDa, and molecular weights of 120 - 90 - 70 - 60 - 47 - 38 - 27 kDa. Each of them is a major protein with some variations. It turned out that proteins with a molecular weight of 90-70-60 kDa were dominant, as they had a greater density than the others did, and they appeared in all eight samples. As for the rest of the molecular weights, their appearance differed from one sample to another.

The results of the Western blotting showed the presence of 6 bands of proteins, and they had molecular weights within the range of 120-27 kDa.The proteins with molecular weights of 90 - 60 - 38 kDa appeared in all eight samples very clearly, which indicates that they are Specific immune reactive proteins in the cysts fluid. The Western blot technique is performed on *Cysticercus tenuicollis* from goats and sheep for the first time in Iraq.

Introduction

Taenia hydatigena is a parasite that is widespread in various countries around the world. They live as adults in the small intestines of dogs and other members of the canine family, such as foxes and wolves. Its larval stage is *Cysticercus tenuicollis* can infect many herbivores, especially ruminants, as they are suitable intermediate hosts. Infections are common among sheep, goats, deer, and pigs. However, in other ruminants, such as herds of cows, it is not widespread. Moreover, it can infect a wide range of mammals, such as camels, rats, and monkeys (Jenkins *et al*., 2014; Scala *et al*., 2015; Foroutan *et al*., 2022; Wakid &Alsulami, 2022).

Definitive hosts, such as dogs, become infected when they eat the larval stage of the parasite along with carcasses or the remains of dead intermediate hosts and their infected organs, as adult worms live in the small intestine of these hosts. The pre-patent period is estimated at a period ranging between 7-9 weeks, as the pregnant pieces are excreted with feces at a rate of approximately 4 pieces per day, each of which carries 6000–43000 eggs. Thus, approximately 100,000 eggs are the average amount excreted by one worm per day (Forbes, 2021).

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(1s), 22-29

The intermediate hosts got infected with the larval stage when they swallow the eggs found in feed and grass contaminated with the faeces of infected dogs. After digesting the egg shell, the embryo penetrates the intestinal wall to reach the liver to settle there or migrate to the mesentery or omentum, where it attaches to these parts and develops into a fluid-filled sac, which is the*Cysticercus tenuicollis*. The infection is severe only if several larvae invade the body as a result of swallowing several eggs. It is worth noting that there is no cure for the disease, and controlling it is a major problem because it requires treating and preventing contact infected dogs (Miller *et al.*, 2012).

In recent years, it has become clear that greater priority and more focus should be given to *C. tenuicollis* due to its economic impact, because the organs or parts of animals infected with the larvae must be destroyed, as well as the accompanying losses such as the cost of disposing of infected parts, especially in the developing countries. Loss in quantity or quality of meat or other organs will have financial implications, with reduced payments for contamination of the carcass or diseased or infected tissue (Anteneh *et al*., 2011; Wondimu *et al*., 2011; Bates, 2011; Jayousi, 2014; Scala *et al*., 2015; Wakid &Alsulami, 2022). In Iraq, this parasite is considered one of the most important and economically influential veterinary parasites in livestock (Hama-Soor *et al*., 2021). It is important to conduct several studies on the immunologically important proteins in the fluid of cysts taken from intermediate hosts in order to accurately identify them, and thus they will be useful in the serological diagnosis of parasite infections in intermediate hosts such as goats (Arunkumar *et al*., 2014). In addition, it's worth mentioning that identifying the proteins of the larval stage of the parasite will be an important guide to determining their role in the biological characteristics of the parasite (Cai *et al*., 2021).

SDS-PAGE is widely used in analyzing proteins within complex extracts. The most commonly used methods are derived from the discontinuous SDS-PAGE system first described by Laemmli (1970). Nowakowski *et al*. (2014) mentioned that it is the most widely used technique for obtaining high-precision analytical separations of protein mixtures.

Western blotting is a well-known technique in molecular biology. It is often used by biologists to detect many features of proteins, and its uses range from simple, such as basic protein analysis, to large goals, such as identifying diseases. This technique is a simple, unique, rapid, and widely used tool that provides easy interpretation and precisely defined results. It is used in various fields of science, research and development, diagnostic laboratories, and hospitals. Its basic working principle is to separate proteins based on the molecular weight and charge they carry (Begum *et al*., 2022). Identification of the specific protein bands of the *C. tenuicollis* in sheep infections using Western blot technology may be useful in the future of vaccines and kits used in diagnostic studies of this tapeworm in its adult and larval stages or for other tapeworms that infect dogs and sheep (Kara *et al.*, 2003). Identification of antigenic segments in the larval stages of Taeniidae parasites is very important for the purpose of diagnosis and vaccine manufacturing (Kordafshari *et al*., 2010).

Materials and methods

In this study, 8 samples were collected from sheep and goats, 4 cysts for each type, collected from slaughterhouses of AL-Muthanna province during the period from January 2023 - February 2023.The cysts were found in the omentum, mesentery, and liver when examining the internal viscera of the slaughtered animal, as they can be easily distinguished by their shape, size, and favorite locations (Arunkumar *et al*., 2014). The diagnosis was confirmed as *Cysticercus tenuicollis* larvae through a microscopic examination of the cysts, and the scolex noticed. These cysts were washed with normal saline PBS solution (pH 7.4), then they were preserved and transported using sterile and inside refrigerated containers and kept under cooling until they were transported to the laboratory in the biology department at the College of Education/University of Al-Qadisiyah, where each cyst was examined and prepared for subsequent operations.

Determine the protein content and perform the SDS-PAGE technique.

The protein content of the study samples was determined using a Nanodrop spectrophotometer. The SDS-PAGE technique was conducted based on the method of (Laemmli, 1970).

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(1s), 22-29

SDS-PAGE steps

Electrophoresis is a method used to separate and analyze large molecules based on their size and charge. It is also known as the process of migration and separation of charged particles (ions) under the influence of an electric field (Fritsch, 2003). The process was conductedas following:

1. Aspirate the fluid from the cyst using a new syringe and under sterile conditions, and then put it in a cooling centrifuge at 9000 rpm for 30 minutes at 4 °C. Then the remaining fluid was filtered through 0.45 µm filter papers, and the remaining fluid was treated as cyst fluid antigens, then Store at -70°C.

2. Prepare the SDS-PAGE device and make sure that it is tightly closed at the bottom to prevent fluid leakage. Then the allocated amount of separating gel solution was poured slowly. After about an hour, this layer polymerized and hardened.

3. Add the stacking gel solution, then placed the comb in its place to make the holes after it hardened. This gel hardens after one hour.

4. After the collection gel hardened, the comb was quietly removed, and we dismantled the device to transfer the cassette containing the hardened gel to the electrophoresis device.

5. Pour the transfer solution, or glycine buffer solution, into the transfer tank.

6. The antigens that were previously prepared were token 15 micrograms of them for each sample and mixed with 5 microliters of loading dye and 2 microliters of 10×35 reducing agent, then placed in a heating block at 100 °C for 5 minutes. At this time, the samples were placed in their places in the holes in the gel placed in the running buffer.

7. Placed the protein marker in one of the empty holes.

8. The process began by running the vertical electrophoresis device at 15 milliampere until the dye passed the stacking gel. Then increased the electrical current to 30 milliampere and 120 volts until it reached the bottom of the resolving gel.

9. Transfered the gel from its plate and washed it with distilled water, then placed it in the staining plate containing the Coomassie blue stain solution and placed it on a shaking incubator for a whole night. This was followed by removing the gel from the staining container, washing it with distilled water twice, and then placing it in another container containing the dye removal solution and also on the shaking incubator for two hours until the excess dye was removed. After that a picture of the gel was taken, and it was stored in a liquid until the Western blot technique was performed.

10. After the bands were visible, we took a picture of them and read the results using graph paper. The previous steps were performed based on the instructions of the kit supplier, Kara *et al*., (2003) and Rafiqi *et al*., (2016).

Western Blot Technique

Western blot (WB) is the process of fixing or transferring proteins onto a solid supporting membrane (after gel electrophoresis) and then analyzing them using immunodetection. The work was done by using nitrocellulose membrane mini-blots. Western blotting was performed to determine the immunereactive proteins of the *C. tenuicollis*. The technique was conducted based on Hnasko & Hnasko, (2015) and the instructions of the company kit.

Preparation of primary antibodies

Serum from study animals infected with the parasite (sheep and goats) was used as a source of primary antibodies. It was drawn from the blood of the animals before slaughtering them, and when it was confirmed that the sample was positive, the tubes carrying the blood were placed in the centrifuge, the serum was separated, and the liquid was then stored at -20 °C until use at the indicated time of the Western blot technique (Goswami *et al*., 2013).

Results

SDS-PAGE technique

SDS-PAGE technology was used to study the active proteins in cyst fluid taken from infected sheep and goats with *C. tenuicollis*, using the Protein Ladder with molecular weights of 10.5 - 14 - 22 - 29 - 42 - 51 - 62 - 70 - 95 - 130 - 175 kDa, the results of the SDS-PAGE

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(1s), 22-29

showed that there are 7 bands of proteins with molecular weight ranged 120 - 27 kDa, and that they had molecular weights of 120 - 90 - 70 - 60 - 47 - 38 - 27 kDa that appeared in the cyst fluidantigens, each of these proteins can be considered a major one with some differences.From Figure (1), it is clear that proteins with a molecular weight of 90-70-60 kDa are dominant, as they have a greater density than others and appeared in all eight samples, while those with a molecular weight of 38-27 kDa were of medium density and also appeared in all samples, while 47 kDa was very weak and appeared only in 5 samples, finally 120 kDa was almost invisible..

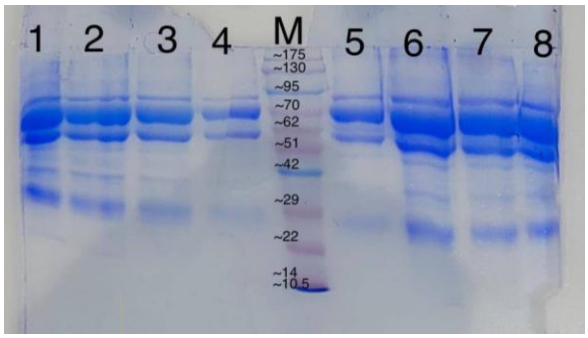

Figure 1 shows the results of the SDS-PAGE, in which we see the protein bands of the study samples. The numbers (1,2,3,4) represent *C. tenuicollis* samples from goats, and (M) represents the Protein Marker Ladder, while the numbers (5,6,7,8) represent *C. tenuicollis* samples from sheep.

Western blotting

After transferring the proteins from the gel to the nitrocellulose membrane and treating them with antibodies from the blood serum of infected animals, then photographing them with X ray, the protein bands with molecular weights of 120 - 47 - 27 kDa did not appear in all samples. As for the other three proteins, which have molecular weights of 90 - 60 - 38 kDa, they appeared in all eight samples very clearly, which indicates that these proteins with molecular weights of 90 - 60 - 38 kDa have specific immune reactivity with antibodies from the blood serum of animals infected with *C. tenuicollis* , which are proteins with special immune activity.

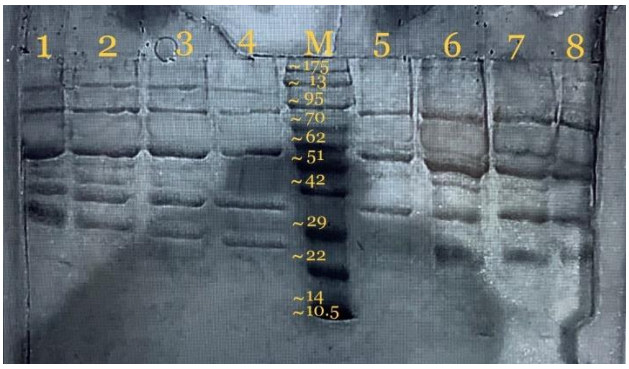

Figure 2 shows the results of the Western blot, in which we see multiple peptide bands for the study samples. The numbers (1, 2, 3, 4) represent *C. tenuicollis* samples from goats, and (M) presents the Protein Marker Ladder. The numbers (5,6,7,8) represent cyst samples from sheep.

Discussion

SDS-PAGE Technique

The results of the SDS-PAGE technique showed the presence of seven bands of proteins whose molecular weight ranged 120 - 27 kDa, and their molecular weights were 120 - 90 - 70 - 60 - 47 - 38 - 27 kDa in the cyst fluid from infected sheep and goats. The study also showed that proteins with a molecular weight of 90-70-60 kDa were dominant, and proteins with a molecular weight of 120-47 kDa were very weak and did not appear in all samples.

The study partly differed from Kara *et al.* (2003) in that their results of SDS-PAGE showed the presence of 16 protein bands, and the range of their molecular weights was between 116-6.1 kDa, which are 116- 66- 55- 35- 36- 27.5- 23- 16- 14- 6.1 kDa, and with the presence of 6 bands appear weak. As for the study by Mathur *et al.* (2003) it targeted proteins from three parts of the parasite, the scolex, the cyst wall, and the cyst fluid of *C. tenuicollis*. It showed the presence of 37 protein bands 3 in the cyst fluid, 12 in the scolex and 22 in the cyst wall. In Iran, a study by Kordafshari *et al.* (2010) showed that the cysts from infected sheep contains 14 protein bands with a molecular weight range of 13-120 kDa, 8 proteins with a range of 12-57 kDa and 11 proteins with a range of 12-100 kDa, from the scolex, cyst fluid and cyst wall, respectively. Thus, the current study differed from it in that regard. While Nath *et al*.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(1s), 22-29

(2011), in India, which targeted cyst membrane antigens from infected goats, showed the presence of 15 proteins ranging of 131-10 kDa, ten of which appeared clearly 90-66-58-44-38-34-31-22-14-10 kDa, thus, it is consistent with the current study, and also showed that proteins with molecular weights of 14-34 kDa are dominant, and recommended that they be used in diagnosing parasite infection in goats.

In the study by Goswami *et al.* (2013) in Iran, it showed the presence of eight protein bands with molecular weights of 149.4-92.9-74.2-63.5-36.2-23.9-15.7-9.6 kDa from antigens of the cyst fluid and wall. The current study also agreed with the study of Arunkumar *et al*. (2014) in India, which showed that prominent protein bands of cyst from infected goats had molecular weights of 60-20-10 kDa and three weakly visible bands with molecular weights of 200-47-35 kDa.

The current study partly differed from the study of Rafiqi *et al.* (2016) in India on cysts taken from infected goats, as that study showed the presence of 6 protein bands within a molecular weight of 28-90 kDa, which are 90-76-68-54- 36-28 kDa, and proteins with molecular weights of 68-54-28 kDa were the most prominent.

The current study differed from the study of Abuseir *et al*. (2018) in Palestine, on the wall of the cyst and its fluid; they found that the proteins were within a molecular weight range of 290-12 kDawhich its 290-270-260-150-130-80-67-55-35-23-14-12 kDa. Their results showed a similarity between the proteins of the larvae of the *T. hydatigena* parasite and the proteins of the larvae of other tapeworms they compared: *T. saginata*, *E. granulosus* and *T. ovis*, thus recommending the possibility of relying on serological tests based on cyst antigens as a diagnostic tool to distinguish between these important species.

Locally, the current study slight agreed with Dirwal *et al*. (2020), in Baghdad, who identified proteins with molecular weights of 70-63-48 kDa as being distinctive in his study on thy cyst from sheep.

In India Jeyathilakan *et al*. (2021) Record the presence of protein bands for cyst fluid antigens from sheep infected with the parasite, with molecular weights 208-172-116-72-42-32-28-24-10 kDa, and for scolex antigens 112-98-72-68-58-49-42-29-12kDa, while the cyst wall antigens were 210-98-72-58-24-16 kDa, and thus they differ from the current study. The current study also differed from the study of Biosa *et al*. (2022), in Italy on sheep, it showed the range of molecular weights between 10-250 kDa and the number of proteins was 679 proteins, of which approximately 61% belonged to the parasite.

Western Blot Technique

The current study aimed to identify the immunodominant proteins in cyst fluid antigens from study animals, sheep and goats, infected with the larval stage of the *Taenia hydatigena*.As we knew previously, the Western blot technique is used to clarify proteins that were previously separated by electrophoresis in the SDS-PAGE technique.

Through the results, it was noted that 6 proteins appeared with molecular weights ranging from 27-120 kDa, and the immune reactive proteins were 38-60-90 kDa. A study by Kara *et al*. (2003) in Turkey showed that the proteins within a molecular weight of 36 kDa are the effective proteins specialized immunologically in animal infections with *C. tenuicollis*, when they were studied on infected sheep. As for the study by Kara & Doğanay, (2005) also in Turkey, when they experimentally infected dogs and examined adult worms, they identified proteins with molecular weights of 38-42.5 kDa as the immunologically active proteins in these worms.

In Nath *et al*. (2011) studies conducted on *C. tenuicollis* from goats infected with the parasite concluded that proteins with molecular weights of 14-34 kDa could be used as diagnostic proteins for *C. tenuicollis*, but they advised additional studies to confirm this. The study of Goswami *et al*. (2013) in Iran, it was found that proteins with molecular weights of 36.2 - 23.9 - 9.6 kDa were the most prominent in all the study samples, with some slight differences, and the source of the proteins was the fluid of the parasite cyst and its wall from infected goats.

The reason for the discrepancy between studies in both SDS-PAGE and Western blot techniques may be due to the difference in the method of reading the bands, as some rely on graph paper and others rely on some

electronic programs such as KODAK 1D Image Analysis or Image J, or the naked eye. In all of them, the process of rounding to the nearest possible number is carried out for bars with similar molecular weights. In addition, the difference in hosts has an impact on the parasite, as some of these studies were on the parasite from dogs or only one type of intermediate host, and the environmental influence and geographical distribution or a difference in the parasite's strains. There is also a difference in the source of proteins, as some of them adopted proteins from the scolex of the bladder cyst, its wall, or the fluid of the parasite cyst, as in the current study.

The difference can be attributed to the fact that complexes of large molecular weight proteins in some tapeworms may dissociate under severe conditions causing them to be reduced to a smaller size for two or more subunits (McManus, 2014). This may also explain the presence in some samples of small proteins that appeared on SDS-PAGE, while in other samples these small proteins were absent or very faint. In addition, Miquel *et al.* (2015) pointed out; different parts of certain proteins may participate in the subsequent development of larvae in some tapeworm infections in the body of the host animal (Abuseir *et al*., 2018).

Conclusions

We conclude that proteins with molecular weights of 90 - 60 - 38 kDa are the immune reactive proteins for the *C. tenuicollis*which infected intermediate host in Iraq, as demonstrated by the results of the Western Blotting, and they can be used to diagnose the parasite and distinguish it from other larval stages of the tapeworm.

References

1. **Abuseir, S., Nagel-Kohl, U., &Strube, C. (2018).** Protein profile of the cysts of *Taenia hydatigena*, *Taenia saginata*, *Echinococcus granulosus* and *Taenia ovis*. Human and Veterinary Medicine, (4), 184-188.
2. **Anteneh, W., Daniel, A., &Yacob, H. (2011).** A study on the prevalence, distribution and economic importance of *Cysticercus tenuicollis*in visceral organs of small ruminants slaughtered at an abattoir in Ethiopia. Journal of Veterinary Medicine and Animal Health, 3(5), 67-74.
3. **Arunkumar, S., Prakash k. R., Balamurugan T. C., & Guru D.V. Pandiyan. (2014).** Electrophoretic analysis of cystic fluid proteins of *Cysticercus tenuicollis*from goats. CIBTech Journal of Microbiology, 3 (4), 52-54.
4. **Bates, P. (2013).**Cysticercosis-controlling tapeworm in dogs and sheep. Veterinary Times, 43(46), 11-12.
5. **Begum, H., Murugesan, P., &Tangutur, A. D. (2022).** Western blotting: a powerful staple in scientific and biomedical research. BioTechniques, 73(1), 58-69.
6. **Biosa, G., Bonelli, P., Pisanu, S., Ghisaura, S., Santucciu, C., Peruzzu, A., ... & Pagnozzi, D. (2022).** Proteomic characterization of *Echinococcus granulosus* sensu stricto, *Taenia hydatigena* and *Taenia multiceps* metacestode cyst fluids. *Acta Tropica*, *226*, 106253.
7. **Cai, M., Li, Y., He, G., Guo, X., Zhang, S., Yan, L., ...& Ding, J. (2021).** Comparative Proteomic Analysis of Different Parts of *Taenia hydatigena*. Frontiers in Veterinary Science, 8, 626579.
8. **Dirwal, A. R., Faleh, I. B., Layj, D. M. (2020).** Manifestation Of *Cysticercus tenuicollis* cyst infected organs of slaughtered sheep with determination of reactive proteins of cyst fluids by SDS-PAGE. Plant Archives. 20 (1), 1072-1076.
9. **Forbes, A. B. (2021).** Parasites of cattle and sheep: a practical guide to their biology and control. pp: 89-165.
10. **Foroutan, H., Moazeni, M., Doroodmand, M. M., &Mootabi-Alavi, A. (2022).** Very low frequency waves as selective probe for *Cysticercus tenuicollis*, Hydatid cyst and *Coenurus cerebralis* bio-analysis using single cell-signal recording. Scientific Reports, 12(1), 20070.
11. **Fritsch, R.J. (2003).** Encyclopedia of Food Sciences and Nutrition || ELECTROPHORESIS. 2055–2062.

12. **Goswami, A., Das, M., &Laha, R. (2013).** Characterization of immunogenic proteins of *Cysticercus tenuicollis*of goats. *Veterinary World*, *6*(5), 267.
13. **Hama-Soor, T. A., Mohammed, A. A., &Weli, S. (2021).** Novel isolate of *Cysticercus tenuicollis*-Kalar isolate has been revealed in Kalar, Iraq. The Journal of Infection in Developing Countries, 15(10), 1532-1538.
14. **Hnasko, T. S., &Hnasko, R. M. (2015).** The western blot. ELISA: Methods and Protocols, 87-96.
15. **Jayousi, A. A. Y. (2014).** Prevalence and Molecular Characterization of *Cysticercus tenuicollis*Cysts in Sheep Slaughtered in Palestine (Doctoral dissertation). pp:1-39.
16. **Jenkins, D. J., Urwin, N. A., Williams, T. M., Mitchell, K. L., Lievaart, J. J., &Armua-Fernandez, M. T. (2014).** Red foxes (*Vulpesvulpes*) and wild dogs (dingoes (*Canis lupus dingo*) and dingo/domestic dog hybrids), as sylvatic hosts for Australian *Taenia hydatigena*and *Taenia ovis*. International Journal for Parasitology: Parasites and Wildlife, 3(2), 75-80.
17. **Jeyathilakan, N., Abdul Basith, S.,John, L., Chandran N. D. J., & Dhinakarraj, G., (2021).** Immunochemical localization of cross-reactive proteins of bladderworms with special reference to detection of specific antigen in cystic echinococcosis of sheep. ind. j. vet. & anim. sci. res., 50 (2) 37-47.
18. **Kara, M. U. R. A. T., Sarimehmetoglu, H. O., &Gonenc, B. (2003).** The determination of immune reactive proteins in *Cysticercus tenuicollis*cyst fluids by SDS-PAGE and western blotting in sheep. *Parasite*, *10*(2), 141-145.
19. **Kara, M., &Doğanay, A. (2005).** Investigation of antigenic specificity against *Cysticercus tenuicollis*cyst fluid antigen in dogs experimentally infected with *Taenia hydatigena*. *Turkish Journal of Veterinary & Animal Sciences*, *29*(3), 835-840.
20. **Kordafshari, S., Hosseini, S.H., Meshgi, B. and Youssefi, M.R. (2010).** Comparison of electrophoretic patterns of larval stages of Taeniidae and determination of specific antigens of hydatid cyst by western blotting technique. Global Veterinaria, 4(6): 601-606.
21. **Laemmli, U. K. (1970).** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. nature, 227(5259), 680-685.
22. **Mathur, V.C., Aiyasami, S.S., Latha, B.R., John, L. (2003).** Western blot analyses of *Cysticercus tenuicollis* antigens. Ind. J. Anim. Sci. 73: 837-839.
23. **McManus, D. P. (2014).**Immunodiagnosis of sheep infections with *Echinococcus granulosus*: in 35 years where have we come?. *Parasite Immunology*, *36*(3), 125-130.
24. **Miller, J. E., Kaplan, R. M., & Pugh, D. G. (2012).** Internal parasites. Sheep and goat medicine, chapter 6. pp:120
25. **Miquel, J., Khallaayoune, K., Azzouz-Maache, S., &Pétavy, A. F. (2015).** Spermatological characteristics of the genus Taenia inferred from the ultrastructural study on *Taenia hydatigena*. *Parasitology research*, *114*, 201-208.
26. **Nath, S., Pal, S., Sanyal, P. K., & Mandal, S. C. (2011).** Determination of immune reactive proteins in cystic membrane of *Cysticercus tenuicollis*of goat origin. *Indian Journal of Animal Sciences*, *81*(8), 822.
27. **Nowakowski, A. B., Wobig, W. J., & Petering, D. H. (2014).** Native SDS-PAGE: high resolution electrophoretic separation of proteins with retention of native properties including bound metal ions. Metallomics, 6(5), 1068-1078.
28. **Rafiqi, S. I., Gupta, S., Chaudhary, R., Kumar, S., Kundave, V. R., & Jawalagatti, V. (2016).** Biochemical characterization of cystic fluid antigens of *cysticercus tenuicollis* collected from bareilly region. International Journal of Science, Environment, Vol. 5, No 3, 930 – 934.
29. **Scala, A., Pipia, A. P., Dore, F., Sanna, G., Tamponi, C., Marrosu, R., Bandino, E., Carmona, C., Boufana, B., &Varcasia, A. (2015).** Epidemiological updates and economic losses due to *Taenia hydatigena*in sheep from

Sardinia, Italy. Parasitology research, 114(8), 3137–3143.

30. **Wakid, M. H., &Alsulami, M. N. (2022).** Genotyping of *Taenia hydatigena*isolated from sheep and goats in KSA based on Cox1 gene. Saudi Journal of Biological Sciences, 29(2), 1270-1275.
31. **Wondimu, A., Abera, D., &Hailu, Y. (2011).** A study on the prevalence, distribution and economic importance of *Cysticercus tenuicollis*in visceral organs of small ruminants slaughtered at an abattoir in Ethiopia. J Vet Med Anim Health, 3(5), 67-74.