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Climatic and environmental risk factors and their role in the prevalence of *Fasciola hepatica* in water buffalo (*Bubalus bubalis*) in Mexico

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Abstract

Fasciolosis is a neglected/re-emerging zoonotic disease caused by the trematode Fasciola hepatica that infects both livestock and humans worldwide, causing severe economic losses and public health problems. The study determined the seroprevalence of F. hepatica infection in buffaloes and its associations with age, sex, and climatic/environmental factors (derived from satellite data) to identify potential risk factors from two tropical climate subtypes in two Mexican states. A total of 725 serum samples were analyzed for the presence of anti-F. hepatica immunoglobulin G (IgG) antibodies using an indirect enzyme-linked immunosorbent assay (ELISA), with excretory-secretory (E/S) products as the antigen. The overall prevalence of the parasite in buffaloes between September 2022 and December 2022 was 10.62% (77 out of 725). The highest rate of F. hepatica infection was detected in Veracruz state 9.18%. In addition, the highest prevalence among the climate regions was found in the tropical rainforest 13.62%. In the two states studied, the highest positivity rate for fasciolosis was observed in young animals aged three years and below 21.52% and in male buffaloes 20.00%. Using multivariate logistic regression analysis, we determined four factors (age, normalized difference vegetation index, land surface temperature day (LST day), and rainfall) to be significantly associated with an increased risk of parasitic infection. Rainfall was the variable most strongly associated with F. hepatica infection (OR 3.434; 95% CI: 2.181-5.406), followed by LST day (OR 2.832; 95% CI: 1.916-4.186). The results indicate that different factors are significantly associated with the prevalence of liver flukes and highlight the importance of continuous monitoring programs for parasite infections to prevent economic losses in buffalo production.

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Introduction

Fasciolosis is a prevalent parasitic and foodborne zoonotic disease that affects many mammals, including humans, mainly grazing animals. Of the causative agents, *Fasciola hepatica* is distributed globally, whereas *F. gigantica* is more prevalent in Africa, the Middle East, and Asia, with a limited distribution of suitable intermediate snail hosts (1,2). More than 180 million people worldwide are at risk of infection, with an estimated 35 to 72 million individuals currently infected with liver flukes; moreover, this number is expected to rise further (3). Fasciolosis causes significant economic losses to the animal husbandry sector, estimated at over USD 3 billion annually (4,5). Previous studies have shown a high prevalence of *F. hepatica* in livestock species, mainly in the Mexican altiplano and southern states (6-8), with an economic loss of USD 119 million annually (8). Numerous serological studies of fasciolosis in buffaloes have reported a prevalence of 33.6%, depending on the climatic region and country (1). Studies in Tabasco, Mexico, reported a prevalence of 5.3% in pregnant buffaloes using the sedimentation test (9). The rapid, early, and accurate diagnosis of infection is essential for the epidemiology, surveillance, and control of the disease. The enzyme-linked immunosorbent assay (ELISA) is a sensitive and cost-effective technique used to detect *F. hepatica*-specific antibodies in milk and sera based on excretory-secretory (E/S) products, somatic antigens, cathepsin L proteinases, and recombinant proteins. The ELISA has been used as a sensitive and inexpensive technique for monitoring and establishing infection status in health management programs (10,11). Climate change is linked to the increase in *F. hepatica* infections due to its impact on the liver fluke's prevalence, intensity, and spatial distribution, which affect the larval stages of the snails that act as intermediate hosts for the liver fluke (12,13). Geographic information systems (GIS) and remote sensing technologies can be utilized to analyze the relationship between climate, environmental data, and disease prevalence rates through regression models, helping to identify risk factors for economically significant infections, improve disease control, and reduce the use of anthelmintics (14-16). However, no studies have been performed thus far on the serum sample prevalence and risk factors of liver fluke infection in buffaloes in different climate regions and states of Mexico.

The objectives of the present study were to investigate the prevalence of *F. hepatica* in two tropical climate subtypes in two states in Mexico by using ELISA with E/S product antigens and perform logistic regression analysis using climatic and environmental variables and the buffaloes' characteristics to identify the potential risk factors for significant parasite infection.

Materials and methods

Ethical approve

This study was approved by the Animal Care and Ethics Committee of the Meritorious Autonomous University of Puebla (458623), and it included the handling and collecting of blood samples by the National Legislation About Animal Health Research.

Study area and climatic data sources

This study was conducted in the Las Choapas district of Veracruz (located in eastern Mexico, specifically Farms 1-3) and the Villahermosa district of Tabasco (located in southeast Mexico, namely Farms 4 and 5) between September and December 2022. The states of Veracruz and Tabasco have a land area of 71,826 km^2 and 24,730 km^2, respectively (Figure 1). The climate of the states of Veracruz and Tabasco is dominated by the summer rainy season from June to September and the summer-autumn rainy season from June to November, with a mean annual temperature of 23.0 °C and 27.1 °C, respectively, and annual precipitation of 1500 mm and 1926 mm, respectively (17). Here, buffaloes roam freely and graze on natural grassland, living in an extensive breeding system. For each farm, the corresponding geographical coordinates were recorded using the global positioning system (GPS) model (Garmin eTrex Vista), and their geographical positions were georeferenced and overlaid in the GIS environment (ArcGIS 10.1, USA) using Köppen climate classification maps modified by Garcia (17). In addition to providing meteorological data, remotely sensed climate data products with global coverage were extracted as monthly means for twelve months before the day of blood collection for the five farms. Monthly rainfall data were obtained from the Tropical Rainfall Measuring Mission (http://disc2.gesdisc.eosdis.nasa.gov) 3B43 satellite product, with a spatial resolution of 0.25° × 0.25°. Satellite measurements of land surface temperature (LST) and the normalized difference vegetation index (NDVI) at a spatial resolution of 0.05° were derived from the Moderate Resolution Imaging Spectroradiometer (MODIS) Terra products, MOD11C3 v061 and MOD13A2 v061. In addition, LST data were used as a proxy for day and night temperature, while the NDVI was used as a proxy for soil moisture (18), and vegetation activity at the land surface. A 1 km resolution Digital Elevation Model Shuttle Radar Topography Mission (SRTM) dataset was used to extract the elevation of the farms.

Sample collection

Farms were selected based on convenience, taking into account the willingness of the owners or managers to participate in the study. A total of 725 blood samples were collected from Veracruz state (n = 490) and Tabasco state (n = 235) by puncturing the jugular vein of the buffaloes. Serum samples from the animals were obtained by a team of one veterinarian and one animal scientist and transported in isothermal boxes to the Laboratory of Molecular Biology and Veterinary Biotechnology, Meritorious Autonomous University of Puebla. Sera were obtained after clotting and centrifuged at 2000 × *g* for 10 min at four °C, following which they were transferred to cryotubes and frozen at −80 °C for ELISA. The *F. hepatica* eggs were identified based on morphology described in a previous study (19). An epidemiology questionnaire was completed for every buffalo during sampling regarding age (in years) and sex (male/female). Buffalo age data were divided into ≤ 3 years, 4 to 6 years, 7 to 9 years, and ≥ ten years.

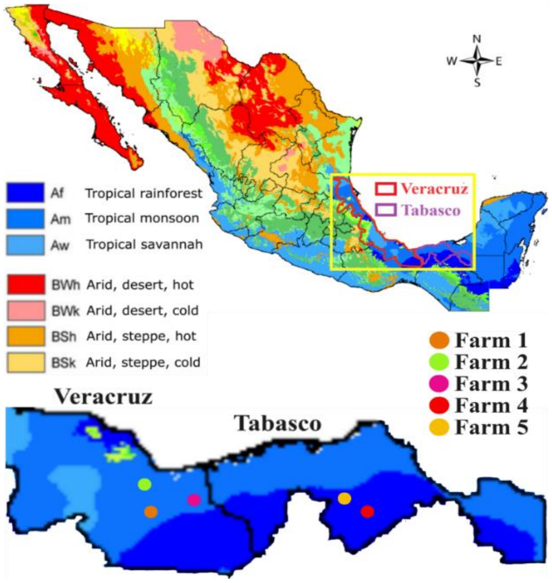

Figure 1: Geographical location of the states of Veracruz and Tabasco in a map of Mexico with the Köppen climate classification system (upper map). Location of the five farms within the states (lower map).

Positive and negative controls for validating IgG ELISA with E/S product antigens

Sixty buffalo serum samples were obtained before slaughter at the local abattoir and visually correlated with flukes in the main bile ducts and gall bladder via post-mortem examination. Adult fluke E/S products of buffalo origin were obtained and prepared (6).

ELISA detection of anti-*F. hepatica* IgG antibodies in serum

Before the final experiments, checkerboard titration with indirect ELISA was used to determine the optimal concentrations of antigen, serum, and the horseradish peroxidase (HRP)-conjugated protein G (M00090, GenScript, China). The optical density (OD) was determined using a microplate reader (BioTek ELx800, USA) at 450 nm. The cut-off value was determined using the mean OD + 3 multiplied by the negative samples' standard deviation (SD). Flat-bottomed polystyrene 96-well microtiter plates (Costar, Corning, USA) were coated with 10 µg/mL of E/S products in 100 µL of phosphate-buffered saline (PBS) and incubated overnight at four °C. After four washes with PBS containing 0.05% Tween 20 (PBS-T), nonspecific binding sites were blocked with 200 µL of 1% bovine serum albumin (BSA) in PBS (w/v) for two h at 37 °C. The plates were rewashed three times with PBS-T. Negative and positive controls and serum samples were used at a dilution of 1:100 in PBS and incubated at 37 °C for one hour. Next, microplates were washed four times with PBS-T and incubated with HRP-protein G (M00090, GenScript, China) diluted at 1:5000 in PBS for one h at 37 °C. Following incubation and washing, color was developed by adding 100 µl per well of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution (Sigma-Aldrich, USA). The enzyme-substrate reaction was stopped with 50 µl of 4 N H_2SO_4. Lastly, OD was measured at 450 nm using an ELISA reader (BioTek ELx800, USA). The values of all serum samples and negative and positive controls were expressed as the mean of the OD tested in triplicate on each plate.

Statistical analysis

Initially, bivariate logistic regression analysis was used to determine the relationship between various climatic/environmental factors and the buffaloes' characteristic-related predictors of *F. hepatica* infection status. Variables with significance levels of $P<0.05$ were entered into a multivariate analysis. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using backward stepwise multivariate logistic regression analysis. The criteria for adding or removing a variable were 5% for inclusion and 10% for exclusion to obtain the most appropriate theoretical model to fit the collected data. A goodness of fit using McFadden's pseudo-R^2 was used for each model to measure overall model fit, and Akaike's information criterion (AIC) was calculated to select the models that best fit the data. All statistical analyses were performed using the IBM SPSS 25 software package for Windows (SPSS Inc., USA).

Results

Prevalence of *F. hepatica* parasites in buffaloes

A total of 725 buffaloes located in two states of Mexico were studied. As measured by indirect ELISA, the prevalence of trematode infection in the five buffalo farms was 10.62% (77 out of 725 samples). The highest prevalence of liver flukes was 10.67% (19 of 178 samples, Farm 3), and the lowest was 8.13% (10 of 123 samples, Farm 1). A summary of the serological detection results using indirect ELISA in buffaloes is presented in table 1. The seroprevalence of parasites in different age groups of buffaloes ranged between 6.03% (7-9 years old) and 21.52% (≤ 3 years old). The seroprevalences of male and female buffaloes were 20.00% and 10.14%, respectively. The highest rates of buffaloes that tested positive for *F. hepatica* were found in Tabasco (13.62%, 32 out of 235 samples) and Veracruz (9.18%, 45 out of 490 samples).

Risk factors of *F. hepatica* infection in buffaloes

The data underwent a two-step analysis: an initial exploration of potentially confounding variables, followed by the development of multivariate logistic regression models. The final model developed for *F. hepatica* is shown in table 2. After screening all variables, four positive variables were retained in the final parasite model as significant risk factors: age, NDVI, LST day, and rainfall. The odds of fasciolosis seropositivity in 4-6-year-old buffaloes were 3.894 times higher than in young animals of 0-3 years old (OR = 3.894; 95% CI: 1.390-10.907; P = 0.010); this was one of the strongest predictors of trematode infection. The climatic and environmental variables NDVI (OR = 1.891; 95% CI: 1.509-2.370; P = < 0.001), LST day (OR = 2.832; 95% CI: 1.916-4.186; P = < 0.001), and rainfall (OR = 3.434; 95% CI: 2.181-5.406; P = < 0.001) at the farm location for 12 months before the day of sampling were identified as risk factors of positive serology for *F. hepatica* infection. The final value of McFadden's pseudo R^2 in the final model was 0.262, indicating a perfect fit (AIC = 734.191; P < 0.001).

Table 1: Prevalence of *Fasciola hepatica* infection rates in water buffaloes in Mexico by indirect ELISA

Risk factor	Overall		Negative samples (OD <0.6)		Positive samples (OD ≥0.6)		
	No. of buffaloes	Mean OD ± SD	No. of samples	Mean OD ± SD	No. of samples	Mean OD ± SD	Prevalence (%)
Veracruz/Las hoapas							
Farm 1	123	0.42 ± 0.27	113	0.42 ± 0.27	10	1.19 ± 0.27	8.13
Farm 2	189	0.44 ± 0.30	173	0.37 ± 0.13	16	1.31 ± 0.24	8.47
Farm 3	178	0.48 ± 0.34	159	0.37 ± 0.14	19	1.35 ± 0.20	10.67
Tabasco/Villahermosa							
Farm 4	121	0.55 ± 0.41	105	0.40 ± 0.12	16	1.52 ± 0.31	13.22
Farm 5	114	0.55 ± 0.44	98	0.54 ± 0.12	16	1.69 ± 0.30	6.93
Total	725	0.48 ± 0.36	648	0.43 ± 0.31	77	1.42 ± 0.31	10.62
Age (years)							
≤3	158	0.62 ± 0.52	124	0.36 ± 0.12	34	1.56 ± 0.21	21.52
4-6	268	0.47 ± 0.35	244	0.38 ± 0.13	24	1.44 ± 0.35	8.96
7-9	199	0.42 ± 0.23	187	0.37 ± 0.12	12	1.20 ± 0.14	6.03
≥10	100	0.47 ± 0.30	93	0.37 ± 0.14	7	1.34 ± 0.37	7.00
Sex							
Male	35	0.65 ± 0.52	28	0.41 ± 0.11	7	1.62 ± 0.32	20.00
Female	690	0.47 ± 0.35	620	0.37 ± 0.13	70	1.40 ± 0.30	10.14
State, climate							
Veracruz, Wet	490	0.45 ± 0.31	445	0.36 ± 0.13	45	1.30 ± 0.24	9.18
Tabasco, Rainforest	235	0.55 ± 0.44	203	0.39 ± 0.12	32	1.58 ± 0.32	13.62

Table 2: Multivariate logistic regression models of buffalo characteristics and climatic and environmental factors associated with the presence/absence of *Fasciola hepatica* infections based on 725 serum samples

Risk factor	B	SE	*P*-value	Odds ratio	95% CI for ORs	
					Lower	Upper
Constant	-517.943	95.049	<0.001*			
Age, year						
0-3				1.000		
4-6	1.360	0.525	0.010	3.894	1.390	10.907
7-9	0.381	0.527	0.523	1.464	0.521	4.117
≥10	-0.020	0.573	0.972	0.980	0.319	3.014
Sex				1.000		
Male						
Female	0.996	0.644	0.122	2.708	0.767	9.566
NDVI	0.637	0.115	<0.001	1.891	1.509	2.370
LST Day	1.041	0.199	<0.001	2.832	1.916	4.186
Rainfall	1.234	0.232	<0.001	3.434	2.181	5.406
State, climate						
Veracruz, Wet				1.000		
Tabasco, Rainforest	-3.719	0.531	<0.001	0.024	0.009	0.069

ORs, odds ratio. CI, confidence interval. LST, land surface temperature; NDVI, normalized difference vegetation index. *Wald: 7.924; AIC, Akaike Information Criterion: 734.191; McFadden's pseudo R^2 0.262.

Discussion

To our knowledge, this study is the first to investigate the prevalence of *F. hepatica* in buffalo farms by detecting serum IgG ELISA with E/S product antigens. Additionally, we investigated the relationship between the presence of parasites and parameters such as buffalo characteristics and climatic and environmental variables in Mexico. Our estimate of the overall prevalence of *F. hepatica* in bubaline farms was 10.62% in regions with a tropical climate, lower than that observed in Bangladesh 18.9 to 73.6% as measured by qualitative coproscopic diagnosis and post-mortem examination for the presence of *Fasciola* spp. (20-24). The present survey also showed a prevalence of *F. hepatica* in the wet tropical climate 9.18% that was lower than that observed in Bangladesh, 22.46%-26.14% using *F. gigantica* fecal egg count (25-27) and 16.7% as per post-mortem examination of each buffalo for *Fasciola* ssp. (28). The prevalence rate found in our study was higher 9.18% than that reported in Bangladesh 5.86% using post-mortem liver inspection for adult *Fasciola* spp. (29). The seroprevalence of *F. hepatica* in Villahermosa district, Tabasco, with a tropical rainforest climate, was 13.62%, higher than that reported in Indonesia 3.5% and Malaysia (8.7%) as determined by fecal egg count (30,31). And that in Malaysia (0.8%) in buffaloes with *Fasciola* ssp. infected livers (31). The climatic subtypes of Tabasco and Veracruz, as classified by the Köppen climate classification modified by Garcia in 2004, were Af (representing a tropical rainforest climate with rainfall throughout the year) and Am (representing a wet tropical climate with rainfall mainly in the summer). One potential reason for these variations in prevalence rates may be the more significant rainfall in Tabasco compared to Veracruz. Likewise, we assumed that the intermediate hosts of *F. hepatica* would be more common in Tabasco.

Our study observed a significant difference in the seroprevalence of liver flukes between the different age categories. The present surveys showed that young buffaloes (≤ 3 years old) had higher seropositivity than old animals (≥ 10 years old). These results contradict previous studies reporting that adult buffaloes show higher seropositivity than calves and young ones (32,33). The multivariate logistic regression model using climatic and risk factors showed that four variables were significantly associated with parasite infection: age, NDVI, LST day, and rainfall. The age of buffaloes was one of the strongest predictors for fasciolosis infection: 4-6-year-old animals had a risk of being infected that was 3.894 times higher than that of old buffaloes of 4-6 years old (OR = 3.894; 95% CI: 1.390-10.907; P = 0.010), and the likelihood of infection decreased with age. Our findings are consistent with previous research in Egypt that used fecal examination, indirect hemagglutination test, and multivariate logistic regression, finding that the prevalence of *F. hepatica* was significantly different in buffaloes according to age (34).

Based on our results, the highest prevalence of *F. hepatica* infection was detected by ELISA in male buffaloes, and the lowest prevalence was observed in female buffaloes (P = 0.122). Considering that male buffaloes were primarily sold and slaughtered for meat, it is possible that the dataset inadequately reflected the prevalence of infection. A similar finding was reported in Egypt using fecal examination and indirect hemagglutination tests, but significant differences were observed between male and female groups (34). In Iraq, previous research found substantial differences in the seropositivity of fasciolosis between the two sexes (32). However, this contrasts with a study from different climate conditions in Indonesia, reporting that infection prevalence was significantly higher in female buffaloes than in male animals (33).

Our study identified NDVI (OR: 1.891; 95% CI: 1.509-2.370) as a statistically significant factor that increases the probability of parasite exposure. The NDVI, obtained from remote sensing data, has been extensively employed to assess the level of vegetation greenness. It combines multiple environmental variables, including land cover, temperature, rainfall, and vapor pressure (35,36). In Mexican cattle herds, NDVI was the most positive predictor of *F. hepatica* infection (8); NDVI was also found to have a consistent relationship with the abattoir prevalence of parasites in Australia (35).

The LST day and average rainfall at the farm location for 12 months before the sampling day were positive predictors of *F. hepatica* infection in buffaloes. El-Tahawy (37) found that temperatures lower than 30 °C and relative humidity below 60% positively affected fasciolosis in buffaloes. Rainfall and potential evapotranspiration are important risk factors affecting the prevalence of the definitive host and eggs, metacercariae, and snail vectors of *F. hepatica* (38,39). The higher prevalence in the three Egyptian governorates studied was explained by the differences in the climatic and environmental factors, such as rainfall and/or farm size, and cattle grazing habits between these governorates. Likewise, the location of the farms, close to the Mediterranean Sea or in areas filled with water and inadequate water management programs, may have favored the development and spread of the intermediate snail hosts responsible for the transmission of fasciolosis (37). Regional variations in climatic and environmental conditions can lead to significant differences in the level of exposure to *F. hepatica* and the growth and spread of the parasite's intermediate molluscan and free-living stages (7,13). The hatching of *F. hepatica* eggs is completed for 2-3 weeks at a temperature range of 23-26 °C. The most favorable temperature range for infection is between 15 and 26 °C (5). A minimum temperature of 10 °C is required for the snail's development, while optimal growth occurs at 18-27 °C. The parasite cercariae undergoes total growth within the snail at 15 °C over 80 days. Shedding requires a minimum temperature of 10 °C, and metacercarial survival is reduced in high temperatures. Cysts can stay viable for three days at 20 °C and a relative humidity of 75-80% (40).

Conclusion

This study showed that *F. hepatica* prevalence was low among buffaloes in two tropical climate subtypes in two Mexican states. In addition, our study identified some factors associated with parasite prevalence, including age, NDVI, LST, rainfall, and climate region, and highlighted the importance of continuous monitoring programs for liver fluke infection. Further studies are necessary to generate more knowledge on shifts in levels of exposure between years and intermediate limnaeid snail hosts.

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Conflict of interest

There is no conflict of interest.

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عوامل الخطر المناخية والبيئية ودورها في انتشار المتورقة الكبدية في جاموس الماء في المكسيك

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الخلاصة

داء المتورقات هو مرض حيواني المنشأ مختفي عاد للظهور تسببه ديدان المتورقة الكبدية التي تصيب الماشية والبشر على حد سواء في جميع أنحاء العالم، مما يتسبب في خسائر اقتصادية شديدة ومشاكل صحية عامة. حددت الدراسة الانتشار المصلي لعدوى المتورقة الكبدية في الجاموس وارتباطاتها بالعمر والجنس والعوامل المناخية والبيئية (المستمدة من بيانات الأقمار الصناعية) لتحديد عوامل الخطر المحتملة من نوعين فرعيين من المناخ المداري في ولايتين مكسيكيتين. تم تحليل ما مجموعه ٧٢٥ عينة مصل لتحديد وجود مضاد المتورقة الكبدية الأجسام المضادة للغلوبولين المناعي الكبدي (IgG) باستخدام مقايسة الممتز المناعي غير المباشر المرتبط بالإنزيم(ELISA) . بلغ معدل الانتشار الإجمالي للطفيلي في الجاموس بين أيلول ٢٠٢٢ كانون الاول ٢٠٢٢ ١٠,٦٢٪ (٧٧ من أصل ٧٢٥). تم الكشف عن أعلى معدل للإصابة بعدوى المتورقة الكبدية في ولاية فيراكروز ٩,١٨٪. بالإضافة إلى ذلك، تم العثور على أعلى معدل انتشار بين المناطق المناخية في الغابات الاستوائية الممطرة ١٣,٦٢٪. في الولايتين اللتين تمت دراستهما، لوحظ أعلى معدل إيجابي لداء المتورقات في الحيوانات الصغيرة التي أعمارها ثلاث سنوات وأقل ٢١,٥٢٪ وفي ذكور الجاموس ٢٠,٠٠٪. باستخدام تحليل الانحدار اللوجستي متعدد المتغيرات، حددنا أربعة عوامل (العمر، ومؤشر الغطاء النباتي للفرق الطبيعي، درجة حرارة سطح الأرض اليومي، وهطول الأمطار) لتكون مرتبطة بشكل كبير بزيادة خطر الإصابة بالعدوى الطفيلية. كان هطول الأمطار هو المتغير الأكثر ارتباطا بالاصابة بالمتورقة الكبدية يليه درجة حرارة سطح الأرض اليومي. أكدت النتائج أن عوامل مختلفة ترتبط بشكل معنوي بانتشار ديدان الكبد وتبرز أهمية برامج المراقبة المستمرة للاصابات الطفيلية لمنع الخسائر الاقتصادية في إنتاجية الجاموس.