



## Molecular Detection of *Schistosoma* spp. in Cattle Urine in Mosul, Iraq

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### Abstract

**T**HIS STUDY conducts to identification the microscopic prevalence of schistosome infection in cattle urine and molecular detection of *Schistosoma bovis* in urine and blood. Totally, 70 anorexic, emaciated and intermittent diarrheic cattle of different ages and sexes were selected and subjected to collection of urine that sediment firstly and tested by light microscope and the samples blood that examined molecularly using the conventional polymerase chain reaction (PCR) assay. Our findings revealed that 11.43% of study animals were positives by microscopy. Targeting the *18S rRNA* gene, the positive findings using the PCR assay detected that (21.43%) of in blood samples were positive; whereas, no positivity was seen in urine. Regarding age, results of microscopy were significantly elevated ( $P < 0.0216$ ) in cattle of  $>1$  year (14.89%) when compared to those of  $<1$  year (4.35%); while molecularly, insignificant variation ( $P < 0.0939$ ) was seen between values of  $<1$  year (21.74%) and  $>1$  year (21.28%). Concerning sex, the findings of microscopy and PCR reported that females (15.39% and 28.21%, respectively) having a higher positivity ( $P < 0.05$ ) than males (6.45% and 12.9%, respectively). In conclusion, this represents the first Iraqi study indicates traditionally the prevalence of bovine schistosomiasis in urine; and molecularly, *S. bovis* in blood. The species-specific DNA detection by PCR appears to be more sensitive than clinical and traditional techniques, in addition to its ability in providing a high valuable data about the prevalence of disease. Moreover studies are recommended be done in other Iraqi areas among other domestic animals to explain their role in transmission of infection.

**Keywords:** *Schistosoma bovis*, Sedimentation, PCR, Sensitivity and specificity, Risk factors.

### Introduction

Urinary parasites play an important role in public health due to direct contact between animals and humans in fields [1]. *Schistosoma* is a snail-borne Trematode parasite which existed endemically in animals as well as humans in many countries including Iraq [2, 3]. In cattle, schistosomiasis considers an important neglected disease that

resulted mainly by several species of *Schistosoma* genus that belongs to Trematoda Class under the Platyhelminthes Phylum [4-6]. The infective stage of *Schistosoma* is fully mature; cercariae which are leave in the snail and swim freely in the water to infect ruminants by penetration of skin or orally [7]. Therefore, the global distribution of the parasite is closely related to marshy pastures, lakes and ponds which existed largely in tropical

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and sub-tropical regions. Additional predisposing factors include environmental conditions such as temperature, moisture grazing system, keeping animals, feeding and drinking areas which can affect greatly on prevalence of the parasite [2, 8].

Despite the disease is more commonly in animals, the risk and the importance of infection are varied largely among animal species since some species are more susceptible than other. In cattle, acute phase of disease occurs during release large number of eggs by adult parasite in mucosal intestine; whereas, the chronic phase occurs when there are damages resulted due to reactions for trapping of eggs within tissue of different organs [9, 10]. In almost cases, subclinical form of schistosomiasis appears in endemic regions, which manifested by great incidence the mild- moderate worm burden in livestock [11]. A number of studies hypothesized that cattle sensitivity to initial infection varies based on species of *Schistosoma* to which it exposed. Additionally, little or no overt clinical symptoms might recognize short terms; but in long term, it has been established that higher rates of chronic infection causing a marked loss in herds [11-13]. Other authors are widely accepted that clinical signs, morbidities and mortalities are obviously associated with severity of disease as calculated with counting of fecal eggs or worms [14-16]. Hence, identification of schistosome infections based on symptoms and / or detection of eggs from infected animals could not usually possible [17, 18]. Several serological diagnostic techniques have been developed to identify the antibodies and antigens of *Schistosoma* in various epidemiological surveys. Although, many of these assays are simple and fast in diagnosis of bovine schistosomiasis, it has little values on practical scale due to false positive reactions [19-21]. Other advanced tests include molecular techniques that modified for diagnosis of different *Schistosoma* species in various samples at a high rate of sensitivity and specificity in both animals and humans [22, 23].

In Iraq, limited available data are done to indicate an occurrence of schistosome infections in cattle using the traditional or molecular assays [24-26]. Thus, current work conducts to evaluate microscopic prevalence of *Schistosoma* infections in urine and molecular detection of *Schistosoma bovis* in urine and blood for first time in Iraq.

## Materials and Methods

### *Ethical approval*

The current work gets a license from the Scientific Committees of the College of Veterinary Medicine in both the University of Mosul and University of Wasit.

### *Samples and data*

Totally, 70 cattle of different ages and sexes which attended to the Veterinary Teaching Hospital (Nineveh province, Iraq) and diagnosed clinically as anorexic, emaciated and intermittent diarrheic cases were selected to the current study during October (2023) and January (2024). Initially, each study cattle was injected furosemide to stimulate of urination and then subjected to collect of urine samples directly into plastic containers to be tested as soon as possible. Then, 2.5 ml of jugular venous blood was drained from each study animal under aseptic conditions into a labeled EDTA-plastic tube and kept frozen until be tested molecularly.

### *Sedimentation and microscopic examination*

As described previously [27], the urine samples were centrifuged firstly (2000 rpm for 5 minutes); and then, 0.5 ml of precipitate was dropped on a glass slide, covered and examined using by the light microscope (MEIJI, Japan) at 40× and 100× to investigate the presence of ova of the parasite.

### *Molecular examination*

Following the manufacturer instructions of protocol (A) in G-Spin™ Total DNA Extraction Kit (Intron, Korea), urine and blood samples were prepared, processed and the DNAs were extracted and examined by Nanodrop System (Thermo Fisher Scientific, UK) for measurement the purity and concentration. Targeting *18S rRNA* gene, a set of primers were designed [F (5'-CGG CTG TGC CTG TTA CAT T-3') and R (5'-CAG CAC CCG TTT AAC ACA GA-3')] based on the GenBank-NCBI *S. bovis* global isolate (AY318828.1). After MasterMax preparation at a final volume of 20 µl, the tubes were transferred to Thermocycler System to subject for optimized conditions. Agarose-gel electrophoresis was carried out as described [28] and the positive product size was identified at 537 bp using the UV transilluminator.

### *Statistical analysis*

GraphPad Prism Software was served to identify significant variation between results of microscopic and molecular assays and association of positivity to risk factor using the *t*-test and Two-

Way ANOVA, respectively, at  $P < 0.05$  (\*) [29, 30].

## **Results and Discussion**

### *Total results*

For microscopy, ova of *Schistosoma* spp. were seen in 11.43% of urine sediments. Targeting the *18S rRNA* gene, molecular examination of blood samples showed that 21.43% of study cattle were positive for conventional PCR assay. However, no positive urine samples were detected by PCR assay (**Table 1, Figures 1 and 2**).

Various epidemiological studies demonstrated that distribution of bovine schistosomiasis is greatly varied between areas and countries, worldwide. In comparison to our findings, the prevalence rate of schistosome infections in Iraqi cattle was 14% [24] in Baghdad and 19.23% in Nineveh [25]; whereas internationally, there was 33.92% in Tanzania [31], 4.5% in Zimbabwe [32], 47.5% in Bangladesh [12], 10% in Nigeria [33], 32.35% in Ethiopia [34], 2.4-5.9% in Côte d'Ivoire [35] and 4.52-12.38% in India [36]. Variation in prevalence rate of bovine schistosomiasis between studies could be caused by differences in epidemiological (draining system and availability of stagnant water) and environmental (climate and agro-ecology) factors, in addition to the samples sizes, sampling periods, type of sample(s), sensitivity and specificity of the diagnostic assay and management system of the studied area.

Variation between our microscopic and molecular results could be because of valuable sensitivities and specificities of molecular assays in addition to facts that cattle harboring *Schistosoma* could not be shown a positivity using traditional techniques as parasitic eggs may be trapped in various tissues and are not given a way to be excreted by feces [9, 37]. On the other hand, cattle of older ages might be acquired a strong specific immunity to suppress worm fecundities as well as releasing of eggs [2, 38].

### *Epidemiological risk factors*

Regarding age, microscopic values were significantly elevated ( $P < 0.0216$ ) in cattle ages  $> 1$  year (14.89%) when compared to those of  $< 1$  year (4.35%); while molecularly, insignificant variation ( $P < 0.0939$ ) was seen between values of  $< 1$  year (21.74%) and  $> 1$  year (21.28%), (**Table 2**).

In an agreement with results of microscopy but not PCR of current study, several researchers have confirmed significant increasing of bovine schistosomiasis in cattle with advancing age

as compared to younger [25, 35]. Aradaib et al. [39] describe the decreasing disease in advanced age occurs because chronically diseased cattle can initiate a specific immune response towards infestation and releasing of eggs. De Bont and Vercruyssen [4] mentioned that immunity against *Schistosoma* acts mainly by reducing the fecundity of worms through decreasing the quantity of fecal eggs in contrast to worm burdens that increased with advancing ages of naturally infected animals. Islam et al. [12] suggested the high prevalence of disease in cattle to a long-time exposure of older cattle that are moved for longer distances to get their food in scarce pastures and water which increase the chance of disease exposure and to infect. Merawe et al. [40] attributed this variation to the false negative results; parasite limited fecundity rate; and performance of a technician. Kerie and Seyoum [34] found the higher rate of schistosome infection in younger animals to absence of immunity in these animals and low ability of them to resist new infection.

Significantly, the findings of microscopy and PCR reported that females (15.39% and 28.21%, respectively) having a higher positivity ( $P < 0.05$ ) than males (6.45% and 12.9%, respectively), (**Table 3**).

For sex, the findings of this study that showed female cattle have a higher positivity than males using the microscopy and PCR assay were in agreement with other studies [8]. Effect of sex on cattle predisposition to *Schistosoma* could be related to genetic susceptibility and hormonal control due to a fact that females exposed to great stress than males due to gestation and milk production. In contrast, other studies have been reported an incidence of bovine schistosome infections in males higher than females attributing this to grazing of males around nutrients than females within areas of study [33, 35]. Islam et al. [12] recorded that reasons seem to associate with social practices of keeping female under the best feeding and management conditions for breeding and milk production whether male cattle usually lack the free graze in pastures and infrequently served to draught purposes under more stressful conditions. However, other studies recorded that no significant differences between females and males and explained that both sexes grazed and drank at same pasture lands and exposed equally to parasite [25, 34].

### Conclusions

This represents the first Iraqi study indicates traditionally the prevalence of bovine schistosomiasis in urine; and molecularly, *S. bovis* in blood. The species-specific DNA detection by PCR appears to be more sensitive than clinical and traditional techniques, in addition to its ability in providing a high valuable data about the prevalence of disease. Moreover studies are recommended be done in other Iraqi areas among other domestic animals to explain their role in transmission of infection.

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### Conflicts of interest

There are no conflicts to declare.

### Funding statement

No external funds were received (private funding).

### Data availability

All data included within the manuscript.

### Authors' contribution

EAA: Collection of urine samples, sedimentation and microscopic examination. HAJG: Collection of blood samples, molecular examination and statistical analysis of obtained results. Both authors were approved the final copy of manuscript.

**TABLE 1. Total results of diagnostic assays for urine and blood of 70 study cattle**

Test	Sample	Total No.	Positive	Negative
Microscopy	Urine	70	8 (11.43%)	62
PCR	Blood	70	15 (21.43%)	55
PCR	Urine	70	0 (0%)	70
p-value			0.0411 *	-

**TABLE 2. Association of positivity of microscopy and PCR to age of study cattle**

Age (Year)	Total No.	Positive		p-value
		Microscopy	PCR	
< 1	23	1 (4.35%)	5 (21.74%)	0.0212 *
> 1	47	7 (14.89%)	10 (21.28%)	0.0493 *
p-value		0.0261 *	0.0939	-

**TABLE 3. Association of positivity of microscopy and PCR to sex of study cattle**

Sex	Total No.	Positive		p-value
		Microscopy	PCR	
Female	39	6 (15.39%)	11 (28.21%)	0.0419 *
Male	31	2 (6.45%)	4 (12.9%)	0.0384 *
p-value		0.0341 *	0.0373 *	-



Fig. 1. Ova of *Schistosoma* spp. detected in urine sediments by microscopy

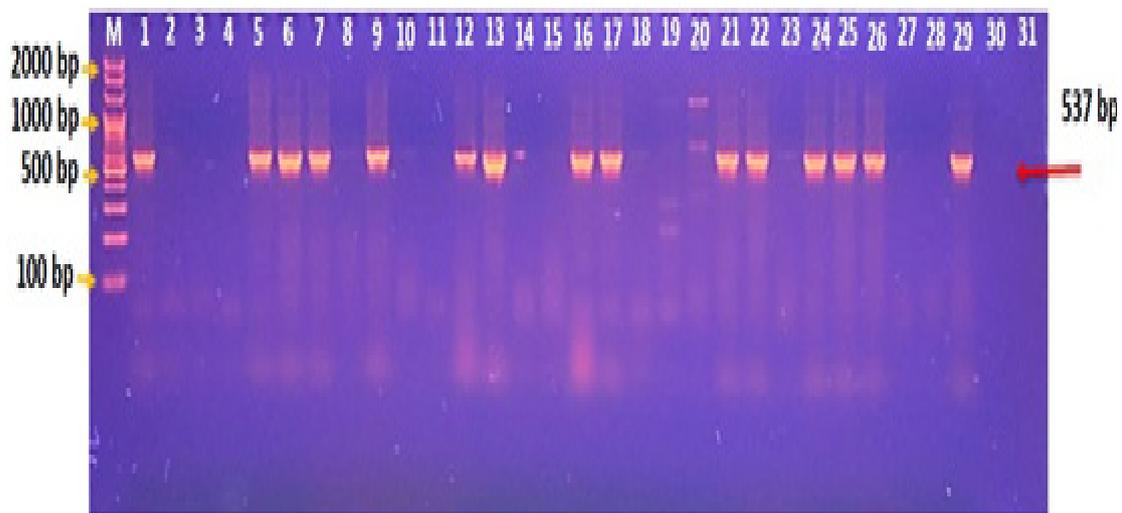


Fig. 2. Agarose gel electrophoresis shows conventional PCR analysis of 16S rRNA gene in PCR products of cattle blood samples to detect *S. bovis*.

(Lane (M): DNA marker ladder (2000-100bp

Lanes (2, 3, 4, 8, 10, 11, 14, 15, 18, 19, 20, 23, 27, 28, 30 and 31): Negative samples

Lanes (1, 5, 6, 7, 9, 12, 13, 16, 17, 21, 22, 24, 25, 26 and 29): Positive samples at 537 bp

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## الكشف الجزيئي للبلهارسيا في بول الأبقار في الموصل ، العراق

إيفا أيسر عجاج<sup>١</sup> و حسنين عبد الحسين جعفر غربان<sup>٢</sup>

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تهدف هذه الدراسة إلى تقييم الانتشار المجهرى لداء البلهارسيات البقري في البول ، والكشف الجزيئي لطفي للبلهارسيا البقرية (*S. bovis*) في الدم . إجمالياً، تم اختيار 70 بقرة تعاني من فقدان الشهية والهزال والإسهال المتقطع من مختلف الأعمار والجنس ، وتم جمع عينات البول والحصول على الراسب أولاً ومن ثم فحصه بالمجهر الضوئي ، في حين خضعت عينات الدم إلى الفحص الجزيئي باستخدام اختبار التفاعل المتسلسل بانزيم البلمرة التقليدي (PCR) . كشفت النتائج التي توصلنا إليها أن ١١,٤٣% من حيوانات الدراسة كانت موجبة بالفحص المجهرى ؛ في حين أظهرت ٢١,٤٣% من الماشية نتائج موجبة باستخدام اختبار PCR الذي أستخدم جين الرنا الريباسي (*18S rRNA*) . فيما يتعلق بالعمر ، أظهرت نتائج الفحص المجهرى ارتفاعاً معنوياً ( $P >$  ٠,٠٢١٦) في داء البلهارسيات في الأبقار بعمر أقل من سنة (١٤,٨٩%) بالمقارنة مع تلك التي بعمر أقل من سنة (٤,٣٥%) ؛ بينما على المستوى الجزيئي ، شوهد تباين غير معنوي ( $P >$  ٠,٠٩٣٩) بين قيم الحيوانات التي تقل أعمارها عن سنة واحدة (٢١,٧٤%) وأقل من سنة واحدة (٢١,٢٨%) . فيما يتعلق بالجنس ، أظهرت نتائج الفحص المجهرى والتفاعل المتسلسل بانزيم البلمرة أن الإناث (١٥,٣٩% و ٢٨,٢١% على التوالي) لديهن نسبة نتائج موجبة أعلى ( $P >$  ٠,٠٥) عند مقارنتها بالذكور (٦,٤٥% و ١٢,٩% على التوالي) . أخيراً ، تمثل هذه الدراسة العراقية الأولى التي أشارت إلى مدى انتشار داء البلهارسيات البقري في البول باستعمال الطرق التقليدية ؛ وجزئياً إلى انتشار طفيلي البلهارسيا البقري في الدم . ويبدو أن الكشف عن الحمض النووي الخاص بالأنواع بواسطة التفاعل المتسلسل بانزيم البلمرة أكثر حساسية من التقنيات السريرية والتقليدية ، بالإضافة إلى قدرته على توفير بيانات عالية القيمة حول مدى انتشار المرض . علاوة على ذلك توصي الدراسة بإجراء المزيد من الدراسات في مناطق عراقية أخرى في الحيوانات الأليفة الأخرى لأيضاح دورها في نقل الأصابة .

**الكلمات الدالة:** البلهارسيا البقرية ، الترسيب ، التفاعل المتسلسل بانزيم البلمرة ، الحساسية والخصوصية ، عوامل