

University of Mosul
College of Veterinary Medicine



Morphological and Molecular Diagnosis of *Fasciola* spp. in Cattle in Nineveh Province

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MSc / Thesis

Veterinary Medicine / Veterinary Parasitology

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Abstract

Fasciola species cause a parasitic disease that poses a significant threat to health and economic productivity of livestock worldwide, including Iraq. The study comprised 400 samples was conducted to diagnose *Fasciola* spp. infection through fecal egg detection by direct and sedimentation methods and through livers inspection as well as confirmation through molecular study by conventional PCR reaction and genetic analysis in cattle in Nineveh province from August 2024 to end of February 2025. According to the data, infection rate was 12.5% in fecal samples of 200 examined cattle and did not reveal a significant difference statistically between sexes at $P\text{-value} < 0.05$, while significantly difference was observed among the age groups with higher rate in older one. There was no significant difference in infection rate recorded among the studied region, as well as there were observed significant differences among the studied months with highest infection rates were recorded at October and November, in contrast to August, no infection rate was recorded. Morphometrically, the dimensions average of 25 estimated eggs was $139.42 \times 81.34 \mu\text{m}$ (length $\times$ width). Infection rate with *Fasciola* spp. in 200 inspected livers was 9.5% while no significant difference was observed between sexes, in contrast to observed significant differences between the ages which was higher in cattle over two years old but there was no significant differences among monthly infection rates.

Morphologically and confidently with morphometry, an examination of isolated flukes subsequently by staining dependence, presence of *Fasciola gigantica* (63.2%) and *Fasciola hepatica* (36.5%) was observed and 42.1% infected heavily but with no mixed infection, furthermore confirmation by molecular study with target genes 28S rRNA and 16S rRNA, proved positively in accurately identifying the species using conventional PCR. Molecular analysis showed 98–100% sequence similarity with known *Fasciola* species. Phylogenetic analysis revealed clear genetic differentiation between *F. gigantica* and *F. hepatica* and significant genetic diversity within *F. gigantica* populations, including newly classified Iraqi isolates (PV344638, PV290745, PV290746 and PV290747). Additionally, two isolates from this study (PV335564 and PV335557) were closely related to known *F. hepatica* populations.

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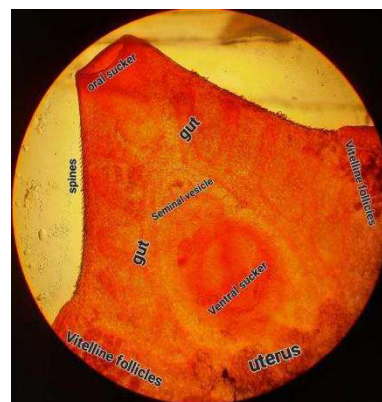
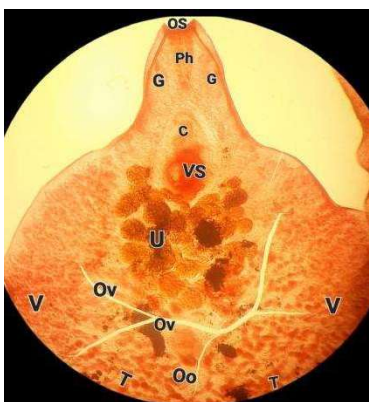
Highlights

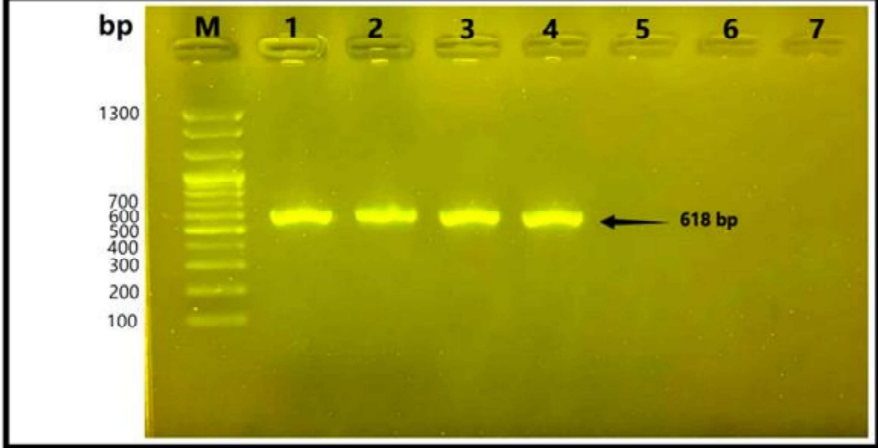
Fasciola spp. cause widespread parasitic disease of cattle and a significant zoonotic disease. An estimated about 2.4–18 million peoples and more than 300 million domestic ruminants are infected worldwide.

The disease have acute, subacute and chronic forms, mostly result in liver damage , haemorrhage and fibrosis lead to anemia, emaciation, odema and complication due to bacterial infection, which usually is fatal.

For prevention and control, the early disease diagnosis is essential; traditional microscopic exams of fecal egg detection and postmortem inspection are initial diagnostic procedures. In addition, using various

Graphical abstract



diagnostic techniques like serological, immunological and coprological surveys.	
Keywords: Fasciola spp. Cattle Infection rate Morphology Molecular study Genetic Tree	<i>Fasciola</i> species cause a parasitic disease that poses a significant threat to health and economic productivity of livestock worldwide, including Iraq. The study comprised 400 samples was conducted to diagnose <i>Fasciola</i> spp. infection through fecal egg detection by direct and sedimentation methods and through livers inspection as well as confirmation through molecular study by conventional PCR reaction and genetic analysis in cattle in Nineveh province from August 2024 to end of February 2025. According to the data, infection rate was 12.5% in fecal samples of 200 examined cattle and did not reveal a significant difference statistically between sexes at P-value < 0.05, while significantly difference was observed among the age groups with higher rate in older one. There was no significant difference in infection rate recorded among the studied region, as well as there were observed significant differences among the studied months with highest infection rates were recorded at October and November, in contrast to August, no infection rate was recorded. Morphometrically, the dimensions average of 25 estimated eggs was $139.42 \times 81.34 \mu\text{m}$ (length $\times$ width). Infection rate with <i>Fasciola</i> spp. in 200 inspected livers was 9.5% while no significant difference was observed between sexes, in contrast to observed significant differences between the ages which

	was higher in cattle over two years old but there was no significant differences among monthly infection rates. Morphologically and confidently with morphometry, an examination of isolated flukes subsequently by staining dependence, presence of <i>Fasciola gigantica</i> (63.2%) and <i>Fasciola hepatica</i> (36.5%) was observed and 42.1% infected heavily but with no mixed infection, furthermore confirmation by molecular study with target genes 28S rRNA and 16S rRNA, proved positively in accurately identifying the species using conventional PCR. Molecular analysis showed 98–100% sequence similarity with known <i>Fasciola</i> species. Phylogenetic analysis revealed clear genetic differentiation between <i>F. gigantica</i> and <i>F. hepatica</i> and significant genetic diversity within <i>F. gigantica</i> populations, including newly classified Iraqi isolates (PV344638, PV290745, PV290746 and PV290747). Additionally, two isolates from this study (PV335564 and PV335557) were closely related to known <i>F. hepatica</i> populations.
	Both species; <i>F. hepatica</i> and <i>F. gigantica</i> were present in Nineveh Province, with <i>Fasciola gigantica</i> being the predominant and more accurately was confirmed by molecular study examination without intrasequence differences in each species. Isolated <i>Fasciola</i> species from this study showed a strong genetic link 98-100% with those from near and far countries and did not find any hybrid forms.



جامعة الموصل
كلية الطب البيطري

التشخيص الشكلي والجزئي لطفيلي المتورقة في الأبقار في محافظة نينوى

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رسالة ماجستير

الطب البيطري / طفيليات البيطرية

بإشراف

الأستاذ الدكتور

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الخلاصة

تسبب أنواع طفيلي المتورقة مرضًا طفيلياً يشكل تهديداً كبيراً لصحة وإنتاجية الثروة الحيوانية في جميع أنحاء العالم، بما في ذلك العراق. تم إجراء الدراسة إلى الكشف عن نسبة الإصابة بطفيلي المتورقة وتقييمه من خلال الكشف عن بيض الطفيلي في البراز بطريقة المباشر والترسيب وايضا لتقييم نسبة الإصابة وتحديد النوع من خلال فحص الكبد، وكذلك تأكيد النوع من خلال الاختبار الجزيئي باستخدام تفاعل البوليميراز المتسلسل (PCR) والتحليل الجيني، في الأبقار في مدينة الموصل خلال الفترة من أغسطس ٢٠٢٤ إلى نهاية فبراير ٢٠٢٥. وفقاً للبيانات، بلغ معدل الإصابة ١٢.٥٪ في عينات البراز من ٢٠٠ رأس ماشية تم فحصها. لم يكن هناك فرق كبير بين الجنسين، ولكن لوحظت فروق كبيرة بين الفئات العمرية، حيث كان المعدل أعلى في الأبقار الأكبر سناً. تم تسجيل أعلى معدل الإصابة في حمام العلي وسوق الأبقار، بينما تم تسجيل أدنى المعدلات في بعشقة وكوكجلي. تم تسجيل أعلى معدلات الإصابة في شهري أكتوبر ونوفمبر، على عكس أغسطس، حيث لم يتم تسجيل أي إصابة.

من الناحية المورفولوجية، كان متوسط أبعاد البيض المقدر البالغ عدده ٢٥ بيضة ١٣٩.٤٢ × ٨١.٣٤ ميكرومتر (الطول × العرض). فيما يخص فحص الأكباد، بلغ معدل انتشار الفاشيولا في ٢٠٠ كبد تم فحصها ٩.٥٪، وكان أعلى في الإناث، دون وجود فرق كبير. ولوحظ أعلى معدل انتشار في الماشية التي يزيد عمرها عن عامين، مع وجود فرق كبير إحصائياً. تم تسجيل أعلى معدلات الإصابة الشهرية في شهري أكتوبر وسبتمبر، ولكن لم يلاحظ أي فرق كبير. كشف التقييم المورفولوجي للديدان المعزولة لاحقاً بناءً على الصبغة، عن وجود *Fasciola gigantica* ٦٣,٢٪ و *Fasciola hepatica* ٣٦,٥٪ علاوة على ذلك، أثبت التأكيد من خلال التحليل الجزيئي باستخدام جينات 28S rRNA و 16S rRNA المستهدفة فعاليته في تحديد الأنواع بدقة باستخدام تفاعل البوليميراز المتسلسل التقليدي. أظهر التحليل الجزيئي تشابهاً في التسلسل مع أنواع الفاشيولا المعروفة بنسبة ٩٨-١٠٠٪. كشف التحليل التطوري عن تمايز وراثي واضح بين *F. hepatica* و *F. gigantica*، وتنوع وراثي كبير ضمن مجموعات *F. gigantica*، بما في ذلك العزلات العراقية المصنفة حديثاً (PV344638، PV290745، PV290745 و PV290747). بالإضافة إلى ذلك، كانت عزلتان من هذه الدراسة (PV335557 و PV335564) وثيقتي الصلة بمجموعات معروفة من *F. hepatica*.