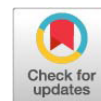


Research Article



Prevalence and Risk Factors of *Haemonchus contortus* Infection along with *In Vitro* Efficacy of a Bio-Anthelmintic against Adult Worms

SANA NOOR PANHWER^{1,2,3}, JAVAID A. GADAH^{3*}, SHAHARBANO MEMON³, SHAHISTA JALBANI¹, BACHAL BHUTTO³, REHANA BURIRO^{1,3}, ZHENGLI CHEN^{2*}

¹Department of Veterinary Parasitology, Faculty of Veterinary Sciences, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Pakistan; ²Laboratory of Animal Disease Model, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China; ³Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan.

Abstract | *Haemonchus contortus*, (barber pole worm), is a significant nematode affecting ruminant's in tropical and subtropical regions. Present study examined the prevalence of *H. contortus* infection in goats in the interior of Sindh, Pakistan, and evaluated the effectiveness of bio-anthelmintics on adult worms. Prevalence was monitored through a survey across five districts, which revealed an overall infection rate of 52.4%, with the highest ($P=0.001$) risk observed in goats aged 8 to 12 months, at 66.11%. In addition, gender base susceptibility revealed males goats were more affected ($P=0.001$), showing a 60% infection compared to 49.14% in females' goats. Seasonal analysis indicated that the summer and monsoon seasons supported a higher ($P=0.000$) prevalence of 64.44% compared to winter, at 21.42%. The study also assessed the efficacy of *Bacillus thuringiensis* purified crystal proteins and spore-crystal mixtures, adult *H. contortus* were treated with these substances at specified concentrations, and motility was measured after 12, 24, and 48 hours. The purified crystal protein at 2 mg/ml reduced worm mobility by 50% after 12 hours, 66.66% after 24 hours, and 83.33% after 48 hours. The spore crystal suspension at 1×10^8 led to a mobility reduction of 16.66% after 12 hours, 25% after 24 hours, and 66.66% after 48 hours. Overall, the findings indicate that *B. thuringiensis* toxins can effectively serve as a bio-anthelmintic for controlling worm burdens in small ruminants.

Keywords | Anthelmintic resistance, Bio-anthelmintic, *Haemonchus contortus*, Prevalence, Sindh-Pakistan

Received | June 08, 2025; **Accepted** | August 30, 2025; **Published** | November 07, 2025

***Correspondence** | Javaid A. Gadahi, Zhengli Chen; Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan; Laboratory of Animal Disease Model, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China; **Email:** chzh-li75@163.com, jagadahi@sau.edu.pk

Citation | Panhwer SN, Gadahi JA, Memon S, Jalbani S, Bhutto B, Buriro R, Chen Z (2025). Prevalence and risk factors of *Haemonchus contortus* infection along with *in vitro* efficacy of a bio-anthelmintic against adult worms. J. Anim. Health Prod. 13(4): 1201-1208.

DOI | <https://dx.doi.org/10.17582/journal.jahp/2025/13.4.1201.1208>

ISSN (Online) | 2308-2801



Copyright: 2025 by the authors. Licensee ResearchersLinks Ltd, England, UK.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

INTRODUCTION

Pakistan is the world's third-largest goat-producing country after China and India. It is rich in goat genetics. Diversity exists in the production system at the phenotypic and genetic levels within and across breeds (Khan *et al.*, 2008; Moaen-ud-Din *et al.*, 2022). Moreover, as far as feeding techniques are concerned, the most prevalent one is grazing or grazing with some forage. However, the

risk of gastrointestinal nematodes, especially *Haemonchus contortus*, is increased on grazing based diet (Bricarello *et al.*, 2023).

H. contortus, a highly pathogenic, blood-sucking parasite that affects small ruminants and is a significant cause of mortality worldwide (Besier *et al.*, 2016; Wei *et al.*, 2025). Additionally, it leads to significant economic losses in terms of production and breeding (Wang *et al.*, 2017; Fayaz *et*

al., 2019). Furthermore, the outbreak of Haemonchosis was based on seasonality, egg output, and worm burden, which increased from late April to late May and decreased immediately until late July, reached the second peak in early August, decreased again, and then remained at a low level till next April (Song *et al.*, 2007). However, gender-wise prevalence is higher in male goats in comparison to female goats (Tasawar *et al.*, 2010).

H. contortus is primarily found in warm, humid countries where sheep and goats are raised (Wei *et al.*, 2025). It was a high-incidence reported nematode in Pakistan (Khan *et al.*, 2010; Lashari *et al.*, 2011; Ayaz *et al.*, 2013). Antiparasitic drug resistance is another serious issue that helps its growth, resistance is produced due to genetic changes in *H. contortus* across different locations and periods, both during and after the implementation of specific treatment or control strategies, as well as due to environmental, management, and climate factors (Yin *et al.*, 2016; Fidèle *et al.*, 2024). Anthelmintic resistance now reaching epidemic levels in small ruminants in the U.S., non-chemical control alternatives are critically needed (Terrill *et al.*, 2012). Furthermore, non-chemical control with *Bacillus thuringiensis* showed activity was similar to that observed by anthelmintic treatment, and it could be used as an alternative biological method for controlling the internal parasites (Lopez *et al.*, 2002; Sinott *et al.*, 2012). In addition, *B. thuringiensis* works through a well-known protein mechanism that causes toxicity, which helps prevent the nematode population and serves as a bioanthelmintic. *B. thuringiensis* proteins titled Cry (crystal produced during a specific growth process), Vip (vegetative insecticidal proteins produced during the vegetative period), and Cyt (cytolytic, pore-forming proteins). These proteins are known for their effectiveness in targeting various insects and nematodes, and have also shown potential in combating certain human cancer cells. These toxins also help to control the *H. contortus* population (Sinott *et al.*, 2016; Lopez-Arellano *et al.*, 2002).

During the present study, goats from Sindh province of Pakistan were selected to monitor the prevalence percentage of *H. contortus*, which is commonly found in small ruminants (Gareh *et al.*, 2021) and produces mortality in goats (Hounzangbe-Adote *et al.*, 2005). In addition, we applied different concentrations of bacterial crystal proteins and spore crystal suspensions to adult *H. contortus* and checked their efficacy.

MATERIALS AND METHODS

EPIDEMIOLOGICAL STUDY

Present study conducted a survey to determine the types of small ruminants preferred by local communities across five

districts in Sindh, Pakistan: Tando Allahyar, Mirpur Khas, Umerkot, Mithi, and Tharparkar. Results indicated that goats outnumber sheep in these areas. Then, we gathered historical data from farmers regarding the rearing methods employed, focusing on whether the animals were raised on a grazing-based diet or through stall feeding. Based on our observations of dietary practices, we selected a grazing-based rearing system.

To identify parasite levels, we looked at clinical signs, used the FAMACHA test, and checked the animals' physical condition. Animals that seemed more likely to be infected were separated, and their fecal samples were collected. We then used the McMaster technique to check for worm infection (Zajac and Conboy, 2012). The present study surveyed about 1,000 animals from different districts, and chose severely infected goats for the faecal sample collection to establish a monospecific flock (Urquhart *et al.*, 1996).

PREVALENCE AND ASSOCIATED RISK FACTORS (AGE, SEX, AND SEASON)

To assess the prevalence of *H. contortus*, we categorized the animals by age, sex, and season, then established three distinct age groups: Group One includes flocks younger than 8 months, Group Two comprises animals aged 8 to 12 months, while Group Three consists of herds that are 12 months or older. Each gender was screened separately to determine the infection rate and to evaluate gender-related differences (Tasawar *et al.*, 2010). We also studied how parasite breeding patterns changed with the seasons, from winter 2021 through summer and the monsoon season of 2022.

PARASITE ANALYSIS

Based on the FAMACHA guide test and McMaster results, we selected 20 severely infected animals to isolate *H. contortus* eggs. Briefly, faecal samples were analyzed by performing EPG using the McMaster technique (Tasawar *et al.*, 2010). A 3-gram sample of pellets was transferred into a saturated saline solution, mixed thoroughly, filtered, and allowed to rest for 30 seconds. The resulting solution was then poured into McMaster chambers, where the gastrointestinal strongyle counts were determined at a magnification of 40x. The mean eggs per gram (EPG) value was calculated from 12 replicates. The Coefficient of Variation (CV) was determined by dividing the standard deviation (S.D.) by the mean EPG value, using the formula $CV = (S.D. / \text{mean EPG}) \times 100$. This same method was applied to high-infection samples (Kaplan *et al.*, 2004). Furthermore, the identification of *H. contortus* eggs was carried out using established identification keys (Zajac and Conboy, 2012). After confirming *H. contortus* infection, animals with egg counts exceeding 1000 eggs per gram of feces were isolated

for final sampling. Fecal samples were collected and cultured for one week at a temperature of 28 °C and 80% humidity in a controlled incubator. After seven days, third-stage larvae (L3) were harvested using the Baermann technique and counted under a microscope at 40x magnification (MRJ microscope). The harvested L3 were quantified, and a total of 8000 L3 were orally administered to a nematode-free experimental flock to establish a monospecific species (Bravo *et al.*, 2011; Iqbal *et al.*, 1993). Adult worms were collected from a monospecific flock.



Figure 1: Stomach collected immediately after slaughter.

EFFICACY OF *B. THURINGIENSIS* PURIFIED CRYSTAL PROTEINS AND SPORE CRYSTAL SUSPENSION AGAINST ADULT *H. CONTORTUS* WORMS *IN VITRO*

APPLICATION OF DIFFERENT DOSAGES

The experimentally infected goats were euthanised. The abomasum was collected immediately after death (Figure 1). The specimen was rinsed with saline and then transferred to a Baermann apparatus containing saline at 37°C. After two hours, the worms that had migrated into the saline were gathered (Figure 2) and quickly placed into a 12-well plate, with ten worms per well. The worms were washed in PBS with 4% penicillin for 1 hour, following standard protocol (Zajac and Conboy, 2012; Tasawar *et al.*, 2010). After washing, all multiwall plates were labelled A-G.

Plate-A was treated with purified crystal proteins diluted in normal saline at a concentration of 2mg/ml, followed by Plate-B, 1.5mg/ml, and Plate-C, 1mg/ml. Furthermore, Plate-D was treated with spore-crystal suspension at a concentration of 1 x 10⁸, followed by Plate-E 1 x 10⁷ and Plate-F 1 x 10⁶ colony-forming units/ml. For control, we use Plate-G and added normal saline (Table 1) (López *et al.*, 2006).



Figure 2: *H. contortus* worms isolated from the Abomasum.

MOBILITY CHECK

All multiwell plates containing varying concentrations were individually placed in a temperature-controlled incubator (Constant Temperature and Humidity Incubator (HWS)) following 12, 24, and 48 hours of treatment with the purified crystal mix and spore crystal suspension. The mobility of the worms was assessed using an MRJ microscope with 40x magnification and compared to the control group (Sinott *et al.*, 2012).

MOBILITY CALCULATION

A mobility index was calculated for each observation by dividing the number of immobile worms by the total number of worms.

BACTERIAL TOXINS ISOLATION AND SPECIES IDENTIFICATION

Previously, we examined *B. thuringiensis* through staining, biochemical tests, and PCR tests. Sequence analysis was

Table 1: Distribution of doses in each 12-well plate.

Application of purified crystal proteins on adult <i>H. contortus</i>				
Protein concentration	Plate-A (2mg/ml)	Plate-B (1.5mg/ml)	Plate-C (1mg/ml)	Plate-G (Control)
Number of worms/well	10	10	10	10
Application of spore crystal suspension on adult <i>H. contortus</i>				
Spore crystal mix concentration	Plate-D (10 ⁸ CFU/ml)	Plate-E (10 ⁷ CFU/ml)	Plate-F (10 ⁶ CFU/ml)	Plate-G (Control)
Number of worms/well	10	10	10	10

conducted to confirm the bacterial species for the isolation of crystal proteins. Following the separation of the crystal proteins from the spores, the protein profile of the crystals was analyzed using SDS-PAGE. This analysis revealed three prominent bands at approximately 70 kDa, 36 kDa, and 15 kDa, in addition to several minor bands (Panhwer *et al.*, 2023).

STATISTICAL ANALYSIS

Statistical analyses were conducted using MedCalc’s statistical software to monitor the odds ratio (95% CI) and p-value. Additionally, worms’ motility percentages were calculated using MS Excel.

RESULTS

EPIDEMIOLOGICAL FINDINGS OF *H. CONTORTUS*

The prevalence of *Haemonchus* spp. in the study area was found to be 52.4%. Affected animals exhibited symptoms including diarrhoea, weakness, anaemia, and weight loss. Anemia levels were assessed using FAMACHA strips. Furthermore, a Fecal Egg Count (FEC) test was conducted utilizing the McMaster technique to confirm the presence of *H. contortus*. This survey also took into account factors such as age, gender, and seasonality.

AGE

We investigated the relationship between age groups and *H. contortus* infection in goats during this study. Our results indicate that the age of the host significantly (P = 0.001; CI: 1.176 - 1.899) affected the infection rate. We categorized the goats into three age groups to identify which group is most susceptible to *H. contortus*. Our findings revealed that goats aged 8 to 12 months were at the highest risk, with an infection rate of 66.11%. In contrast, goats younger than 8 months had an infection rate of 47.22%, while those aged 12 months and older experienced a slightly lower rate of 43.51% (Table 2).

GENDER

The prevalence of *Haemonchus contortus* was observed to be higher in male goats, with an infection rate of 60%, compared to 49.14% in female goats (P= 0.001; CI: 0.490 - 0.848) (Table 2). Present study indicates that male goats are at a greater risk of infection than female goats.

SEASON

Seasons play a crucial role in the growth of various nematode species. In this study, we examined the winter season of 2021 and the summer and monsoon seasons of 2022, during which all five districts experienced flooding. Our findings indicate that the summer and monsoon seasons significantly (P= 0.000; CI: 4.808 – 9.186) enhanced the nematode population, with a prevalence percentage of 64.44% compared to 21.42% in winter (Table 2).

HAEMONCHUS IDENTIFICATION

Conventional identification of *H. contortus* was performed based on physical appearance, and eggs were identified using published keys.

BIO-ANTHELMINTIC PROPERTIES OF *B. THURINGIENSIS* PURIFIED CRYSTAL PROTEINS AND SPORE CRYSTAL SUSPENSION ON ADULT *H. CONTORTUS* WORMS *IN VITRO* WORM MOTILITY

The study aimed to control adult *H. contortus* using bio-anthelmintic *in vitro*. Purified crystal proteins and spore crystal suspensions of *B. thuringiensis* were administered to adult worms to evaluate motility at various time intervals. The results indicated that purified crystal proteins significantly impacted worm motility at a concentration of 2 mg/ml, showing rapid effects with 50% mortality after 12 hours of treatment, 66.66% after 24 hours, and 83.33% after 48 hours. In comparison, 1.5 mg/ml concentration resulted 33.33% mortality at 12 hours, followed by 41.66% at 24 hours, and 75% at 48 hours. Furthermore, 1 mg/ml showed 4.1% mortality at 12 hours, 16.66% at 24 hours,

Table 2: Risk factors and prevalence of haemonchosis in goats.

Variable	No. examined	Positive No. (%)	Negative No. (%)	Odds ratio, (95% C.I) p-value
Age				
≤8 month	360	170(47.22)	190 (52.77)	1.494, (1.176 - 1.899) P = 0.0010
8-12 months	180	119 (66.11)	61 (33.88)	
>12month	540	235 (43.51)	305 (56.48)	
Sex				
Female	700	344 (49.14)	356(50.85)	0.644, (0.490 - 0.848) P = 0.0017
Male	300	180 (60)	120 (40)	
Season				
Summer (rainy)	720	464 (64.44)	256 (35.55)	6.646, (4.808 - 9.186) P < 0.0001
Winter	280	60 (21.42)	220 (78.57)	
Total	1000	524 (52.4)	476 (47.6)	P-value < 0.00001

Table 3: Worm mobility percentage after application of different concentrations of Purified crystal proteins and spore crystal mix.

Treatment type	Concentration	12 h	24 h	48 h
Normal saline	0.9%	0 (0.00%)	10 (8.33%)	80 (66.66%)
Purified crystal proteins	2ml	60 (50.00%)	80 (66.66%)	100 (83.33%)
	1.5ml	40 (33.33%)	50 (41.66%)	90 (75.00%)
	1ml	5 (4.16%)	20 (16.66%)	70 (58.33%)
Spore crystal suspension	10 ⁸ CFU/ml	20 (16.66%)	30 (25.00%)	80 (66.66%)
	10 ⁷ CFU/ml	0 (0.00%)	10 (8.33)	70 (58.33%)
	10 ⁶ CFU/ml	0 (0.00%)	5 (4.16%)	75 (62.50%)

and 58.33% at 48 hours. The second treatment contains spore crystal suspension, at a concentration of 1×10^8 exhibited slight effects on worm motility, with 16.66% mortality at 12 hours, 25% at 24 hours, and 66.66% at 48 hours. In addition, 1×10^7 demonstrated no mortality at 12 hours, followed by 8.33% at 24 hours and 58.33% at 48 hours. whereas 1×10^6 did not impact motility at 12 hours, with subsequent mortality rates of 4.1% at 24 hours and 62.5% at 48 hours, in comparison of control group (Table 3).

The findings of this study indicate that purified crystal proteins at a concentration of 2 mg/ml are highly effective in controlling *H. contortus* motility. Additional observations revealed that as the duration of treatment increased, worm motility decreased, ultimately resulting in mortality across all treatments, including the control group.

DISCUSSION

During the present study, goats from various localities in Sindh, Pakistan, were selected to monitor the prevalence and epidemiology of *H. contortus*. Five different districts in the interior of Sindh were chosen. Various studies indicate that *H. contortus* is commonly found in small ruminants (Lopez et al., 2002). Based on clinical signs and the FAMACHA anemia guide test, it was determined that most animals in these areas are infected with Haemonchosis. Other findings also revealed that *H. contortus* is one of the most frequently reported nematodes in Pakistan (Fayaz et al., 2019; Lashari, 2011; Ayaz et al., 2013). The current study examined the prevalence of *H. contortus*, which was found to be 52.4% among goat populations in Sindh, Pakistan. Additionally, we monitored the animals based on factors such as age, gender, and seasonal variation, as summarized in Table 2. Our findings indicated that male goats are at a higher risk of *H. contortus* infection, with an infection rate of 60% compared to female goats 49.14%. These findings align with previous research conducted by other scholars (Mandonnet et al., 2003; Tasawar et al., 2010). During the current study, we observed a significant incidence of disease, predominantly occurring in the summer and

monsoon seasons, accounting for 64.44% of cases, in contrast to 21.42% during winter. This aligns with previous research, which highlighted a 90.10% infection rate during the monsoon season compared to 83.84% in summer (Singh et al., 2017). Additionally, our findings suggest that infants aged 8 to 12 months are especially vulnerable, likely due to their developing immune systems. Similarly, weaker immunity in both younger and older animals may explain the higher prevalence of Haemonchosis (Regassa et al., 2024).

Another aspect of the current study focuses on Biological control, a method utilized in agriculture for years to manage pests without relying on chemical pesticides (Betz et al., 2000). We are applying this approach to combat the highly pathogenic gastrointestinal parasite *H. contortus*. Another study reported that nearly all helminths, as well as ecto- and endoparasites, have natural enemies, effective management of these natural antagonists can lead to better control of parasites (Ikele et al., 2024; Mahr and Ridgway, 1993; Widiarso, 2018).

Bacillus thuringiensis is recognized as an environmentally friendly bacterium (Zajac and Conboy, 2012) and has shown significant effects on nematodes compared to traditional anthelmintic medications. The problem of anthelmintic resistance is becoming increasingly critical globally, with multidrug resistance (MDR) in nematodes impacting the productivity of both small and large ruminants. This underscores the pressing need to develop innovative solutions to address this challenge (Kumar et al., 2008).

The protein produced by *B. thuringiensis* interacts with parasitic worm proteins, allowing them to bind to receptors on the gut lining. This binding results in the formation of holes in the gut, leading to cell death (Kumar et al., 2008). Our focus on Bacillus strains, particularly *B. thuringiensis*, emphasizes their effectiveness in controlling the *H. contortus* population. Present study uncovered in vitro nematocidal properties of *B. thuringiensis* crystal proteins (Btcp's) and the *B. thuringiensis* spore-crystal mix.

We observed that Btcp's at a concentration of 2 mg/ml resulted in a significant reduction (50%) in worm motility over 12 hours, in contrast, a spore-crystal suspension at a concentration of 1×10^8 -CFU/ml only achieved a 16.66% reduction in motility during the same time frame. Detailed findings can be found in Table 3. Other studies support these result on reduction of *H. contortus* larval development (Sinott *et al*, 2012; Panhwer *et al.*, 2023).

The results indicated that the purified crystal proteins were more toxic to adult *H. contortus*. Other findings revealed that the *B. thuringiensis* crystal Cry5B exhibited excellent efficacy in vitro against larval stages (Sanders *et al.*, 2020), and the Cry11Aa toxin also demonstrated the same ability (DE *et al.*, 2016).

CONCLUSION

In conclusion, our findings suggest that toxins of *Bacillus thuringiensis* have great potency as an alternative source of bioanthelmintic. The purified crystal proteins derived from isolated *Bacillus thuringiensis* exhibit a toxic effect on adult *Haemonchus contortus* worms at a concentration of 2 mg/ml. We propose that these toxins could provide a viable treatment option for small ruminants facing the challenge of anthelmintic resistance.

ACKNOWLEDGEMENTS

Department of Veterinary Parasitology, Sindh Agriculture University, Tandojam. Laboratory of Animal Disease Model, College of Veterinary Medicine, Sichuan Agricultural University. University, Chengdu, Sichuan, 611130. Central Veterinary Diagnostic Laboratory and Vaccines Production Unit Government of Sindh, Pakistan.

NOVELTY STATEMENT

For the first time, we applied *Bacillus thuringiensis* as biological control against *Haemonchus contortus* in this study area. This innovative approach aims to enhance our understanding of its effects on *Haemonchus contortus*.

AUTHOR'S CONTRIBUTION

Conceptualisation: JAG.
Field survey, methodology and writing original draft: SNP.
Formal analysis: MBB, ZC.
Investigation, Validation: SNP, SJ.
Resources: RSB.
Supervision: JAG and ZC.
Visualization: SB.
Writing review and editing: ZC, JAG, SB.

FUNDING

This research was not supported by any funding organization.

ETHICS STATEMENT

Animal experiments were conducted in accordance with the guidelines set by the Institutional Ethics Committee at Sindh Agricultural University, Tandojam, Pakistan (Institutional Ethical Committee No. AH.06.2020, dated August 5, 2020).

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

We used Grammarly for writing assistance.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

- Ayaz MM, Raza MA, Murtaza S, Akhtar S (2013). Epidemiological survey of helminths of goats in southern Punjab, Pakistan. *Trop. Biomed.*, 30(1): 62-71.
- Besier, R. B., Kahn, L. P., Sargison, N. D., Van Wyk, J. A. (2016). Diagnosis, treatment and management of *Haemonchus contortus* in small ruminants. *Advances in parasitology*, 93, 181-238.
- Betz FS, Hammond BG, Fuchs RL (2000). Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. *Regulat. Toxicol. Pharmacol.*, 32(2): 156-173. <https://doi.org/10.1006/rtp.2000.1426>
- Bravo A, Likitvivatanavong S, Gill SS, Soberón M (2011). *Bacillus thuringiensis*: A story of a successful bioinsecticide. *Insect Biochem. Mol. Biol.*, 41(7): 423-431. <https://doi.org/10.1016/j.ibmb.2011.02.006>
- Bricarello PA, Longo C, da Rocha RA, Hötzel MJ (2023). Understanding animal-plant-parasite interactions to improve the management of gastrointestinal nematodes in grazing ruminants. *Pathogens*, 12(4): 531. <https://doi.org/10.3390/pathogens12040531>
- DE APDSS, Lorenzon LB, Vianna AM, Santos FDS, Pinto LS, Berne MEA, Leite, FPL (2016). Larvicidal activity of *Bacillus thuringiensis* var. israelensis Cry11Aa toxin against *Haemonchus contortus*. *Parasitology*, 143(12): 1665-1671. <https://doi.org/10.1017/S0031182016001451>
- Fayaz MR, Abbas RZ, Abbas A, Khan MK, Raza MA, Israr M, Zaman MA (2019). Potential of botanical driven essential oils against *Haemonchus contortus* in small ruminants. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, 18(6).
- Fidèle TD, Roland MNT, Geneviève Z, Benjamin K, Adama K, Hamidou TH, Gaston BAM (2024). Phytochemical composition and in vitro anthelmintic activity of *Combretum*

- micranthum* g. Don leaf extracts on *Haemonchus contortus* of small ruminants. J. Anim. Health Prod., 12(1): 85-92. <https://doi.org/10.17582/journal.jahp/2024/12.1.85.92>
- Gareh A, Elhawary NM, Tahoun A, Ramez AM, El-Shewehy DM, Elbaz E, Elmahallawy EK (2021). Epidemiological, morphological, and morphometric study on *Haemonchus* spp. recovered from goats in Egypt. Front. Vet. Sci., 8: 705619. <https://doi.org/10.3389/fvets.2021.705619>
- Hounzangbe-Adote MS, Paolini V, Fouraste I, Moutairou K, Hoste H (2005). *In vitro* effects of four tropical plants on three life-cycle stages of the parasitic nematode, *Haemonchus contortus*. Res. Vet. Sci., 78(2): 155-160. <https://doi.org/10.1016/j.rvsc.2004.05.009>
- Ikele CB, Uju UV, Ikele CF (2024). Anti-parasite activity of different plant leaf extracts against infective stage theront of *Ichthyophthirius multifiliis*. J. Anim. Health Prod., 12(2): 196-204.
- Iqbal Z, Akhtar M, Khan MN, Riaz M (1993). Prevalence and economic significance of haemonchosis in sheep and goats slaughtered at Faisalabad abattoir. Pak. J. Agric. Sci., 30(1).
- Kaplan RM, Burke JM, Terrill TH, Miller JE, Getz WR, Mobini, S, Vatta AF (2004). Validation of the FAMACHA® eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. Vet. Parasitol., 123(1-2): 105-120. <https://doi.org/10.1016/j.vetpar.2004.06.005>
- Khan MN, Sajid MS, Khan MK, Iqbal Z, Hussain A. (2010). Gastrointestinal helminthiasis: Prevalence and associated determinants in domestic ruminants of district Toba Tek Singh, Punjab, Pakistan. Parasitol. Res., 107(4): 787-794. <https://doi.org/10.1007/s11605-010-1294-8>
- Khan MS, Khan MA, Sultan M (2008). Genetic resources and diversity in Pakistani goats. Int. J. Agric. Biol., 10(2): 227-231.
- Kumar S, Chandra A, Pandey KC (2008). *Bacillus thuringiensis* (Bt) transgenic crop: An environment friendly insect-pest management strategy. J. Environ. Biol., 29(5): 641-653.
- Lashari MH, Zahida Tasawar ZT (2011). Prevalence of some gastrointestinal parasites in sheep in Southern Punjab, Pakistan.
- López ME, Flores J, Mendoza P, Vázquez V, Liebano E, Bravo A, Zamudio F (2006). Use of *Bacillus thuringiensis* toxin as an alternative method of control against *Haemonchus contortus*. Ann. N. Y. Acad. Sci., 1081(1): 347-354. <https://doi.org/10.1196/annals.1373.049>
- López-Arellano ME, Flores-Crespo J, Mendoza de Gives P, Bravo de la Parra A, Herrera-Rodríguez D, Liébano-Hernández E, Vargas-Urióstegui P (2002). *In vitro* lethal activity of *Bacillus thuringiensis* toxins against *Haemonchus contortus* eggs and infective larvae.
- López-Arellano ME, Flores-Crespo J, Mendoza de Gives P, Bravo de la Parra A, Herrera-Rodríguez D, Liébano-Hernández E, Vargas-Urióstegui P (2002). *In vitro* lethal activity of *Bacillus thuringiensis* toxins against *Haemonchus contortus* eggs and infective larvae.
- Mahar, D., Ridgway, N. (1993). An introduction to beneficial natural enemies and their use in pest management. N. Cen. Regional Pub., 481, 36.
- Mandonnet N, Ducrocq V, Arquet R, Aumont G (2003). Mortality of Creole kids during infection with gastrointestinal strongyles: A survival analysis. J. Anim. Sci., 81(10): 2401-2408. <https://doi.org/10.2527/2003.81102401x>
- Moaeen-ud-Din M, Danish Muner R, Khan MS (2022). Genome wide association study identifies novel candidate genes for growth and body conformation traits in goats. Sci. Rep., 12(1): 9891. <https://doi.org/10.1038/s41598-022-14018-y>
- Panhwer SN, Gadahi JA, Luo Q, Huang C, Liu W, Jia L, Chen Z (2023). The anthelmintic potential of *Bacillus thuringiensis* to counter the anthelmintic resistance against *Haemonchus contortus*. Exp. Parasitol., 250: 108533. <https://doi.org/10.1016/j.exppara.2023.108533>
- Regassa, D., Bihane, A., Getahun, Y., Chalchisa, A. (2024). Prevalence and Associated Risk Factors of *Haemonchus contortus* in Sheep Slaughtered at Bahir Dar Municipal Abattoir, Ethiopia. Journal of Parasitology Research, 2024, 1-6. <https://doi.org/10.1155/2024/1433264>
- Sanders J, Xie Y, Gazzola D, Li H, Abraham A, Flanagan K, Aroian RV (2020). A new paraprobiotic-based treatment for control of *Haemonchus contortus* in sheep. Int. J. Parasitol. Drugs Drug Resist., 14: 230-236. <https://doi.org/10.1016/j.ijpddr.2020.11.004>
- Singh E, Kaur P, Singla LD, Bal MS (2017). Prevalence of gastrointestinal parasitism in small ruminants in western zone of Punjab, India. Vet. World, 10(1): 61. <https://doi.org/10.14202/vetworld.2017.61-66>
- Sinott MC, Cunha Filho NA, Castro LLD, Lorenzon LB, Pinto NB, Capella GA, Leite FPL (2012). *Bacillus* spp. toxicity against *Haemonchus contortus* larvae in sheep fecal cultures. Exp. Parasitol., 132(2): 103-108. <https://doi.org/10.1016/j.exppara.2012.05.015>
- Sinott MC, de Castro LD, Leite FLL, Gallina T, De-Souza MT, Santos DFL, Leite FPL (2016). Larvicidal activity of *Bacillus circulans* against the gastrointestinal nematode *Haemonchus contortus* in sheep. J. Helminthol., 90(1): 68-73. <https://doi.org/10.1017/S0022149X14000844>
- Song MingXin SM, Ma GuangPeng MG, Han CaiXia, HC, Zhu JiangWei ZJ, Yuan JinQian YJ, Zhu YanMei ZY, Lu YiXin LY (2007). Observation of seasonal incidence of egg output and adult worm burden of gastro-intestinal nematodes in sheep in Songhuajiang area in Heilongjiang Province of China.
- Su L, Kamboh AA, Matar AM, Leghari RA, Kumar C (2024). Assessment of gastrointestinal nematode infections in dairy cattle and the associated risk factors in Northern China. J. Vet. Sci., 25(6): e73. <https://doi.org/10.4142/jvs.24173>
- Tasawar Z, Ahmad S, Lashari MH, Chaudhary SH (2010). Prevalence of *Haemonchus contortus* in sheep at research centre for conservation of Sahiwal cattle (RCCSC) Jehangirabad District Khanewal, Punjab, Pakistan. Pak. J. Zool., 42(6).
- Terrill TH, Miller JE, Burke JM, Mosjidis JA, Kaplan RM (2012). Experiences with integrated concepts for the control of *Haemonchus contortus* in sheep and goats in the United States. Vet. Parasitol., 186(1-2): 28-37. <https://doi.org/10.1016/j.vetpar.2011.11.043>
- Urquhart G, Armour J, Duncan J, Dunn A, Jennings F (1996). Veterinary parasitology 2nd Edition. University of Oxford.
- Waller PJ, Chandrawathani P (2005). *Haemonchus contortus*: parasite problem No. 1 from tropics-Polar Circle. Problems and prospects for control based on epidemiology. Trop. Biomed., 22(2): 131-137.
- Wang C, Li F, Zhang Z, Yang X, Ahmad AA, Li X, Hu M (2017). Recent research progress in China on *Haemonchus contortus*. Front. Microbiol., 8: 1509. <https://doi.org/10.3389/fmicb.2017.01509>
- Wei W, Lan Z, Li Z, Wang R (2025). Prevalence and risk

- factors of *Haemonchus contortus* in small ruminants in China: A systematic review and meta-analysis. Small Rumin. Res., 107486. <https://doi.org/10.1016/j.smallrumres.2025.107486>
- Widiarso BP, Kurniasih K, Prastowo J, Nurcahyo W (2018). Morphology and morphometry of *Haemonchus contortus* exposed to *Gigantochloa* apus crude aqueous extract. Vet. World, 11(7): 921. <https://doi.org/10.14202/vetworld.2018.921-925>
- Yin F, Gasser RB, Li F, Bao M, Huang W, Zou F, Hu M (2016). Population structure of *Haemonchus contortus* from seven geographical regions in China, determined on the basis of microsatellite markers. Parasit. Vectors, 9(1): 586. <https://doi.org/10.1186/s13071-016-1864-z>
- Zajac AM, Conboy GA (2012). Veterinary clinical parasitology. A John Wiley and Sons. Inc., Publication.