



EPIDEMIOLOGY OF FASCIOLOSIS IN NILI RAVI AND KUNDI BUFFALOES AND ITS EFFECT ON HEMATOLOGICAL PARAMETERS

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ABSTRACT:

Fasciola hepatica is a common parasite of grazing livestock. Fasciolosis is a burning problem for the livestock and dairy industry in Pakistan. The coproscopic examination was done for fasciolosis and associated risk factors were explored in Nili Ravi and Kundi buffaloes from various dairy farms in the locality of district Mardan. For this purpose, a total of 450 buffalo communities were chosen for random research faecal and blood sampling. A subset of 450 (N=450) buffaloes from these farms were divided into 2 groups based on species. Group A (n = 225) Nili Ravi, and Group B (n = 225) Kundi. 5 grams of fecal material was collected from each individual and tightly packed in a polyethene bags in the month of September 2023. After proper collection of samples, it was stored in refrigerator at 4C till processing. All the groups examined in the laboratory one by one carefully by using dissecting microscope in the Lab of laboratory of Clinical Medicine and Pathology in the month of September to October 2023. From the current research work district Mardan KP, showed overall 38% (171/450) incidence rates. Out of which 20% were Nili-Ravi and 18% were Kundi Buffalo. The site which favors more prevalence was Khaow 56.14% and lowest Toru 16.4% the most hygienic habitat. During copro-scopic study two species of *Fasciola* name *F.hepatica* and *F.gigantica* and their eggs were identified using





Medical Parasitology key. Blood sample was collected in EDTA tube. Positive cases analyzed in a hygienic laboratory. Hematological parameters were checked in infected buffalo then compared to healthy one. The changes recorded shown significant reduction in various blood cells count. The proposed framework applied and evaluated on two buffalo breeds, namely, Nili Ravi and Kundi. At the end of study, statistical analysis applied on collected data such as percentile, ANOVA and t-test will be applied on prevalence, risk factors analysis and blood parameters respectively. The result should be made correct significantly at (P<0.05).

KEYWORDS: Buffalo, Blood Parameters, Fasciolosis, Nili-Ravi, Mardan, Kundi,

INTRODUCTION

Pakistan has 29.9 million buffaloes and 33.0 million cattle, of which Punjab accounts for 65% and 49%, respectively. Pakistan has five buffalo breeds: Nili, Ravi, Nili-Ravi, Kundhi, and Azakhali (Bilal, Suleman et al. 2006; Khan, Ahmad *et al.* 2007) in addition to some of the best breeds of cattle (Bos indicus), including crosses between Sahiwal and Freesian that yield milk and meat, as well as Sahiwal, Bhagnari, Red Sindhi, Cholistani, Dhanni, Thari, Rojhan, Lohani and Dajal (Rehman, Khan *et al.* 2014). The buffalo population in Pakistan is 29.9 million strong. The main breeds found in Pakistan are Aza-kheli, Nili-Ravi, Ravi, Kundi, and Nile. Ninety million people are thought to be at risk and 2.4–7 million people to be infected. Worldwide, over 700M pet animals are in danger, and the annual economic losses exceed US\$ 2 billion (Reichel, Ayanegui-Alcérreca *et al.* 2013).

One of the biggest and most significant issues facing the world today is *fasciolosis*, which is primarily caused by animal death, the high expense of diagnosing and treating the diseased liver, decreased production of milk and meat, infertility, and drug resistance to the disease (Mehmood, Zhang et al. 2017). *Fasciola hepatica*, sometimes referred to as sheep liver fluke or common liver fluke, causes a condition known as *fascioliasis*, which is also referred to as *fasciolosis*. The global *species Fasciola hepatica* has been causing significant economic losses for several hundred years. It may be the most well-known Trematode species due to its size and financial value, having been the focus of numerous scientific studies (Alba, Grech-Angelini *et al.* 2023).

Fasciolasis is a significant helminth because it has a broad host range that includes humans, livestock, and wild animals (Rondelaud, Vignoles et al. 2001). Fasciola hepatica along with





Fasciola gigantic are the parasites that cause *fasciolosis*, a disease that affects human health and can result in significant financial losses. (Anas, Farooq *et al.* 2023). According to Soulsby (1982), two major prevalent species are *Fasciola* (*F.*) *hepatica* along with *F. gigantica*, which infect about 300 million animals each year (Boray, Fraser *et al.* 1985).

Of every species, *F. hepatica*—often referred to as the "liver fluke"—is a significant livestock pathogen that affects animals globally and can cause acute or long-term infections (Mahanty, Maclean *et al.* 2011). Fasciolosis is a significant illness caused by zoonotic helminthes globally (Haridy and Ibrahim 1999). The two species of liver flukes are thought to be economically significant because they infect a variety of mammals, particularly sheep and cattle (Mitchell 2002). Different animal species in different locations in Pakistan have reported varying rates of *fascioliasis* prevalence: Bahawalpur (23.97%), Punjab (17.68%), Lahore, Multan (10.48%) and Peshawar (55%)(Hasanat, Sial et al. 2014). Khan *et al.* (2009) recorded maximum *fascioliasis* incidence ratio in buffaloes (43.96%) followed by population of cattle (36.67%) (Khan, Sajid *et al.* 2009).

Over 600 million livestock worldwide are infected with *fasciolosis*, which is estimated to cost rural farming communities and business owners \$200 million annually in lost animal productivity. F. hepatica can have an incidence of up to 77% in developed nations. With a reported prevalence of 30–90%, fasciolosis is regarded as the most significant helminthes infection in cattle in tropical countries. The sudden onset or prolonged *fasciolosis* in domestic ruminants can have negative effects on meat and dairy production, fertility, and veterinary expenses (Ali, Khan *et al.* 2013) Many scientists from all over the world, including those in England, Malawi, the United States, Spain, Brazil, Peru, India, Tabasco, Zambia, and Iran, have established a link between climatic factors and the spread of disease. They showed that a temperature increase of more than 10-15 0C creates an environment that is conducive to the emergence of eggs and larvae (Phiri, Phiri *et al.* 2005; Moazeni, Ansari-Lari *et al.* 2010). Yet, *fascioliasis* has been discovered to be endemic all year round in a number of different regions of the world (Conceição, Durao *et al.* 2004; Phiri, Phiri *et al.* 2005). It has been observed that livestock contract the infection during the winter and reach maturity in ten to fifteen weeks. When there is enough moisture present, which is essential for parasite development, the springtime is when egg production peaks. The abundance



of water available at this time and the existence of infected snails carrying *Fasciola* cercariae could be additional factors contributing to the disease's high magnitude (Ortiz, Claxton *et al.* 2000). In a same vein, Phiri et al. (2005) in Zambia indicated maximum fluke existence in the post-rainy season livers as well as the eggs in the faeces. An investigation into Iranian slaughterhouses over a five-year period found that in dry and hot weather, up to 4% of livers are condemned due to *fascioliasis* (Roberts, Widjayanti *et al.* 1997; Conceição, Durao *et al.* 2004).

There have been reports of fasciolosis prevalence in various provinces in Pakistan. (Shahzad, Mehmood *et al.* 2012). Previous research relied less on precise location and more on morphology, incidence, or worm populations that originated from larger areas.

There is currently a dearth of epidemiological data regarding *fasciolosis* in KP's district Mardan. Nevertheless, epidemiological research results from other nations is available and can be a source of knowledge and assistance; sadly, these methods are not applicable to our particular situation. Pakistan has experienced climatic changes, which have impacted the prevalence of fasciolosis. Thus, it is imperative to assess the epidemiological pattern that *fasciolosis* in the various Mardan regions, where large ruminants play a significant economic role, particularly for the farmers who depend on raising buffalo and cattle for a living and thereby make a substantial economic contribution to the country. The agroclimatic conditions in the Mardan district are especially conducive to the growth and survival of a variety of freshwater snail species that serve as intermediate hosts. Therefore, in order to evaluate the epidemiological nature of *fasciolosis* in the Mardan geographical area of KP, Pakistan, up-to-date information about the current situation is required.

METHODOLOGY

Ethical Approval

At Abdul Wali Khan University, Mardan, Pakistan's (CVS & AH) College of Veterinary Sciences and Animal Husbandry, all experimental and research protocols and procedures have been approved by the research ethics committee. All pertinent samples were obtained from the lambs and sheep in compliance with the guidelines and directives provided by the Medicine & Parasitology Laboratory.

Study Area



Pakistan is located at latitude 30° 00 North and longitude 70° 00 East in South Asia. Pakistan's total land area is 803, 940 square kilometers. This area has a predominantly hot climate with a vast arid topography. Pakistan's southern shoreline stretches 1,046 kilometers along the Arabian Sea, while its western boundaries are shared by Afghanistan and Iran, its eastern boundary is shared with India, and its far northeastern neighbor is China.

Mardan is the second-largest city in Khyber Pakhtunkhwa, with 404,436 residents, according to the Pakistani Census of 2023. With 51,429 households, these people were dispersed throughout the city, giving Mardan an average household size of 7.89. Mardan's population increased fivefold in a span of 50 years during the last half of the 20th century, exhibiting explosive growth. However, the city's rate of growth has significantly slowed down over time; between 1998 and 2017, the population of the city increased by only 2% annually. Mardan is situated at 34°12'0N 72°1'60E, or the southwest of the district, at an elevation of 283 meters (928 feet)

Climate of Mardan

The steppe climate of the surrounding area influences Mardan's hot, semi-arid climate (Köppen BSh). The average annual temperature of Mardan is 22.2 °C, with an average of 559 mm of precipitation. With an average rainfall of 12 mm, October is the driest month, and August is the wettest with 122 mm on average. June is the the warmest month of the year, with an average mean temperature of 33.2 °C. The coldest month, January, has an average temperature of 10.0 °C.

Morphological Characterization

3.4.1 Nili-Ravi

Nili-Ravi is regarded as Pakistan's "black gold." The Nili-Ravi breed's ancestral home is located between the Sutlaj and Ravi rivers. This breed is known as "Panj Kalian" because of the five white markings on its udder, lower legs, forehead, muzzle, and tail switch.

3.4.2 Kundi





The Kundi homeland stretches from Kashmore to Sindh to Shah Bandar (Sindh) on both banks of the Indus River. The body is jet-black in color, with medium-sized ears, a short horn, a broad forehead, and a short neck. The name "kundi" comes from the broad base of the horns that taper upwards and inwards to resemble a fish hook.

3.5 Sampling Area

The current study carried out between October 2023 February 2024 to predict the coproscopic identification, prevalence and risk factor associated with fasciolosis in the Gastro-intestinal tract of buffalo in dairy farms of Mardan locality. All the buffalo (N = 450) residing in 10 farms were selected in the area of Nawan Kaly, Toru and Khaow.

3.5.1 Number of Sample

In the current study a total of 450 buffalo 225 from each breed has been chosen from 10 dairy farms. Out of 225 the number of male were 20 (n=20), calf were 25(n=20) while 180 were female buffalo of various age.

3.6 Sample Collection

During collection fecal were kept in the sterile free container each tag with farm name, number, date, age, gender. After complete collection of the samples. 5 gram of fecal sample was retained in the container for further processing.

3.7 Fixation of sample and Laboratory Examination

10ml of Formalin solution added to each container and kept in the refrigerator for next laboratory examination. The fecal samples were examined and examined at the AWKUM College of Animal Husbandry and Veterinary Sciences' Laboratory of Medicine and Public Health.

3.8 Differential Flotation Technique and Coproscopy

A stool sample weighing about 4 grams was put in a test tube with 10 milliliters of zinc sulphate solution in order to identify *Fasciola hepatica*. After gently grinding the material with a spatula, the liquid was strained through a tea filter. After adding the filtrate solution, a 10 ml centrifuge tube has been filled with a zinc sulphate solution and centrifuged for five minutes at 2000 rpm. To create a convex surface at the tube's apex, more concentrated sodium chloride was utilized after





centrifugation. After adding a single drop of methylene blue, the cover slip was fastened for five minutes before being taken off and put on a slide so that it could be examined using 10X and 40X objectives. Photographs and identifications of parasite eggs were made using morphometry, which measures color, shape, and size. (Soulsby, 1982).

3.8.1 Sedimentation Technique

Using a sedemantation method, fasciolosis was discovered. Subsequently the floatation component was examined and the salt solution that was saturated was carefully taken out of the test tube, the resulting sediment portion was poured into the watch glass along with the mixture was carefully mixed. A tiny amount of this mixture has been utilized to prepare the second slide. The specimen was stained using iodine wet mounts solution. In this way, two slides in a single sample—one from sedimentation and the other from floating—were made, and they were subsequently checked for eggs under microscopes using 10X and 40X objectives. (Soulsby, 1982)

3.8.2 Stool Examination

The feces samples was gathered, transported to the lab in preservatives, and kept cold—4C. The sedimentation technique, stoll's counting method, and differential flotation technique will all be used to examine the stool samples.

9 Fecal Qualitative Examination

Applying zinc sulphate (ZnSO4.7H2O) with a specific gravity of 1.27 as a flotation solution, a fecal qualitative investigation will be carried out with the aid of the fecal flotation technique. (Zajac, Conboy et al. 2021).

3.10 Stoll's counting method

Without using McMaster, this is the most straightforward quantitative approach to count the eggs in the field. Using a microscope, the species-specific helminth parasite eggs on the slide have been examined and will be tallied. Fasciola hepatica eggs were identified and tallied using a parasitology key. The entire number of eggs determines the quantity of eggs for each gram of feces.

Prevalence rate



The prevalence rate of an infection in sheep was calculated using the following formula, expressed as a percentage:

No. of Buffalo (Positive cases) Prevalence (%) = ----- × 100 No. of Buffalo at Risk (Total)

OR

Percent prevalence = $d/n \times 100$

where n denotes the total number of sheep tested who tested positive for Fasciola *hepatica* at that particular time, and d & n represent the total number of sheep/lambs infected (at a specific time and place).

Data analysis statistically

Microsoft Excel was used to store the data and analyze basic descriptive statistics. We will compute descriptive statistics with IBM-SPSS version 24.0. A P-value of 0.05 or lower were considered significant for any variation in prevalence in the statistical analysis. Multiple Logistic Regression and the Pearson Chi Square Test were used to statistically analyze the data. With SAS statistical software, the correlation between prevalence and potential influencing factors was calculated at a 95% confidence level using Odd's ratio.

RESULTS

Detail of Dairy Farms and Sites

The current study was conducted for the prevalence of *fasciolosis*, its effect on blood parameter and its associated risk factors in the district Mardan. The following 10 farms from 3 major sites were selected for the study.



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		Asghar Dairy Farm
		Ishaq Dairy Farm
		Saif Alam Dairy Farm
2	Khaow	Abdul Salam Dairy Farm
		Khurshid Dairy Farm
		Nasir Dairy Farm
3	Nawan Kaly	Rahat Saleem Dairy Farm
		Shafiq Dairy Farm
		Karim Dairy Farm

Table 4.1 Dairy Farms Details and Sites

4.2 Total Animals and Distribution

The following buffalo of different gender was selected from the mentioned area for the detection of fasciolosis. (Table 4.2)

S.	Female		Males	Calves	Total
No					
	Nili-Ravi	Kundi			
1	18	18	04 (2 each breed)	04 (2 each	44
				breed)	
2	18	18	04 (2 each	04 (2 each	44
			breed)	breed)	
3	18	18	04 (2 each	04 (2 each	44
			breed)	breed)	
4	18	18	04 (2 each	04 (2 each	44
			breed)	breed)	

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5	18	18	04 (2 each	04 (2 each	44
			breed)	breed)	
6	19	19	04 (2 each	04 (2 each	46
			breed)	breed)	
7	19	19	04 (2 each	04 (2 each	46
			breed)	breed)	
8	19	19	04 (2 each	04 (2 each	46
			breed)	breed)	
9	19	19	04 (2 each	04 (2 each	46
			breed)	breed)	
10	19	19	04 (2 each	04 (2 each	46
			breed)	breed)	
Total	185	185	40	40	450



Fig. 4.3 Pi-Chart of overall Prevalence



Fig4.4 Area Wise Prevalence

No. of infected Nili-Ravi

Prevalence (%) = ----- × 100

No of Buffalo at risk

n (female + male + calves)

% age = ----- × 100

N (Nili-Ravi + Kundi)

90 (78 + 0	08 +	04)
%age	=	× 100

450

Prevalence = 20%



4.5.2 Prevalence in Kundi

Kundi showed overall prevalence of 18% (81/450). Female buffalo were 85.18% (69/81) 2-4 years old were 44.88% (35/78) while 4-7 years were 55.12% (43/78), male from both breed were 8.6% (07/81) 4.4% (04/90) and calves were 7.4% (06/81). 13.33% (12/90). On the other hand in Kundi breed 2-3 years were 19/81 (23.4%), 3-5 years were 23/81(28.3%) and 5-7 years were 39/81(48.1%).

No. of infected Kundi Prevalence (%) = -----× 100

No of Buffalo at risk

· · · · · ·

81 (69 + 07 + 06) %age = -----× 100

450

Prevalence = 18%



Fig. 4.5 Breed Wise Graph

S.No.		1	2	3
Age		06-24 Months	2-4 Years	4-7 Years
Breed	Nili-Ravi	13.33% (12/90).	38.88% (35/90)	47.77% (43/90)
	Kundi	6.17% (05/81)	39.50% (32/81)	45.6% (37/81)
Total Prevalence		19.5%	78.38%	93.37%
	Kundi	6.17% (05/81)	39.50% (32/81)	45.6% (37/81)



Fig.4.6 Age Wise Prevalence



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Infected Animals (n/N)	Pre	valence (%) = n/N ×100
(171/450)	389	
Associated Determinants	Levels	Prevalence/%age
Sex	Female (147/171)	85%
	Male (07/171)	4.09%
	Calves (18/171)	10.52%
Age	06-24 Months	06-13%
	02-04 Years	30-39%
	04-07 Years	45-47.77%
Breed	Nili-Ravi (90/171)	52.63%
	Kundi (81/171)	47.36%
Parasite Species	Fasciola hepatica 118/171	69%
	Fasciola gigantic 53/171	30.99%

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Feeding System	Grazing			
	Ground			
	Through			
Water System	River water			
	Pond Water			
Regions	Khaow 96/171	56.1%		
	Nawy Kaly 47/171	27.48%		
	Toru 28/171	16.37%		

Hematological Parameter

From the result of infected buffalo it was diagnosed that *Fasciola hepatica* and *Fasciola gigantica* has affect hematocytes count. Due to infection Red Blood Cells destruction occurs which cause anemia in buffalo also lead to weakness of the body. Various cell parameters using Hemolyzer® 3 NG | Analyticon Biotechnologies AG are below;

Blood Parameters	Unit	Infected (n=171)	Healthy Buffalo
RBCs	× 10 ¹² /l	4.7 - 8.3	12.83 - 13.01
WBCs	× 10 ⁹ /l	13.54 – 15.3	10.05- 11.0
Lymphocytes	× 10 ⁹ /l	4.73- 5.2	6.03- 7.59
Eosinophils	× 10 ⁹ /l	2.01-3.7	0.23-0.29

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Monocytes	$\times 10^{9}/1$	0.17- 0.23	0.17-0.18
Basophils	$\times 10^{9}/l$	0.010.01	0.01 - 0.01

Hematological Parameters

DISCUSSION

In the population of two buffalo breeds, Nili-Ravi and Kundi, the epidemiology of fasciolosis, its impact on blood parameters, and related risk factors were investigated in the current study. It was discovered during a cross-sectional study on coproscopy and hematology analysis that the overall prevalence in the chosen area remained at 38%. The Khaow had the greatest prevalence, followed by Nway Kelly, and the Toru site had the lowest. Three different agro-ecological sites' data were compared. Using SPSS software, all the data showed statistical significance (P<0.05).

Two members of the genus *Fasciola, F. gigantica* and *F. hepatica*, are responsible for the serious trematode infection known as fasciolisis, which affects goats and sheep. The genus *Fasciola* is a member of the phylum Platyhelminthes' class Trematoda. Sampaio-Silva and associates, 1996). The WHO has added the illness to its index of neglected tropical diseases due to its significance as a zoonotic disease. The WHO estimates that millions of people worldwide have *fascioliasis* and that 180 million are at risk of developing the disease. It is becoming more common among people and has been documented in 70 different nations worldwide. Digeneans are known to involve one or two intermediate hosts in their life cycle. Snails serve as an intermediate host, and their presence is necessary for fluke transmission. Because *fascioliasis* can easily colonize both its ruminant host and vector species, it has a high potential for proliferation (Mas-Coma et al., 2003). The disease's geographic range is restricted to regions with snail populations. *Fascioloasis* can be maintained and spread in intermediate hosts in areas with higher rainfall each year, poor drainage, and certain types of soil that retain moisture (Malone *et al.*, 1994; Asrat, 2004).



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According to Iqbal et al. (2007), the highest frequency of F. hepatica disease was found in milkproducing dairy animals bred in Punjab, Pakistan. Appropriate anthelmintic treatment was recommended as a control measure. During the current study, similar observations were made at three different Mardan sites. When compared to healthy buffalo, the buffalo's daily output was lower due to their poor health.

Another study by Khan *et al.* (2009) discovered that the incidence of bovine *fasciolosis* was 25.46 percent in the five districts at Punjab Province, via *F. gigantica* (22.40 per cent) having been more prevalent compared with F. hepatica (3.06 percent). The prevalence of *fascioliosis* was highest in the winter (39.08 percent), spring (29.50 percent), and summer (12.92 percent) seasons. It was widespread in different parts of Sargodha (40.31 percent) as well occasionally in Layyah (11.77 percent). When compared with cattle (20.42 percent), buffaloes (30.50 percent) displayed higher rates of infection. Given that a recent study was done during the winter, the Punjab region agreed with these winter data. These months' similar climates encourage the same prevalence rate.

The association between age and disease prevalence was further supported by Maqbool et al. (2002), who found that disease prevalence was lower (11.36%) in younger animals and higher (18.45%) in older animals. On the other hand, it has been noted that the incidence of sudden infection with a high cercariae load peaks between the ages of two and three, and then declines with aging, possibly as a result of partial resistance that has evolved. However, it has been noted that adult animals frequently have a persistent Fasciola infection.

The current study showed 38% overall prevalence in three regions of Mardan namely Khaow, Nawan Kaly and Toru. The highest ratio was observed in the khaow site was identified as the highest prevalent 56.14% while lowest was Toru 16.37%. Upon research data analysis and questionnaire interviews from local form owner it confirmed that improper vaccination and unhygienic condition favor *fasciolosis* infection. The agro-ecology has been shown the crucial role in the incidence of the disease. Also it was proven that older and Nili-Ravi showed slightly more prevalence due to its exposure to comparatively heterogeneous grazing habitat and weak immunity response. From coprological and hematological diagnosis it was spoted that *fasciolosis* not only cause weakness, however it also effect the daily milk product and poor health condition that lead buffalo to death in many cases. It also become secondarily available for slaughter house due to





less milk production which cause loss of economy of farmers. Blood diagnosis showed reduced RBCs in the clinical positive cases.

Associated Risks with Fasciolosis

Cattle productivity and health are severely impacted by *fasciolosis*, which is brought on by the liver fluke parasite *Fasciola hepatica*. Let's investigate its impacts:

Reduced Growth and Productivity:

- > Cattle with *fascioliosis* experience growth retardation.
- > It results in less meat and milk being produced.
- > Pregnant cows may experience weight loss, which can lead to weaker calves and low-grade milk.
- Cattle herds, particularly those that calve in the spring, suffer negative consequences on calf health and productivity.

Liver Damage:

- > Adult flukes cause inflammation and biliary obstruction because they live in the bile ducts.
- > The parasite's presence causes damage to liver tissue.
- In afflicted cattle, elevated liver enzymes like gamma glutamyl transferase (GGT) and aspartate aminotransferase (AST) signal liver damage

Subclinical Impact:

- ➤ *Fasciolosis* frequently exhibits subclinical symptoms, or no symptoms at all.
- Despite this, it lowers milk yield, butterfat content, and general body condition, which has an impact on dairy herds.
- A small number of liver flukes can significantly affect the amount of milk produced by highyielding dairy herds.

Epidemiology and Control:

- > Comprehending the epidemiology of the illness is imperative for efficient management.
- > The parasite population on pastures is influenced by temperature and rainfall.
- > The parasite benefits from climate change, which has an impact on snail survival and spread.
- > One way to lessen the spread is to fence off or drain high-risk pastures

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