



جامعة الموصل

كلية الطب البيطري

التشخيص المجهرى والجزيئى لطفيلي الابواغ الخبية في الأغنام في محافظة نينوى/العراق

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رسالة ماجستير

الطب البيطري / الطفيليات البيطرية

بإشراف

الأستاذ الدكتورة

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الخلاصة

أجريت هذه الدراسة في محافظة نينوى على 200 عينة جمعت من براز الأغنام و50 عينة حليب جمعت من النعاج في الفترة من شهر آب 2024 ولغاية نهاية شهر شباط 2025، وفحصت العينات في مختبر الطفيليات في فرع الاحياء المجهرية في كلية الطب البيطري/ جامعة الموصل إذ جرى تحليل النماذج بطريقة الفحص المجهرى وطريقة الفحص الجزيئي وكانت نسبة الإصابة الإجمالية في الفحص المجهرى 52% من مجموع 200 عينة براز. وأظهرت نتائج الفحص المجهرى أن أكياس بيض طفيلي الأبواغ الخبيثة ظهرت عند استخدام الصبغة الصامدة للحامض بشكل كروي أو دائري وذات لون أحمر على خلفية زرقاء، أما عند استخدام صبغة الأكردين البرتقالية ظهرت أكياس بيض الطفيلي بأشكال دائرية أو كروية ذات لون برتقالي على خلفية خضراء، وكذلك ظهرت هذه عند استخدام صبغة اليود بشكل كروي أو بيضوي محاطة بهالة شفافة. وكانت نسبة تواجد أكياس بيض الطفيلي 56% من مجموع 50 عينة حليب. وأكدت نتائج هذه الدراسة أن الإصابة بطفيلي الأبواغ الخبيثة لها علاقة بأشهر الدراسة إذ بلغت أعلى نسبة 85.7% في شهر آب أما أقل نسبة سجلت في شهر كانون الثاني بلغت 20% وإن لعمر الحيوان تأثيراً معنوياً على نسبة الإصابة بطفيلي الأبواغ الخبيثة وإن كل الفئات العمرية معرضة للإصابة بهذا الطفيلي إذ بلغت نسبة الإصابة بالحملان من (1-3) شهر 95.45% وإن نسبة الإصابة تتناقص مع تقدم عمر الحيوان وكذلك أظهرت الدراسة وجود اختلاف معنوي بنسبة الإصابة بين الذكور والإناث فكان معدل الإصابة 75.5% و 32.7% في الذكور والإناث على التوالي. وأشارت النتائج أن عدم وجود الإسهال لا يستثني تواجد الطفيلي إذ كانت نسبة الإصابة في البراز الطبيعي 52% ومن حالات الإسهال كانت نسبة الإصابة 50% فضلاً عن ذلك أكدت نتائج الدراسة أن هناك عدة عوامل لها تأثير معنوي على نسبة الإصابة بطفيلي الأبواغ الخبيثة مثل حجم القطيع ونوع التربية وحالة النعاج بوجود حمل أو عدم وجود حمل وطبيعة الأرضية التي تربي عليها الحيوانات، وأكدت نتائج الفحص المجهرى بواسطة إجراء الفحص الجزيئي للعينات الشديدة الإصابة من خلال استخلاص الحمض النووي من أكياس بيض الطفيلي المطروحة في براز الأغنام. وأظهرت نتائج تسلسل الحمض النووي باستخدام جين *18srRNA* وجود ثلاثة أنواع من طفيلي الأبواغ الخبيثة وهي *C. parvum* و *C. hominis* و *C. ubiquitum* وتعتبر هذه الدراسة الأولى في تسجيل أنواع طفيلي الأبواغ الخبيثة في الأغنام في محافظة نينوى وقد سجلت ست عزلات تحت اسم RFMN-2024 وحملت رقم التسجيل في بنك الجينات. كما أكد تحليل الشجرة

التطورية الوراثية للسلاسل المعزولة وجود تقارب بين السلاسل المحلية للطيفي المعزولة مع بقية السلاسل المسجلة في بنك الجينات والمعزولة في بعض محافظات العراق وكذلك وجود تقارب مع بقية العترات المعزولة بدول أخرى حول العالم.

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Microscopic and Molecular Diagnosis of The *Cryptosporidium* Parasite in Sheep in Nineveh Governorate/ Iraq

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MSc/Thesis

Veterinary Medicine / Veterinary Parasitology

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Abstract

This study was conducted in Nineveh Province on 200 fecal samples collected from sheep and 50 milk samples collected from ewes during the period from August 2024 until the end of February 2025. The samples were examined at the Parasitology Laboratory, Department of Microbiology, College of Veterinary Medicine, University of Mosul, using both microscopic and molecular diagnostic techniques. The overall infection rate detected by microscopy was 52% among the 200 fecal samples. Microscopic analysis revealed that *Cryptosporidium* oocysts appeared spherical or round and stained red against a blue background when using the acid-fast stain. When using acridine orange stain, the oocysts appeared as circular or spherical orange structures on a green background, while with iodine staining they appeared spherical or oval surrounded by a clear halo. The presence of *Cryptosporidium* oocysts was also detected in 56% of the 50 milk samples.

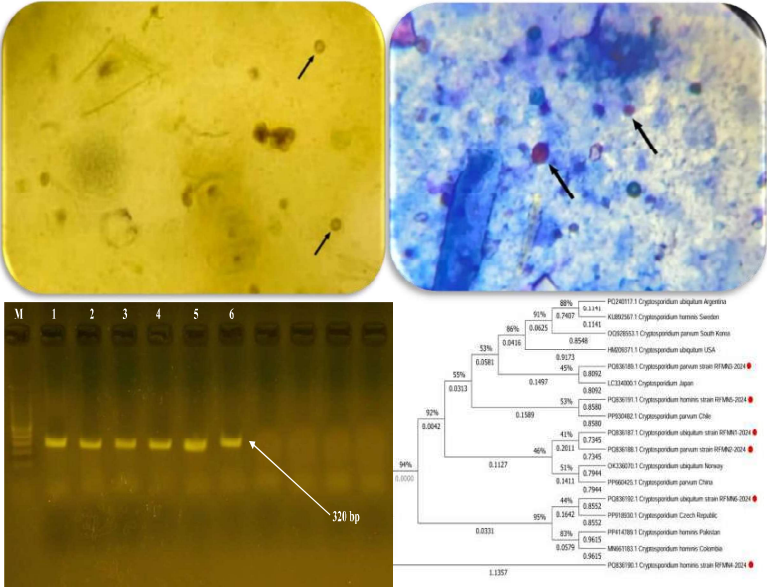
The results of this study confirmed a correlation between the month of sampling and the infection rate, with the highest prevalence recorded in August (85.7%) and the lowest in January (20%). Animal age had a significant effect on infection prevalence, as all age groups were susceptible to infection. The highest rate (95.45%) was observed in lambs aged 1–3 months, and the infection rate decreased progressively with age. A significant difference was also found between sexes, with males showing an infection rate of 75.5% compared to 32.7% in females.

The study also showed that the absence of diarrhea did not exclude the presence of the parasite, since 52% of normal fecal samples and 50% of diarrheic samples were positive. In addition, several factors showed significant effects on infection prevalence, including herd size, type of husbandry, pregnancy status of ewes, and the nature of the housing floor.

Microscopic findings were further confirmed by molecular testing of highly infected samples through DNA extraction from *Cryptosporidium* oocysts shed in sheep feces. Sequencing of the 18S rRNA gene revealed three species of *Cryptosporidium*: *C. parvum*, *C. hominis*, and *C. ubiquitum*. This is the first study to report these *Cryptosporidium* species in sheep in Nineveh Province. Six isolates were deposited under the name RFMN-2024 and registered in the GenBank database. Phylogenetic analysis demonstrated a genetic similarity between the local isolates and strains previously recorded in other Iraqi provinces as well as strains isolated from other countries around the world.

Microscopic and Molecular Diagnosis of The Cryptosporidium Parasite in Sheep in Nineveh Governorate/ Iraq

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HIGHLIGHTS	GRAPHICAL ABSTRACT
• This study was conducted in Nineveh Province on 200 fecal samples collected from sheep and 50 milk samples collected from ewes during the period from August 2024 until the end of February 2025. • The overall infection rate detected by microscopy was 52% among the 200 fecal samples. Microscopic analysis revealed that <i>Cryptosporidium</i> oocysts appeared spherical or round and stained red against a blue background when using the acid-fast stain. • The study also showed that the absence of diarrhea did not exclude the presence of the parasite, since 52% of normal fecal samples and 50% of diarrheic samples were positive. • Six isolates were deposited under the name RFMN-2024 and registered in the GenBank database.	 The graphical abstract consists of three main components. On the left, there are two microscopic images: the top one shows oocysts stained red against a blue background (acid-fast stain), and the bottom one shows oocysts as circular or spherical structures on a green background (acridine orange stain). In the center, there is a gel electrophoresis image showing six lanes labeled M, 1, 2, 3, 4, 5, and 6. A white arrow points to a band in lane 6, labeled '320 bp'. On the right, there is a phylogenetic tree showing the relationship between the six isolates (RFMN-2024) and other <i>Cryptosporidium</i> species from various countries, with bootstrap values indicated at the nodes.
Keywords: PCR Nineveh Province <i>Cryptosporidium</i> Phylogenetic analysis Acid Fast Stain	ABSTRACT This study was conducted in Nineveh Province on 200 fecal samples collected from sheep and 50 milk samples collected from ewes during the period from August 2024 until the end of February 2025. The samples were examined at the Parasitology Laboratory, Department of Microbiology, College of Veterinary Medicine, University of Mosul, using both microscopic and molecular diagnostic techniques. The overall infection rate detected by microscopy was 52% among the 200 fecal samples. Microscopic analysis revealed that <i>Cryptosporidium</i> oocysts appeared spherical or round and stained red against a blue background when using the acid-fast stain. When using acridine orange stain, the oocysts appeared as circular or spherical orange structures on a green background, while with iodine staining they appeared spherical or oval surrounded by a clear halo. The presence of <i>Cryptosporidium</i> oocysts was also detected in 56% of the 50 milk samples. The results of this study confirmed a correlation between the month of sampling and the infection rate, with the highest prevalence recorded in August (85.7%) and the lowest in January (20%). Animal age had a significant effect on infection prevalence, as all age groups were susceptible to infection. The highest rate (95.45%) was observed in lambs aged 1–3 months, and the infection rate decreased progressively with age. A significant difference was also found between sexes, with males showing an infection rate of 75.5% compared to 32.7% in females. The study also showed that the absence of diarrhea did not exclude the presence of the parasite, since 52% of normal fecal samples and 50% of diarrheic samples were positive. In addition, several factors showed significant effects on infection prevalence, including herd size, type of husbandry, pregnancy status of ewes, and the nature of the housing floor. Microscopic findings were further confirmed by molecular testing of highly infected samples through DNA extraction from <i>Cryptosporidium</i> oocysts shed in sheep feces. Sequencing of the 18S rRNA gene revealed three species of <i>Cryptosporidium</i>: <i>C. parvum</i>, <i>C. hominis</i>, and <i>C. ubiquitum</i>. This is the first study to report these <i>Cryptosporidium</i> species in sheep in Nineveh Province. Six isolates were deposited under the name RFMN-2024 and registered in the GenBank database. Phylogenetic analysis demonstrated a genetic similarity between the local isolates and strains previously recorded in other Iraqi provinces as well as strains isolated from other countries around the world. 2024 M.Sc. Thesis @Univ. of Mosul, College of Veterinary Medicine. (https://www.uomosul.edu.iq/).