



Protoscolex metabolites of *Coenurus cerebralis* as antigenic-produced humoral immune response in sheep

S.S. Alkhuzaie 

Department of Veterinary Microbiology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah, Iraq

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Correspondence:

S.S. Alkhuzaie

sora.hamada@qu.edu.iq

Abstract

The purpose of the current experiment was to test the immunization against *Coenurus cerebralis* in sheep. Sixteen animals (6 months old, from 1 October 2020 to 30 March 2021 in Najaf city) were recruited to perform the experiment, in which eight of them were injected twice at 21-day interval using the cellular metabolic antigen of *C. cerebralis* protoscolex cultivated and then emulsified by complete Freund's adjuvant (CFA). The shots were injected intramuscularly at a dose of 1 ml (15 mg of antigenic protein determined in a separate experiment). The second group of eight sheep served as controls (injected intramuscularly with 1 ml sterile saline only at the days of injections). Blood samples were collected from all animals at day-0 (before injection) and at day 10, 18, and 24 after the first injection, and at day 10, 16, 26, 40, 48, 53, 61, 80, 85, and 89 after the second injection. Serum activity was studied by indirect enzyme-linked immunosorbent assay (iELISA). The findings, by iELISA, revealed that the cellular antigen of *C. cerebralis* protoscolices is an active stimulator of antibody response. Day-10 (after the first injection) showed significantly ($P < 0.05$) 3.4 to 9.9 time-higher antibody levels compared to those from day-0. This elevation in the titer of antibodies was increased after receiving the second dose showing 6.3 to 12 time-higher antibody presence even at the final days of blood collection compared to those from day-0. No changes were noticed in the sera of the control animals. The obtained data allow us to conclude that metabolites synthesized by cultivation are active immunogenic components that activate the humoral part of the immune system manifested by the increases in the antibody titers. This gives a solid ground for future work regarding alternative methods of discovering immunization techniques against cestodes.

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Introduction

Taenia multiceps (*Multiceps multiceps*) is a taeniid cestode which infests the small intestine of dogs, coyotes, foxes, and jackals as final host during its adult phase (1). *C. cerebralis* larval stage is commonly located in the nervous systems of small ruminants such as sheep, goats and large ruminants such as buffaloes, cattle, camels, and equids leading to cerebral coenurosis marked by neurological abnormalities (2,3). Non-cerebral coenurosis has also been identified in other parts of the body such as in

the muscular tissues and visceral organs of the affected intermediate hosts (4,5). Small ruminants have a very high infestation risk with *C. cerebralis* (6). In small ruminants, coenurosis is linked to disease burden, fatality and major economic losses (7). Reports of human infection have been identified in Egypt, Canada, France, and the United States. Infection occurs by the fecal-oral path, which involves contaminated food and/or water with the infective stage (eggs) (6).

Clinical signs, epidemiological occurrences, histological defects and morphological characteristics of *C. cerebralis*

have generally been used to diagnose taeniid tapeworms (8). The important aspects for morphometric recognition are hook number, size and shape (9); nevertheless, these parameters are subject to errors particularly in the situation of sterile, young or degraded coenurior also in the maturity phase. Molecular tests have been extensively utilized in research relating to population biology, epidemiological and phylogenetic studies as well as in distinguishing the identity of cysts of different taeniids (10).

Small ruminant production is regarded as a critical component of long-term economic growth in the developing countries. Nevertheless, these infections have been identified as a significant serious obstacle for the livestock farming industries leading to stagnant economic growth (11). The purpose of the current experiment was to test the immunization against *Coenurus cerebralis* in sheep.

Materials and methods

Samples and experimental design

Sixteen sheep (6 months old) (From 1 October 2020 to 30 March 2021 in Najaf city) were recruited to perform the experiment, in which eight of them were injected twice at 21-day interval using the cellular metabolic antigen of *C. cerebralis* protoscolex. The second group of eight sheep served as controls (12). Blood samples were collected from all animals at day-0 (before injection) and at day 10, 18, and 24 after the first injection, and at day 10, 16, 26, 40, 48, 53, 61, 80, 85, and 89 after the second injection.

Production of protoscolex metabolites

Metabolites for experimental antigenicity were obtained by cultivating *C. cerebralis* protoscolex cells in a Heraeus CO₂ based incubator at 37°C using automated control of the gases (CO₂ - 5%, O₂ - 95%) at 70% humidity in RPMI-1640 medium. The antigens were then emulsified by CFA. The metabolites synthesized in the process of cultivation of

C. cerebralis protoscolex cells are active immunogens that activate the humoral component of immune system, which is manifested by synthesis of specific antibodies and gives grounds to speak about an alternative method of obtaining immune drugs from cestodes.

Experiment

The shots were injected intramuscularly at a dose of 1 ml (15 mg of antigenic protein determined in a separate experiment which discussed in production of protoscolex metabolites) in the treatment groups. The control sheep were injected intramuscularly with 1 ml sterile saline only at the days of injections.

IELISA

Serum activity was studied by iELISA. After determining the optimal antigen concentration, solid phase sensitization was performed using the cellular antigen. The conjugate in the reaction was antibodies based on peroxidase labeling prepared against sheep serum IgG (13). The reaction was evaluated using an automated colorimetric enzyme-immunoassay analyzer 340/ATC (STL-Labsystems, Austria), at 492 nm of a wavelength to measure the optical density (OD) (14).

Results

The findings, by iELISA, revealed that the cellular antigen of *C. cerebralis* protoscolices is an active stimulator of antibody response. Day-10 (after the first injection) showed significantly ($P < 0.05$) 1.6 to 2.1 time-higher antibody levels compared to those from day-0 (Table 1).

This elevation in the titer of antibodies was increased after receiving the second dose showing 0.151 to 0.291 time-higher antibody presences even at the final days of blood collection compared to those from day-0. No changes were noticed in the sera of the control animals (Table 2).

Table 1: Antibody titers in sheep injected with cellular antigens of *C. cerebralis* protoscoleces

Days of study	Optical density in IER* in experimental animals			Mean ± SE
10**	2.107	1.822	1.611	2.383±0.05
18	2.322	2.011	1.980	2.104±0.01
24	2.111	2.321	2.032	2.154±0.04
31\10***	2.211	2.377	1.911	2.166±0.016
37\16	2.011	1.711	1.924	1.882±0.052
47\26	2.110	2.011	2.111	2.077±0.061
61\40	2.326	2.222	2.300	2.282±0.043
69\48	2.439	2.441	2.654	2.511±0.053
74\53	2.654	2.352	2.454	2.486±0.034
82\61	2.591	2.524	2.311	2.475±0.054
101\80	2.422	2.655	2.611	2.562±0.044
106\85	2.510	2.811	2.721	2.680±0.056

* IER: Immunoenzymatic reaction ($P \leq 0.001$). ** First Injection. *** Second injection.

Table 2: IER values with sera of control sheep

Days of study	Optical density in IER* in experimental animals			Mean±SE
0	0.241	0.211	0.196	0.216±0.033
15	0.251	0.234	0.211	0.227±0.051
29	0.291	0.218	0.244	0.251±0.045
38	0.284	0.204	0.214	0.234±0.044
43	0.231	0.233	0.197	0.220±0.036
51	0.288	0.190	0.188	0.224±0.043
58	0.289	0.241	0.231	0.253±0.045
77	0.281	0.151	0.244	0.225±0.056
110	0.280	0.177	0.213	0.223±0.046

* IER: Immunoenzymatic reaction ($P \leq 0.001$).

Discussion

Even through these species-specific variations, cestode has been revealed to convert/regulate the host reaction to their body parts (immunoregulation). These organisms, nevertheless, may change the immune reaction to antigens, such as vaccinations with long-term persistent infection. They've also been linked to a change in the incidence of inflammatory bowel disease, diabetes, and arthritis (15-20). Since parasites have the ability to control the host immune response, which can be partly restored by anthelmintic medications, there has been a lot of curiosity more about the pathways behind helminth-induced immune modulations and the parasite-encoded compounds that could be causing it. The excretory/secretory products of helminth parasites have gotten the most interest, since they may be used as alternative therapeutics for inflammatory and autoimmune diseases or as candidates for anthelmintic vaccinations, diagnostic tools, and medications (21).

Our results indicate high antibody response as it identified by the ELISA method. This agrees with the fact that metabolites of the *C. cerebralis* protoscolex cells may provide a strong immunogenic agent that could give a solid protection against the cystic stage of the cestode (21). Coenurosis is a lethal central nervous system condition triggered by the larval coenurus of the tapeworm *T. multiceps* in both sheep and humans. Despite the availability of medication and preventive services, controlling coenurosis remains a difficult task. Li *et al* (22) have exhibited a significant genome sequence of *T. multiceps*, length of 44.8 Mb and an effective assembly of 240 Mb 96% of the genome utilizing Pacbio single-molecule real-time and Hi-C results. Furthermore, they have discovered many genes that encode proteins participating in proglottid development and associations with the host central nervous system, which may help *T. multiceps* adjust to its parasitic lifestyle. They have suggested that their research not only sheds light on the genetics and development of *T. multiceps*, but also establishes a range of species-specific gene targets that could be used to establish new coenurosis treatments and

controls. Exactly, this agrees with our findings in which metabolites of the cells belong to the *C. cerebralis* protoscolex have led to the production of immune responses represented by the increases in the circulating antibodies.

The identification of helminth-secreted extracellular vesicles (EVs) has proposed a new model in the research of host-parasite communication. EVs are generated by a wide range of cell types and organisms, such as parasites. EVs are a cell-to-cell exchange system that happens at homeostasis by the movement of genetic information, proteins, lipids, and signals. Some studies have identified those vesicles as immunogenic materials to produce protection against certain parasites (21-26).

Conclusion

The obtained data allow us to conclude that metabolites synthesized by cultivation are active immunogenic components that activate the humoral part of the immune system manifested by the increases in the antibody titers. This gives a solid ground for future work regarding alternative methods of discovering immunization techniques against cestodes.

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Conflict of interests

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نواتج أيض الرؤيسات للطور اليرقي للرأسفة المخية كموااء مسؤضفة للاستجابة المناعفة الخطفة فف الأغانم

سرى سعد حماءة الخزاعف

فرع الأءفاء المءهرفة البفرفة، كلفة الطب البفررف، ءامعة القاءسفة،
مءافظة الءفوانفة، العزاق.

الءلاصة

كان الغرض من الأءربة الءالفة هو أءءار الأءصفن ضد أكفاس
الرأسفة المخفة فف الأغانم. تم اسؤءام سؤة عشر ءفوانا (بعمرف ٦
أشهر) للفترة من بءافة شهر ئشرفن الأول ٢٠٢٠ ولءافة نءافة شهر
أءار ٢٠٢١ فف مءفنة النءف) لإءراء الأءربة، ءفء تم ءفن ءمانفة
منهم مرؤفن بفاصل ٢١ فوما باسؤءام مسؤضء الأءمفل العءائف الءلوف
لرؤفساء الرأسفة المخفة المزروع والمسؤءب بواسؤة المرسب
الءامل. تم الءفن العسلف بءرعة ١ مل (١٥ ملء من البروؤفن
المسؤضءف تم الأءفء الأءرفز فف ءءربة منفصلفة). المءموة الأءففة من
ءمانفة أغانم كانؤ بمؤافة مءموة سفررة (ءفنؤ عسلفا بمءلوف ملءف
مءقم ١ مل فقط فف أفاام الءفن). تم ءمع عفناء الءم من ءمفع
الءفواناء فف الفوم ٠ (قبل الءفن) وفف الفوم ١٠ و ١٨ و ٢٤ بعء
الءفن الأول وفف الفوم ١٠ و ١٦ و ٢٦ و ٤٠ و ٤٨ و ٥٣ و ٦١ و ٨٠
و ٨٥ بعء الءفن الأءف. تمؤ ءراسة نساؤ المصل عن طررف
فءص الامؤصاص المناعف ءفر المباشرف المرؤبب بالإنزفم. كئشفؤ
النءاء أن المسؤضء الءلوف لرؤفساء الرأسفة المخفة هو مءفر نساؤ

حيوانات السيطرة. تسمح لنا البيانات التي تم الحصول عليها بالاستنتاج أن المستضدات التي يتم تحضيرها عن طريق الزراعة هي مكونات مناعية نشطة والتي تنشط الجزء الخلطي من الجهاز المناعي الذي يتجلى في الزيادة في عيار الأجسام المضادة. وهذا يعطي أرضية صلبة للعمل المستقبلي فيما يتعلق بالطرق البديلة لاكتشاف تقنيات التحصين ضد الديدان الشريطية.

لاستجابة الأجسام المضادة. أظهر اليوم ١٠ (بعد الحقن الأول) مستويات أضعاف أعلى بشكل ملحوظ (>0.05) ٣,٤ إلى ٩,٩ مرة مقارنة بتلك الموجودة في اليوم صفر. لوحظت زيادة هذا الارتفاع في عيار الأجسام المضادة بعد تلقي الجرعة الثانية مما أظهر وجود أجسام مضادة أعلى من ٦,٣ إلى ١٢ مرة حتى في الأيام الأخيرة من جمع الدم مقارنة بتلك الموجودة في اليوم ٠. لم يلاحظ أي تغييرات في مصل