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Dynamics of *Haemonchus Contortus* Coproantigen Appearance in Feces of Experimentally Infected Sheep

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Abstract

HAEMONCHOSIS is one of the most important parasitic diseases due to its economic effect on the productivity of small ruminants. Accordingly, there is a significant need for the development of accurate immunological assays for early diagnosis. Little is known about coproantigen's dynamics in the feces of *Haemonchus contortus* (*H. contortus*)- infected sheep before egg shedding. In this study, two immunodiagnostic techniques were used: antigen capture-Enzyme Linked Immunosorbent Assay (ELISA) and Enzyme-Linked Immunoelectrotransfer Blot (EITB). At 5-8 days post infection (DPI), antigen capture- ELISA detected *H. contortus* coproantigen in feces of the experimentally infected lambs. Diagnostic detection of the antigen using capture ELISA was recorded four days earlier than detection with indirect ELISA. EITB was more accurate in the early detection of coproantigen in fecal supernatants at 2nd DPI. Two specific polypeptide bands of 42 and 126 KDa strongly reacted with the sera of experimentally infected lambs. Additionally, two specific polypeptide bands of *H. contortus* coproantigen with molecular weights of 54 and 59 KDa might be considered a reliable parameter in detecting the early phase of infection before egg shedding. Reactivity against these bands was observed beginning with the 8th DPI and lasting until the experiment ended. In conclusion, *H. contortus* coproantigen could be used as a diagnostic antigen in sheep to detect haemonchosis during the prepatent period.

Keywords: *Haemonchus contortus,* coproantigen, capture ELISA, EITB, sheep.

Introduction

Haemonchosis caused by infection with *Haemonchus contortus* (*H. Contortus*) is one of the major economic and health problems affecting wild and domestic ruminants worldwide. The animals acquire the infection through ingestion of the infective larvae (L3) from infested pasture. The warm moist environment is the most favorable condition for survival of the worm. The disease is common in many areas especially in tropical and subtropical regions [1,2]. In Egypt, it is one of the main nematodes infecting sheep and goat populations and negatively affects adult livestock performance and productivity [3]. Acute infection of lambs can cause death. More frequently, chronic infection causes considerable productivity losses [4,5] because of the blood feeding nature of the parasite. The most recorded clinical signs for infected animals were anaemia, ill thrift, and subcutaneous oedema (bottle jaw); there is usually no diarrhea [6].

Historically, diagnosis of haemonchosis has been dependent on an evaluation of clinical symptoms and fecal examinations of the suspected cases. However, each of these two approaches has its drawbacks. To start with, the clinical symptoms usually appear when the infection is beginning to resolve. Additionally, *H. contortus* has a short prepatent phase of around 21-28 days (about 4 weeks), after which the infection progresses, and eggs can be detected in feces. Currently, the damage

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is already done to the animal, as the average daily blood loss in infected sheep is about 0.03 ml/parasite [7,8]. Therefore, the development of reliable schemes for controlling the disease before the exaggeration of its destructive effect on the host tissues requires an accurate early method of diagnosis, especially during the prepatent period of the disease. Immunodiagnostic techniques could be used either for antigen or antibody detection in the infected serum. Precise selection of the used antigen can assist in accurate early diagnosis of the diseases [9-12] . The possibility that diverse gastrointestinal infections might be diagnosed within feces rather than serum was suggested by Sykes and McCarthy [13]. Mainly, whole parasite crude extract specific antibodies were developed, and coated on microtiter plates. Subsequently, coproantigen was apprehended and recognized using the same or a second parasite-specific antibody in a capture assay. Immunodiagnostic assays used for antigen detection have better superiority over others, as they have precise and sensitive ability for early detection of the infection at the prepatent period. Moreover, the detection of coproantigen remains a promising tool for usage in large-scale laboratories or epidemiological studies as it overcomes the problem of cross-reactivity, false negativity, and could be used to monitor the efficacy of treatment of such diseases [14-17]. Even though diverse diagnostic techniques for haemonchosis were narrated using different types of *H. contortus* antigen in enzyme linked immunosorbent assay (ELISA) [8,18], a knowledge gap about characterization and usage of coproantigen for early diagnosis is still present. Therefore, this work aimed to characterize the initial presence and continuing dynamics of *H. contortus* coproantigen in experimentally infected sheep feces using antigen capture ELISA and immunoblotting and validate their usage as serodiagnostic tools for early detection of haemonchosis.

Material and Methods

Ethical approval

The International Animal Ethics Committee and the Ethical Committee of the National Research Centre and the current Egyptian Law and Regulations for the protection of experimental animals approved the animals' experiments and protocols in this study under the certificate No. (19150).

Haemonchus contortus larvae culture

The adult *H. contortus* females were obtained from abattoir slaughtered sheep and homogenized for egg liberations and larval culture processing to get the required L3 for infection of the experimental lambs under study [19].

Experimental infection of lambs

Five lambs of Rahmani native breed were 3-month-old, helminths-free, and hygienically maintained in individual stalls. Four lambs were experimentally infected with 20.000 L3, and the fifth lamb was kept as a negative control. One hundred grams of fecal matter and 4 ml of blood were simultaneously collected at 0, 2, 5, 8, 11, 14, and 17 DPI. The fecal matter was used in preparation of the coproantigen corresponding to the days of collection. Sera was isolated from the blood by centrifugation at 2500 rpm for 10 min and kept at -20 °C for immunological investigation.

Harmonchus contortus antigen preparation

For the preparation of coproantigen, the previously collected fecal matter was individually processed according to El-Bahi *et al.* [14]. Briefly, 6-8 KDa dialysis bags and 36 KDa polyvinylpyrrolidone were used for concentration of fecal supernatants to the required volume. For the adult somatic antigen preparation, *H. contortus* adults were recovered from abomasa of slaughtered sheep at El-Monieb abattoir according to the previously described standard procedures [20]. The collected *H. contortus* worms were used for adult somatic antigen preparation [18]. The protein concentrations were estimated according to the method of Lowry *et al.*[21].

Preparation of hyperimmune serum

For the preparation of monospecific antibodies against the adult crude *H. contortus* extract, four healthy male rabbits with a body weight of about 2 kg each were obtained. Two rabbits were subcutaneously immunized with 400 µg of the adult somatic *H. contortus* antigen used per kg of rabbit body weight, plus the same amount of complete Freund's adjuvant stirred with the antigen before inoculation. Two weeks later, two booster doses were applied with a week interval. The animals were boosted with subcutaneous injections containing the same antigen stirred with a matching amount of incomplete Freund's adjuvant. The monospecific antibodies raised against the adult somatic *H. contortus* antigen were obtained, and stored as monospecific anti-adult somatic *H. contortus* sera at -20 °C. The other two rabbits were kept as a negative control group [8].

Immunodiagnostic techniques

Indirect ELISA

To track the appearance of antibodies in the experimentally infected sheep sera during infection, adult somatic *H. contortus* antigen-based ELISA was carried out according to Kandil *et al.* [8].

Optimal dilutions of reagents were first determined by checkerboard titrations. Flat-bottomed polystyrene microtitre plates (Linbro, Flow Laboratories, Connecticut, USA) were incubated with 4µg/ well of the adult somatic *H. contortus* antigen diluted in 100 µl of coating buffer, pH 9.6, for 1 h at 37 °C, then overnight at 4 °C. After washing three times with PBS-T 0.05%, the plates were blocked with 300 µl/well of blocking buffer containing 2% non-fat milk for 2 h at 37 °C. After blocking, the previously obtained serum samples at 0, 2, 5, 8, 11, 14, and 17 DPI were diluted at 1:50 in diluting buffer and 100 µl was loaded per well and incubated for 2 h at 37 °C. After washing again as above, 100 µl/well of anti-sheep IgG (whole molecule) peroxidase antibody conjugate produced in donkey (Sigma-Aldrich, USA) was diluted at 1:1000 in diluting buffer and was applied for 1 hr at 37 °C. Finally, 100 µl substrate solution containing 0.04% (w/v) ortho-phenylenediamine (Sigma), was added to all the wells, incubated for 15 min at 37 °C, and the reaction was stopped by the addition of 100 µl of stopping buffer. The sera were considered positive when the absorbance values were more than the cut-off value (mean value plus three times the standard deviation of OD value of negative control sera). OD was read at 450 nm wavelength with an ELISA reader (BIO-TEK, Inc., ELx, 800 UV)

Coproantigen capture ELISA

Antigen-capture ELISA was performed to monitor the kinetics of appearance of the *H. contortus* coproantigen in feces of the experimentally infected lambs according to the experimental design of Shalaby *et al.* [15]. Ninety-six well microtiter plates were incubated with 100 µl/ well with rabbit monospecific anti- *H. contortus* serum at the dilution 1:20 in coating buffer, PH 9.6, at 37 °C for 1 hr, then at 4 °C overnight. After washing three times with PBS-T 0.05%, the plates were blocked with 300 µl of 2% non-fat milk dissolved in the coating buffer and incubated at 37 °C for 1 hr. After blocking, each well was incubated with 100 µl of the undiluted fecal supernatant (*H. contortus* coproantigen) at 37 °C for 1 hr, then held at 4°C overnight. The adult somatic *H. contortus* antigen was used as a control positive as follows: 100 µl of 1: 20 diluted control positive lamb sera were added to the wells and incubated for 2 hr at 37 °C. Then, 100 µl/well anti-sheep IgG secondary antibodies were diluted at 1:1000 and incubated for 1 hr at 37 °C. Finally, 100 µl substrate solution containing 0.04% (w/v) ortho-phenylenediamine (Sigma), was added to each well and the procedure was completed as explained previously.

SDS-PAGE and EITB Technique

For a more accurate determination of the dynamics of the appearance of *H. contortus* coproantigen that disseminated in the feces of the experimentally infected lambs, the fecal supernatants were extracted at 0, 2, 5, 8, 11, 14, and 17 DPI. 50ug from each extract were fractionated using 12 % acrylamide/bis-acrylamide gels under reducing conditions according to Laemmli [22] and blotted on nitrocellulose membranes for EITB [23]. After blocking the blotted nitrocellulose membranes for 1 h in 1% non-fat milk dissolved in PBS pH 7.2, primary antibody incubations were conducted overnight using positive and negative control sera diluted 1:100 in tris-buffered saline (TBS) containing 0.5% bovine serum albumin (BSA). After washing, anti-sheep immunoglobulin G (whole molecule) peroxidase antibody conjugate (Sigma-Aldrich, USA) was diluted at 1:1000 in diluting buffer (0.5% BSA in TBS) and added to the membranes for 1 hour. A final wash was completed, and the membranes were developed in a substrate solution for 5 minutes. To make the substrate solution, 30 mg 4-Chloro-1-Naphthol (Sigma-Aldrich, USA) was dissolved in 1 ml methanol. Then, 10 ml methanol was added, the solution was made up to 50 ml with TBS, and 30ul 30% H_2O_2 was added. After developing the membranes, the reactive bands were visualized using a gel documentation system (Bio-Rad, Hercules, USA).

Results

The dynamics of the emergence of *H. contortus* coproantigen in the feces of the experimentally infected lambs before egg shedding were studied. The results of coproantigen capture ELISA as in Fig.1 demonstrated that *H. contortus* coproantigen could be detected within 5 DPI in three of the four infected lambs. Within 8 DPI, coproantigen was detected in all four infected lambs. The coproantigen was consistently present during the subsequent observation period until the 17th DPI, showing slight week-to-week fluctuations during the experiment (Fig.1). On days 5 – 17 PI, the mean levels of coproantigen (measured as mean absorbance values at OD_{450}), were positive and varied from 0.48 to 0.51, above the cut-off value of 0.39.

Regarding the emergence of anti-*Haemonchus* antibodies in the lambs that were experimentally infected throughout 17 days PI (Fig. 2), the mean absorbance values (OD_{450}) revealed haemonchosis at 8 DPI in three of the four infected lambs and at 11 DPI in the last fourth lambs with mean OD_{450} value of 0.43. Then, the mean antibody levels increased slightly to 0.46 on 17 DPI.

During the first week PI, *H. contortus* coproantigens were found in the feces of 75% of the infected lambs. By the 8th DPI, this antigen had been found in every infected lamb. Furthermore, all

infected lamb sera were found to contain anti-*Haemonchus* antibodies by the 11th DPI. The comparison between coproantigen and antibody levels indicated that *H. contortus* infected lambs had detectable amounts of coproantigen four days earlier than that of antibody.

The EITB was performed to determine the antigenically active components in *H. contortus* coproantigens and establish the dynamics of their appearance at different intervals post infection with sera of experimentally infected lambs. As shown in Fig.3, the non-infected fecal supernatants (0 DPI) displayed a pattern of non-specific faint reaction with two polypeptide bands at 37 and 187 KDa. As the infection progressed, the number of recognized bands increased. Four additional polypeptide bands at molecular weights of 42, 54, 59 and 126 KDa were recognized at 2nd DPI. The reactivity against the 42 and 126 KDa bands was strong and recognized only at 2nd DPI, while a faint reaction was recognized with the 54 and 59 KDa bands. The reactivity against these bands increased as the infection progressed. Beginning at 8th DPI, this increase became apparent and lasted until the experiment's completion (17th DPI). One more doublet band at an approximate molecular weight of 45 KDa was recognized at 5th and 17th DPI.

On comparing the performance of the EITB assay with the coproantigen capture ELISA, a correlation could be discerned between the number of bands developed by the EITB and OD values disclosed by ELISA. Moreover, two specific polypeptide bands of *H. contortus* coproantigen with molecular weights of 54 and 59 KDa might be considered reliable parameters in detecting the early phase of infection before egg shedding.

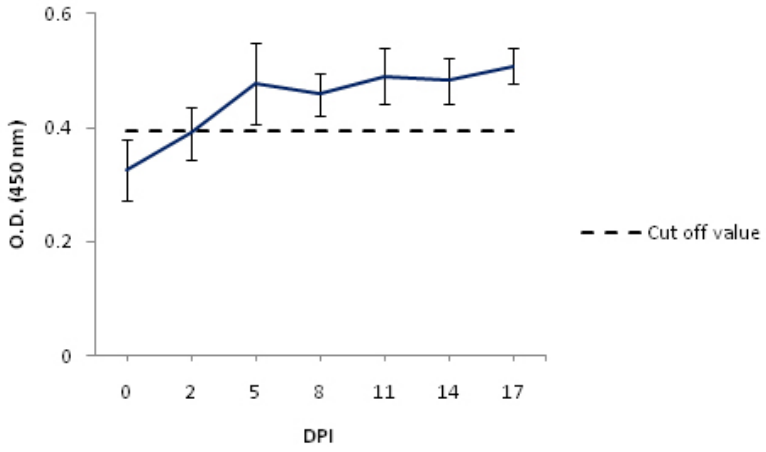

Fig.1. Mean OD_{450} values (± SD) obtained in coproantigen capture ELISA from *H. contortus* experimentally infected lambs at different times after infection (days).

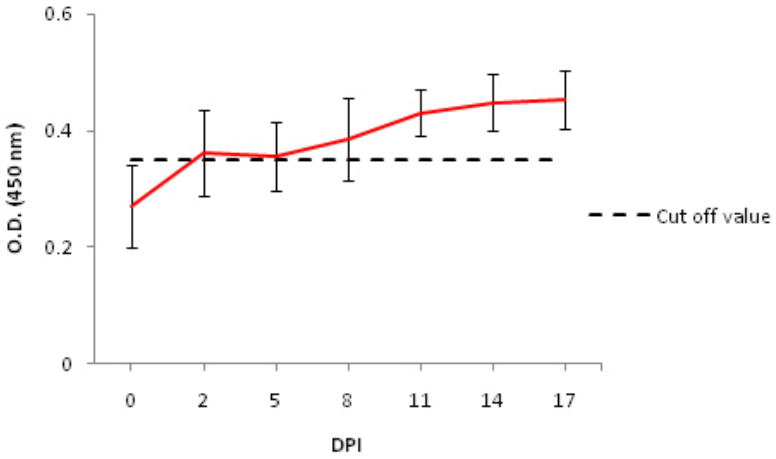

Fig. 2. Mean OD_{450} values (± SD) obtained in antibody detection ELISA from *H. contortus* experimentally infected lambs at different times after infection (days).

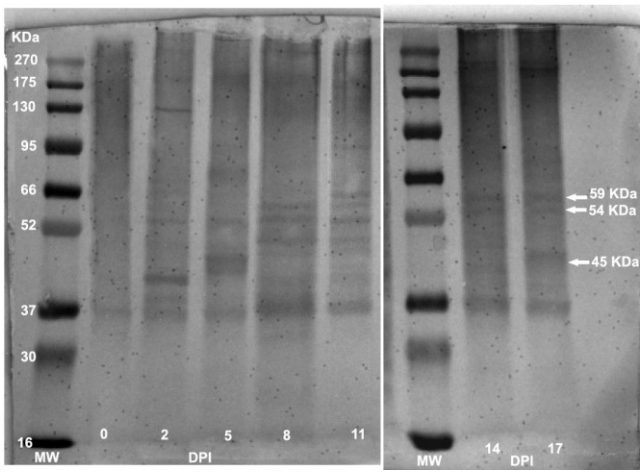

Fig. 3. Immunoblot reaction demonstrating protein bands of *Haemonchus* coproantigen in feces of infected lambs at different intervals post-infection.

Discussion

Current haemonchosis diagnosis is mainly based on microscopic examination of fecal samples for the detection of *H. contortus* eggs, which are difficult to recognize in the absence of larval identification. However, the larval identification is not without constraints [24]. Serological tests also have a limited diagnostic importance due to the persistence of antibody titers after curing. In the interval of 21 days after infection, *H. contortus* eggs in feces might be difficult to detect [19]. Even before the infection manifests any symptoms, serious damage has already been done. To get around these restrictions, there is a perceptive need for developing a reliable immunological assay like coproantigen capture for early detection of active infection. Coproantigen detection has the advantage of early diagnosis of many parasitic diseas es before shedding of the diagnostic stage in feces [25]. Although several diagnostic assays for animal gastrointestinal nematode infections had been described by the detection of coproantigen [26,27], little is known about the dynamics of the appearance of *H. contortus* coproantigen in feces of experimentally infected lamb before egg shedding. In the present study, two immunodiagnostic techniques were used for this purpose: antigen capture ELISA and EITB. Although both techniques were simple and easily applied, EITB was more accurate and sensitive in the early detection of *H. contortus* coproantigen. In the coproantigen capture ELISA, the *H. contortus* coproantigen was detected in lamb's feces within 5-8 DPI. There had previously been one record demonstrating coproantigen detection for *H. contortus*. In this study, the *H. contortus* antigen, which is expressed on the parasite's surface cuticle, was detected in the feces of infected sheep using an antigen detection ELISA method [24]. Furthermore, Nageswaran *et al.* [28] examined the potential for coproantigen detection in rats experimentally infected with *Strongyloides ratti* for the diagnosis of strongyloidiasis. Five days after infection, a capture ELISA identified the coproantigen, which concurred with the appearance of eggs and larvae in the feces. Peak antigen levels were found 9 days after infection and stayed relatively high until day 25. In a closely related nematode, *Teladorsagia circumcincta*, a capture ELISA for the detection of *T. circumcincta* coproantigen in sheep was developed, and it demonstrated high sensitivity for detecting 0.25 µg of parasite ES per ml of fecal supernatant [26].

In the current study, the *H. contortus* infected lambs had detectable amounts of coproantigen four days earlier than that of the antibody. This finding might be related to the exsheathing of the infective third stage (L3) after ingestion by a grazing host, such as sheep, in the forestomach and travel to the abomasum, where they commence to feed. After 1.5 – 2 days post infection, the larvae then moult to the fourth stage (L4), growing quickly and feeding on blood [19]. The coproantigen capture ELISA in this work gave a positive reaction from the 5th DPI and remained positive during the subsequent observation period until the 17th DPI showing slight week to week fluctuations during the experiment. These fluctuations in the antigen level might be reflected in the development of the nematode parasite [29].

For more accurate determination of coproantigen appearance dynamics in feces of the infected lambs, five polypeptide bands with molecular weights of 42, 45, 54, 59, and 126 KDa were found in fractionated fecal supernatants at various intervals post infection and were recognised by sera of experimentally infected lambs; these bands were not found in non-infected fecal supernatants. The reactivity against the 42 and 126 KDa bands was strong and recognized only at 2nd DPI. These

detected bands could be associated with exsheathing of L3 and molting to L4. As the infection progressed, the reactivity against the 54 and 59 KDa bands increased. The increase was clear starting at the 8th DPI and lasting until the experiment ended. This increase in band reactivity might be related to the larval development and the beginning of blood feeding that occurred on the 11th DPI [30]. In this context, Ellis *et al.* [24] characterized the *H. contortus* coproantigen that was expressed on the parasite cuticle revealing three polypeptide bands at molecular weights of 49, 65, and 122 KDa. Kandil *et al.* [18] recorded two immune-dominant polypeptide bands at molecular weights of 57 and 59 KDa in *H. contortus* larval, adult somatic, and excretory-secretory antigens.

Conclosions

According to the findings of this study, coproantigen is an effective immunodiagnostic tool for early diagnosis of haemonchosis in sheep. Its determination in feces four days earlier than antibody detection in the serum of infected lambs is an advantage over the conventional serological assays. Moreover, two specific polypeptide bands of *H. contortus* coproantigen with molecular weights of 54 and 59 KDa might be considered a reliable parameter in detecting the early phase of infection before egg shedding.

Conflict of interest

The authors declare that there is no conflict of interests.

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Authors' contributions

HAS, OMK, SHMH, shared in conceptualization and research design. All authors contributed to experimental work and data analysis. HAS, SHMH, BSME, OMK, HMA, AHE and EBA shared in preparation and writing of the manuscript. All authors contributed to the critical review and approval of the final manuscript.

References

1. Adduci, I., Sajovitz, F., Hinney, B., Lichtmannsperger, K., Joachim, A., Wittek, T. and Yan, S. Haemonchosis in Sheep and Goats, Control Strategies and Development of Vaccines against *Haemonchus contortus*. *Animals,* **12,** 2339 (2022). doi:10.3390/ani12182339
2. Carson, A., Reichel, R., Bell, S., Collins, R., Smith, J. and Bartley, D. Haemonchus contortus : an overview. *Veterinary Record,* **192**,26–28 (2023). doi:10.1002/vetr.2613.
3. Kandil, O., Shalaby, H.A., Hendawy, S.H., Abdelfattah, M.S., Sedky, D., Hassan, N.M., El Namaky, A.H., Ashry, H.M., Abu EL Ezz, N.M., Mahmoud, M.S. and Mahmoud, A.A. In vitro and in vivo anthelmintic efficacy of condensed tannins extracted from the seeds of alfalfa (Medicago sativa L.) against *Haemonchus contortus* infection. *Iraqi Journal of Veterinary Science,* **37**,229–237(2022). doi:10.33899/ijvs.2022.133537.2247.
4. Hassan, N.M.F., Aboelsoued, D., Farag, T.K., Hassan, S.E. and Abu El Ezz, N.M.T. Assessment of Haemonchus contortus larval and adult somatic antigens in sero-diagnosis of haemonchosis in naturally infected sheep and goats. *Journal of Parasitic Diseases,* **43** ,718–725 (2019). doi:10.1007/s12639-019-01152-0
5. Hendawy, S.H.M. Immunity to gastrointestinal nematodes in ruminants: effector cell mechanisms and cytokines. *Journal of Parasitic Diseases,* **42**,471–482 (2018). doi:10.1007/s12639-018-1023-x
6. Besier, R.B., Kahn, L.P., Sargison, N.D. and Van Wyk, J.A. Diagnosis, Treatment and Management of Haemonchus contortus in Small Ruminants. *Advanced Parasitology,* **93**,181–238 (2016). doi:10.1016/bs.apar. 2016.02.024
7. Herath, H.M.P.D., Taki, A.C., Sleebs, B.E., Hofmann, A., Nguyen, N., Preston, S., Davis, R.A., Jabbar, A. and Gasser, R.B.,. Advances in the discovery and development of anthelmintics by harnessing natural product scaffolds. *Advanced In Parasitology,* **111,**203–251 (2021). doi:10.1016/bs.apar.2020.10.002
8. Kandil, O.M., Gamil, I.S., Hendawy, S.H.M., Medhat, F. and El-Habit, O.H.. Efficacy of glutathione-S-transferase purified antigen of the gastro-intestinal nematode *Haemonchus contortus* in diagnosis of sheep haemonchosis. *Journal of Parasitic Diseases,* **41**,968–975 (2017a). doi:10.1007/s12639-017-0920-8
9. Hade, B.F., Al-Biatee, S.T. and Al-Rubaie, H.M. Traditional and molecular diagnosis of *Haemonchus contortus* in sheep in Babylon province, Iraq. *Iraqi Journal of Veterinary Science,* **36** (2),479-481 (2022). doi:10.33899/ijvs.2021.130533.1842
10. Birhan, M., Gesses, T., Kenubih, A., Dejene, H. and Yayeh, M. Evaluation of anthelmintic activity of tropical *tani ferous* plant extracts against *Haemonchus contortus*. *Veterinary Medicine (Auckl),* **11**, 109-117 (2020). doi: 10.2147/VMRR.S225717
11. Kandil, O., Hassan, N., Sedky, D. and Beshir Ata, E. Studies on the specific immunodiagnosis of cystic echinococcosis in camels using enzyme-linked immunosorbent assay. *Bulgarian Journal of Veterinary Medecine,* **22**,305–313 (2019). doi:10.15547/bjvm.2136

12. Kandil, O.M.O.M., Abdelrahman, K.A.A., Mahmoud, M.S.S., Mahdy, O.A.O.A., Ata, E.B.E.B., Aloufi, A.S.A.S.A.S. and Al-Olayan, E. Cystic echinococcosis: Development of an intermediate host rabbit model for using in vaccination studies. *Expermintal Parasitology,* **208**,107800 (2020). doi:10.1016/j.exppara.2019.107800

13. Sykes, A.M. and McCarthy, J.S. A coproantigen diagnostic test for Strongyloides infection. *PLoS Neglected Tropical Diseases,* **5**, e955 (2011). doi:10.1371/journal.pntd.0000955

14. El-Bahi, M.M., Malone, J.B., Todd, W.J. and Schnorr, K.L. Detection of stable diagnostic antigen from bile and feces of *Fasciola hepatica* infected cattle. *Veterinary Parasitology,* **45**,157–67 (1992). doi:10.1016/0304-4017(92)90039-c

15. Shalaby, S., El-Bahy, M., Hassan, A., Shalaby, H. and Gupta, N. Diagnotic value of some *Fasciola gigantica* antigens. *Journal of Parasitic Diseases,* **39**, 479–86 (2015). doi:10.1007/s12639-013-0379-1

16. Shalaby, S.I., El-Bahy, M., Shalaby, S.I.A., Shalaby, H., Gupta, N. and Gupta, D.K. Detection of Coproantigens by Sandwich ELISA in Rabbits Experimentally Infected with *Fasciola gigantica*. *Iranian Journal of Parasitology,* **9**, 374–81 (2014).

17. Mutombo, P.N., Man, N.W.Y., Nejsum, P., Ricketson, R., Gordon, C.A., Robertson, G., Clements, A.C.A., Chacón-Fonseca, N., Nissapatorn, V., Webster, J.P. and McLaws, M.-L. Diagnosis and drug resistance of human soil-transmitted helminth infections: A public health perspective. *Advanced Parasitology,* **104**, 247–326(2019). doi:10.1016/bs.apar.2019.02.004

18. Kandil, O.M., Hendawy, S.H.M., El Namaky, A.H., Gabrashanska, M.P. and Nanev, V.N. Evaluation of different *Haemonchus contortus* antigens for diagnosis of sheep haemonchosis by ELISA and their cross reactivity with other helminthes. *Journal of Parasitic Diseases,* **41**, 678–683 (2017b). doi:10.1007/s12639-016-0865-3

19. Soulsby, E.J.L. Helminths, arthropods and protozoa of domesticated animals (7th edition), Transactions of the Royal Society of Tropical Medicine and Hygiene. Bailliere Tindall, London (1986).

20. Maff, H. M. Manual of Veterinary Parasitological Techniques. London, UK, in: Technical Bulletin. Her Majesty's Stationary Office, London, United Kingdom (1986).

21. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry,* **193**, 265–275(1951).

22. Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685 (1970). doi:10.1038/227680a0

23. Towbin, H., Staehelin, T. and Gordon, J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Science,* **76**, 4350–4354 (1979). doi:10.1073/pnas.76.9.4350

24. Ellis, T.M., Gregory, A., Turnor, R., Kalkhoven, M. and Wroth, R.H. Detection of Haemonchus contortus surface antigen in faeces from infected sheep. *Veterinary Parasitology,* **51**, 85–97(1993). doi:10.1016/0304-4017(93)90199-w

25. Allan, J.C., Avila, G., Garcia Noval, J., Flisser, A. and Craig, P.S. Immunodiagnosis of taeniasis by coproantigen detection. *Parasitology,* **101** (Pt 3), 473–477 (1990). doi:10.1017/s0031182000060686

26. Johnson, D.A., Behnke, J.M. and Coles, G.C. Copro-antigen capture ELISA for the detection of Teladorsagia (Ostertagia) circumcincta in sheep: improvement of specificity by heat treatment. *Parasitology,* **129**, 115–126 (2004). doi:10.1017/s0031182004005256

27. Johnson, M.J., Behnke, J.M. and Coles, G.C. Detection of gastrointestinal nematodes by a coproantigen capture ELISA. *Research In Veterinary Science,* **60**,7–12(1996). doi:10.1016/s0034-5288(96)90122-8

28. Nageswaran, C., Craig, P.S. and Devaney, E. Coproantigen detection in rats experimentally infected with Strongyloides ratti. *Parasitology,* **108**,335–42. (1994). doi:10.1017/s0031182000076174

29. Nonaka, N., Iida, M., Yagi, K., Ito, T., Ooi, H.K., Oku, Y. and Kamiya, M. Time course of coproantigen excretion in Echinococcus multilocularis infections in foxes and an alternative definitive host, golden hamsters. *International Journal of Parasitology,* **26**,1271–1278 (1996). doi:10.1016/s0020-7519(96)00117-8

30. Rodríguez, A. V, Goldberg, V., Viotti, H. and Ciappesoni, G. Early detection of Haemonchus contortus infection in sheep using three different faecal occult blood tests. *Open Veterinart Journal,* **5,**90–97 (2015).

ديناميكيه ظهور مستضد الهمونكس كونترتس في روث الاغنام المصابة تجريبيا

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يعتبر مرض الهمونكوس احد أهم الأمراض الطفيلية نظرا لتأثيره على إنتاجية المجترات الصغيرة . وبناء على ذلك، هناك احتياج مهم لتطوير تقنيات مناعية دقيقة للتشخيص المبكر للمرض. لا يُعرف الا القليل عن ديناميكه ظهور المستضد البرازي (كوبروانتيجن) لديدان الهمونكس كونترتس قبل ظهور البيض في روث الاغنام المصابة بالعدوي. في هذه الدراسة، تم استخدام تقنيتين للتشخيص المناعي عند 5 الي 8 ايام من العدوي التجريبية بديدان الهمونكس كونترتس للحملان:

In Direct-ELISA) اختبار الامتزاز المناعي المرتبط بالانزيم (الاليزا، EITB)-

- اختبار الطبع المناعي المرتبط بالإنزيم (اوضحت الدراسة ظهور المستضد البرازي (كوبروانتيجين) باستخدام اختبار الامتزاز المناعي الملتقط وذلك مبكرا قبل اربعه ايام عند استخدام ولكن قد كان اختبار الطبع المناعي المرتبط بالإنزيم اكثر دقه في الكشف (In Direct-ELISA) اختبار الامتزاز المناعي المرتبط بالإنزيم المبكر لظهور المستضد البرازي (كوبروانتيجن) في مستخلص روث الحملان المصابة تجريبيا كما ظهرت عدد 2 حزمه بروتينيه عند الوزن الجزيئي 42 و 126 كيلو دالتون عند تفاعل ضد مصل الحملان المصابة تجريبيا . بالإضافة الي ظهور عدد 2 حزمه بروتينيه اخري عند الوزن الجزيئي 54 و 59 كيلو دالتون التي يمكن ان تعتبر مقياس للكشف المبكر للعدوي بديدان الهمونكس كونترتس في مرحله ما قبل ظهور البيض حيث ظهرت هذه الحزم البروتينيه من اليوم الثامن للعدوي التجريبية واستمرت حتي نهاية التجربة .

بالملخص ، يمكن اعتبار المستضد البرازي (كوبرو انتيجن) لديدان الهمونكس كونترتس مستضد تشخيصي للكشف المبكر لمرض الهمونكوزس في الاغنام في مرحله ما قبل ظهور الاعراض السريريه.

الكلمات الدالة: همونكس كونترتس، مستضد ،اليزا، الاغنام.