

RESEARCH ARTICLE

Standardized protocol for laboratory rearing and breeding of the Lymnaeidae snail, *Radix natalensis* (Krauss, 1848)

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Abstract

Freshwater lymnaeid snails are involved in the transmission of fascioliasis in tropical and subtropical Africa, Asia, as well as in temperate regions. This study improved and standardized laboratory rearing and breeding of first-generation (F_1) *R. natalensis* using field-collected F_0 snails. Ninety field-collected *R. natalensis* adult snails with shell heights of 4–5 mm were divided into three experimental treatment groups: A, B, and C. Each experimental treatment group comprised of ten (10) 2L containers, with each containing 3 snails. Group A, the control, was fed *Elodea* sp. weed powder and *Cyperus papyrus* twigs from snail-sampling sites as oviposition material. Group A containers were filled with water from the snail sample sites. Spring-watered snails in experimental group B were fed with dried lettuce, fish flakes, and eggshells. In experimental group C, snails were fed with algal wafers and trout pellets in dechlorinated water. Groups B and C used polystyrene strips for oviposition. Daily snail mortality and egg mass counts were obtained. Experimental group B snails produced 69 egg masses and 500 F_1 offspring with the lowest snail mortality (13%). Group C produced 60 egg masses and 450 F_1 offspring. The mortality rate in this group was 20%. Group A control snails laid 10 eggs and 48 F_1 offspring. Also, mortality (66%) was higher in this group. Mean egg masses differed significantly between groups A and B (Group A: 0.85 ± 0.22 egg masses; Group B: 2.33 ± 0.53 , $p = 0.034$) and A and C (Group A: 0.85 ± 0.22 egg masses; Group C: 2.16 ± 0.48 , $p = 0.041$), but not between groups B and C. Treatment differences explained 11.4% ($F_{1,25} = 4.36$, $p = 0.047$) of egg mass variability. The median snail survival in group B was 8.11 days versus 4.57 days in group A. Significant differences in median survival time were observed between experimental groups (Log Rank $X^2 = 9.87$, $p = 0.007$). Group B had

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Data availability statement: All relevant data are within the manuscript and its [Supporting Information](#) files.

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the highest fecundity and lowest mortality among the treatment groups. However, the use of spring water increased the costs of mass breeding of snails using this approach. On the other hand, experimental group C produced a comparable number of egg masses. Thus, for mass breeding of *R. natalensis*, the use of an experimental approach from group C would be recommended as it is cheaper.

1. Background

Lymnaeid snails are freshwater gastropods widely distributed globally. These snails are important intermediate hosts (IHS) of trematode parasites [1]. Globally, about 20 species of lymnaeid snails have been described as potential IHS of *Fasciola* sp. [2,3]. Several lymnaeid snails serve as intermediate host snails of *Fasciola hepatica* in temperate regions of Europe, Asia, Australia, and the Americas. These include *Galba truncatula*, *Austropeplea (Lymnaea) tomentosa*, *Lymnaea viatrix*, *Lymnaea viridis*, *Galba cubensis*, and *Pseudosuccinea columella* [2]. In contrast, *Radix auricularia*, *Radix acuminata*, *Pseudosuccinea columella*, and *Radix natalensis* transmit *F. gigantica* in the tropical and subtropical regions of Asia and Africa [2,4]. Understanding the transmission dynamics of fascioliasis requires knowledge of its chain of transmission and the ecology of IHS involved in the transmission cycle. Understanding the ecology of these snails may also help in the improvement of control programmes [5].

Conducting laboratory mechanistic experiments to understand the ecology of snails has become an important aspect in veterinary research and public health [5]. A key requirement for conducting mechanistic experiments is raising a clean, laboratory-bred snail colony that is free from field trematode infection. When developing and rearing a lymnaeid snail colony, several factors are considered, including environmental factors such as temperature, humidity, light, and water quality, which need to be strictly monitored to ensure snail survival and reproductive success [6]. Furthermore, feed is another important factor to consider. Earlier studies have suggested lettuce and algae, supplemented with trout pellets, tropical fish flakes, and calcium for shell development, as ideal feeds for snails [7]. Most snails are hermaphrodites and reproduce through self-fertilization [8,9]. Thus, egg laying and hatching in addition to survival should be continuously monitored. Breeding of snails is also important because snails are used as hosts for research into drugs and vaccines, such as those for parasitic diseases like trematodiasis [10,11]. Laboratory-bred snails are also commonly used in controlled investigations for snail-borne disease surveillance and risk assessment [11], developing predictive disease transmission models and investigating how snails respond to various biotic and abiotic factors. Earlier malacological studies reported that *Radix natalensis*, the main intermediate host snail for *Fasciola gigantica* in the tropical and subtropical regions is difficult to culture in laboratory conditions [1]. Furthermore, breeding snails in the laboratory is critical for completing the parasite life cycles, especially when studying complicated trematodes with many hosts [1,2,12].

Little work has been done on the breeding of *R. natalensis* snails for further laboratory experimental work. To the best of our knowledge, three experimental works have been previously done to determine the influence of *F. gigantica* on the life history traits of *R. natalensis* in East and West Africa [13–15]. Earlier studies provide important insights on how to breed snails using dried lettuce, trout pellets, and crushed chicken eggshells as snail feed, as well as the use of dechlorinated water in researches conducted in Kenya and Nigeria by [7,14]. However, differences in altitude between the regions where these studies were conducted and our study area suggest that assessing the breeding feasibility of locally available snails would improve preparedness for fascioliasis outbreaks, particularly in Southern Africa. An earlier study on the breeding of *Bulinus globosus* snails suggested that the use of field-collected snails in laboratory studies may lead to inaccurate and non-reproducible results, due to the change in conditions and inadequate acclimatization time for snails [5]. *R. natalensis* is a major IHS of fascioliasis, a disease of public and veterinary health importance that affects livestock and humans in the tropics and subtropics regions worldwide [16–19]. In livestock, infection leads to animal weight loss, reduced productivity and fertility, and reduced milk production, leading to economic losses [20]. Bovine fascioliasis is of concern in livestock production in South Africa [21,22]. Furthermore, humans get accidentally infected by consuming metacercariae-contaminated water crest [18]. In South Africa, human fascioliasis is not a burden, only 3 cases were reported in Gauteng (1956) and Western Cape Province (1964) [23]. To enable current and future laboratory mass breeding of *R. natalensis* for laboratory experiments, we reported results from a cost-effective standardized rearing and breeding protocol for mass-producing F_1 *R. natalensis* snails.

2. Materials and methods

2.1. Snail collection and identification

To enable the development of laboratory breeding F_1 *R. natalensis*, an F_0 parental generation of snails was collected from Kwafik'suthe dam (−29.671, 29.875) in Impendle municipality and The Plains farm (−29.149; 30.008) in Mpofana municipality in uMgungundlovu district in KwaZulu-Natal, South Africa. Snails for the study were identified using morphological identification keys designed by Brown (1994) [24] and Appleton (1996) [25]. *R. natalensis* were identified with the shells being dextral and oblong and four rapidly increasing whorls, basal whorl usually markedly swollen. The columellar margin of the aperture twisted, and the sharp outer lip. The spire was depressed and about half the height of the aperture. The shells were colorless, yellow or dark.

2.2. Culturing of snails

One hundred and twenty snails were collected from the field. However, during acclimatization to laboratory conditions, 30 snails died before allocation to treatment groups. The remaining ninety snails were randomly assigned to three experimental groups, with the experiments being carried out at the Biomedical Resource Unit (BRU) laboratory, Westville campus of the University of KwaZulu-Natal, South Africa. Each treatment group consisted of 10 transparent 2L plastic containers (26.65 cm × 19.3 cm × 6.45 cm), with three snails per container, totaling 30 snails per group. Group A (control): fed with *Elodea* weed powder; *Cyperus papyrus* twigs provided oviposition substrate; field-collected water was used, Group B: fed with dry lettuce, fish flakes, and crushed eggshells; spring water was used, and Group C: fed with algae wafers and trout pellets; dechlorinated tap water was used (left to stand for four days). All groups were fed three times per week, and water was changed at feeding to prevent waste buildup. Snail mortalities and egg masses were recorded daily. Egg masses laid on substrates or container walls were carefully removed and transferred to new containers containing the same water type as the original group to ensure consistency. Ambient and water temperatures were monitored daily and maintained at 26 ± 1 °C (air) and 22 ± 1 °C (water), respectively. Artificial light was provided in the breeding room using a 4 × 100watt fluorescent tube. The breeding room maintained a 12-hour day and night. No artificial aeration was done in the breeding containers. Furthermore, water pH was maintained between 7.40 and 9 and checked using a Hanna multiparameter water testing meter.

Ethical standards

Research permits were obtained through the Animal Research Council Committee (AREC) of the University of KwaZulu-Natal. Protocol Reference number: AREC/00005642/2023(00020552) in accordance with the South African national guidelines on animal care, handling, and use for biomedical research.

2.3. Statistical analysis

Data analysis was performed using StataNow/SE 18. Data on egg masses was square root transformed to enhance homogeneity of variance [5]. A one-way ANOVA was used to determine if the treatment to which snails had been exposed to had an effect on the square root transformed number of egg masses laid. Furthermore, a linear regression model on transformed egg masses was used to determine the influence of treatment on the number of egg masses laid. Kaplan Meier survival analysis was used to determine the effect of the differences that treatments had on snail survival, while the survival times were compared between the treatments using the Log Rank (Mantel-cox) tests. For all statistical tests the level of significance cut off was set at $p < 0.05$.

3. Results

Group A snails (fed *Elodea* weed powder, with papyrus twigs as oviposition substrate and field water) experienced a high mortality rate (67%), followed by Group C (20%), and Group B (13%) (Table 1). Group B snails (fed dry lettuce, fish flakes, eggshell powder, with white polystyrene strips as oviposition substrate and spring water) produced the most egg masses ($n = 69$), followed by Group C ($n = 59$) and Group A ($n = 10$). Group B produced the highest number of hatched juveniles ($n = 500$), followed by Group C ($n = 450$) and Group A ($n = 48$) (Table 1).

3.1. Fecundity

Overall, 138 egg masses were collected from the experimental groups. Group A snails laid the least number of egg masses ($n = 10$) 7.24%, followed by those in group B ($n = 69$) 50%, and group C ($n = 59$) 42.76%. The overall mean number of egg masses across all treatments was 5.11 (95%CI: 2.78–7.45). Fig 1 shows the mean number of egg masses laid across treatment groups.

Statistical differences in the mean number of eggs laid across the three treatments were observed ($F_{2,24} = 3.29$, $p = 0.047$). The mean number of egg masses laid by snails maintained in experimental group A (0.85 ± 0.22 egg masses) was lower than that of snails in experimental groups B (2.33 ± 0.53 egg masses, $p = 0.034$) and C (2.16 ± 0.48 egg masses, $p = 0.041$). On the other hand, no statistical differences in the mean number of egg masses laid were observed between experimental groups B and C ($p = 0.989$) (Table 2). Furthermore, treatment type influenced the mean number of eggs laid (Coeff: 0.657, $p = 0.047$), and also accounted for 11.4% of the total variability observed in the number of egg masses laid.

Table 1. Summarized results of indicators performance of snails in different treatments.

Group	Group A (Control)	Group B	Group C
Feed type	<i>Elodea</i> weed	Dry lettuce, fish flakes, eggshell powder	Algae wafers, and trout pellets
Water type	Dam water (source water)	spring water	Dechlorinated water
Egg laying substratum	<i>C. papyrus</i> twigs	White polystyrene strips	White polystyrene strips
Initial number of snails per group	30	30	30
Number of dead snails	20	4	6
Number of surviving snails	10	26	24
Percentage mortality	67	13	20
Egg masses collected	10	69	59
Number of hatched juveniles (F1 snails)	48	500	450

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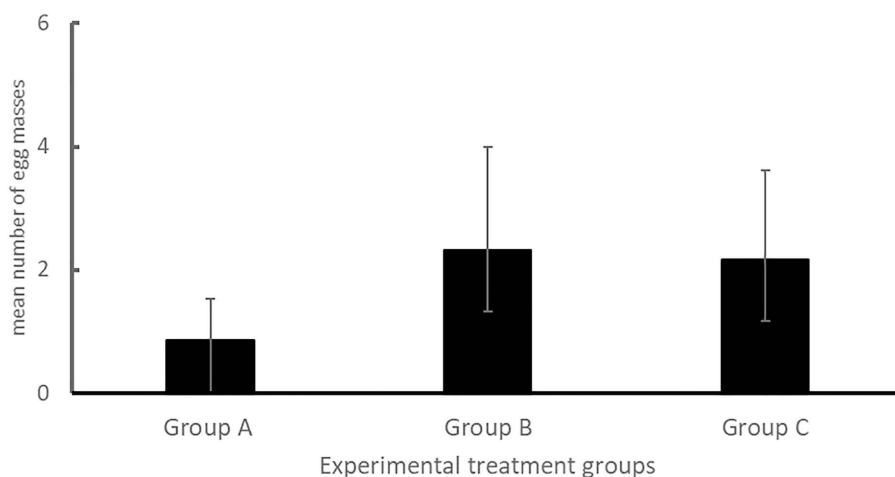


Fig 1. Mean number of egg masses laid per group treatment.

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Table 2. Regression outcomes of group treatment on egg masses.

Variable	Coefficient	Standard error	t	P	95% CI
Groups	0.6571054	0.3148534	2.09	0.047*	0.0086526 –1.305558
Constants	0.465311	0.6801611	0.69	0.499	–0.9342869–1.867349

*Statistically significant at $p < 0.05$.

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3.2. Survival

[Fig 2](#) displays the Kaplan-Meier survival plot. Experimental group A (blue line) had the highest mortality rate compared with experimental groups B (red line) and C (green line). Experimental group B (red line) had the highest survival rate. Overall, statistical differences in the survival time were observed ($p < 0.001$).

The Cox proportional hazards model reveals that snails from experimental groups B and C had a significantly lower risk of mortality compared to experimental group A ([Table 3](#)). When compared to experimental group A, which was the control, there was an 88.3% reduction in the mortality of snails in experimental group B (Hazard Ratio: 0.117) ([Table 3](#)). No

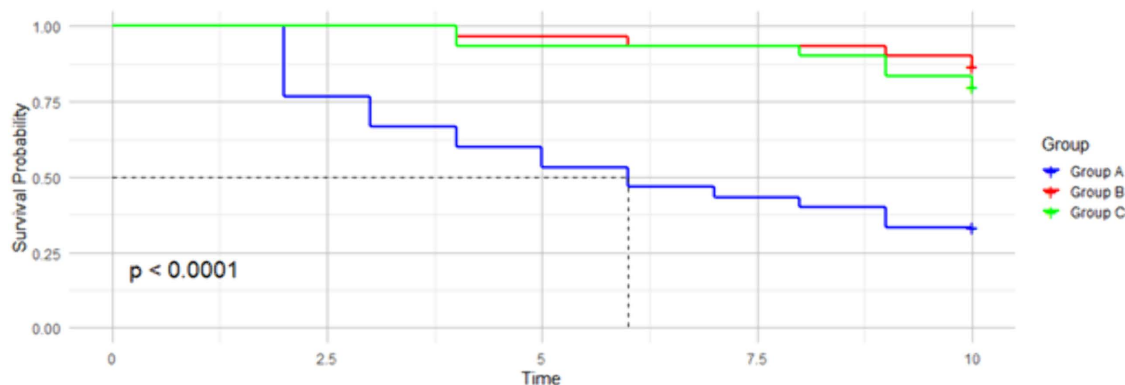


Fig 2. Kaplan-Meier survival plot.

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Table 3. Cox proportional hazards model results for snail mortality across treatment levels.

Treatments	Coefficient	Hazard Ratio	SE	z-value	p-value	95%CI
Group B	-2.143	0.117	0.551	-3.892	<0.001*	(0.040-0.345)
Group C	-1.712	0.181	0.468	-3.655	<0.001*	(0.072-0.452)

*Statistically significant at $p < 0.05$.

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difference in the survival rate was observed between snails maintained in experimental groups B and Group C ($p = 0.779$). The model's concordance statistic was 0.749.

4. Discussion

Our findings highlight the critical role of laboratory conditions, particularly food type, water quality, and oviposition substrate in maintaining wild-caught F_0 generation *R. natalensis* and optimizing its reproductive output F_1 generation. Compared to the control (group A), our study showed that snails bred under the conditions of groups B and C produced a considerable number of egg masses and juvenile snails. Our improved protocol thus suggests that the experimental groups B or C approaches can be used, depending on the availability of resources for a particular laboratory. For laboratories from low-income settings that may seek to mass breed *R. natalensis* for parasitological assessment, we believe that the protocol following the approach of the experimental C protocol would be the best because the resources needed, such as dechlorinated water, can be easily accessible compared to buying commercial spring water that was observed to produce the best results in this study. Despite numerical differences in survival, number of eggs laid, and juveniles hatching, the absence of a statistical difference between experimental groups B and C suggests that the outcome from the two approaches is comparable.

Many studies have described protocols for laboratory mass breeding of lymnaeid snails for improved understanding of the life history traits of *Fasciola* and potentially improving policy and enhancing livestock productivity [1,7,14,15,26,27]. However, these studies have also reported challenges in the breeding of *R. natalensis*, thus limiting our understanding of the ecology of these snails, especially in areas where climate change-induced emerging and re-emerging cases of fascioliasis occur. Hence, our study intended to improve the breeding protocols for *R. natalensis* under laboratory conditions. Our study considered previously published protocols for *R. natalensis* [7,14], *G. truncatula* [26], and *P. columella* [27] to come up with an improved and cost-effective version for mass snail production.

Our results showed that *Elodea*-fed snails in field water, which was designed to mimic the environmental conditions from which the snails were obtained, exhibited the lowest returns in terms of survival, egg masses laid, and number of juveniles laid. The high mortality rate and low fecundity observed in group A might be due to the natural water used for breeding that could have been contaminated by parasites that we did not screen and that may have interfered with the monitored parameters [28]. The potential presence of molds in the water and the vegetation that was used as substrate led to snail mortality. A study by Madson [7] suggests that mold affects adult snails, leading to a reduction in egg masses' hatchability and the survival of juvenile F_1 snails [7]. Snails in the wild are used to flowing water and a continuous replenishment of food, unlike in our study, where water was changed three times a week. The time lag in the changing of water may have also affected the feeding of snails due to poor water quality. The natural feed was inferior, particularly for protein content, to the artificial feed used for other groups. An earlier study suggested that *R. natalensis* prefers clean and clear water [28,29] and any slight change in the water quality affects snail survival. We also believe that the quality of the water may have been made worse by the *Elodea* sp. weed powder which was used as food and the *C. pyrus* twigs which were used as oviposition substrate. These factors might have been key in affecting snail survival and oviposition. These results highlight the importance of carefully selecting husbandry conditions in experimental protocols involving *R. natalensis*, particularly in studies on parasite life cycle maintenance.

5. Conclusions

This study underscores the trade-offs between biological efficacy and cost efficiency in the mass breeding of *R. natalensis*. While snails in experimental group B demonstrated superior outcomes, laboratories in low-income settings may have a comparable number of snails if they chose to use the experimental group B approach for their work. This approach gives considerable number of eggs and juvenile snails at a minimum cost.

Supporting information

S1 File. Statistical analysis output for reproduction.

(TXT)

S2 File. Breeding data set.

(XLSX)

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Project administration: Moses John Chimbari.

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Visualization: Chester Kalinda.

Writing – original draft: Agrippa Dube.

Writing – review & editing: Agrippa Dube, Chester Kalinda, Tawanda Manyangadze, Tendai Makoni, Moses John Chimbari.

References

1. Dreyfuss G, Vignoles P, Rondelaud D, Sánchez J, Vázquez AA. Laboratory cultures of Lymnaeidae for parasitological experiments. The Lymnaeidae: A Handbook on Their Natural History and Parasitological Significance. Springer; 2023. p. 395–414.
2. Dube A, Kalinda C, Manyangadze T, Mindu T, Chimbari MJ. Effects of temperature on the life history traits of intermediate host snails of fascioliasis: A systematic review. PLoS Negl Trop Dis. 2023;17(12):e0011812. <https://doi.org/10.1371/journal.pntd.0011812> PMID: 38048345
3. Claxton JR, Sutherst J, Ortiz P, Clarkson MJ. The effect of cyclic temperatures on the growth of *Fasciola hepatica* and *Lymnaea viatrix*. Vet J. 1999;157(2):166–71. <https://doi.org/10.1053/tvj.1998.0293> PMID: 10204412
4. Mas-Coma S, Valero MA, Bargues MD. Human and Animal Fascioliasis: Origins and Worldwide Evolving Scenario. Clin Microbiol Rev. 2022;35(4):e0008819. <https://doi.org/10.1128/cmr.00088-19> PMID: 36468877

5. Kalinda C, Chimbari MJ, Mukaratirwa S. Effect of temperature on the *Bulinus globosus* - *Schistosoma haematobium* system. Infect Dis Poverty. 2017;6(1):57. <https://doi.org/10.1186/s40249-017-0260-z> PMID: 28457230
6. Oso OG, Sunday JO, Odaibo AB. Temporal modelling of *Lymnaea natalensis* (Krauss, 1848) in tropical aquatic habitats. Onderstepoort J Vet Res. 2023;90(1):e1–13. <https://doi.org/10.4102/ojvr.v90i1.2023> PMID: 37265142
7. Madsen H, Monrad J. A method for laboratory maintenance of *Lymnaea natalensis* and for mass production of *Fasciola gigantica* metacercariae. J Parasitol. 1981;67(5):735–7. <https://doi.org/10.2307/3280458> PMID: 7299589
8. Stothard JR, Brémond P, Andriamaro L, Loxton NJ, Sellin B, Sellin E, et al. Molecular characterization of the freshwater snail *Lymnaea natalensis* (Gastropoda: Lymnaeidae) on Madagascar with an observation of an unusual polymorphism in ribosomal small subunit genes. Journal of Zoology. 2000;252(3):303–15. <https://doi.org/10.1111/j.1469-7998.2000.tb00625.x>
9. Jansen van Rensburg C. Snail borne larval trematodes of the Okavango Delta, Botswana. 2001.
10. Dhiman V, Pant D. Human health and snails. J Immunoassay Immunochem. 2021;42(3):211–35. <https://doi.org/10.1080/15321819.2020.1844751> PMID: 33226881
11. Svgruha R, Fodor I, Padisak J, Pirger Z. Progesterone-induced alterations and their ecological relevance in different embryonic and adult behaviours of an invertebrate model species, the great pond snail (*Lymnaea stagnalis*). Environ Sci Pollut Res Int. 2021;28(42):59391–402. <https://doi.org/10.1007/s11356-020-12094-z> PMID: 33349911
12. Modabbernia G, Meshgi B, Kinsley AC. Climatic variations and *Fasciola*: a review of impacts across the parasite life cycle. Parasitol Res. 2024;123(8):300. <https://doi.org/10.1007/s00436-024-08319-6> PMID: 39145846
13. Madsen H, Stauffer JR. Zoonotic trematode infections; their biology, intermediate hosts and control. 2022.
14. Adediran O, Uwalaka E. Laboratory breeding of *Lymnaea natalensis* (Krauss, 1848), intermediate host of *Fasciola gigantica* (Cobbold, 1856). 2013.
15. Bitakaramire PK. *Lymnaea natalensis* laboratory culture and production of *Fasciola gigantica* metacercariae. Parasitology. 1968;58(3):653–6. <https://doi.org/10.1017/S0031182000028948> PMID: 4905987
16. Jaja IF, Mushonga B, Green E, Muchenje V. Seasonal prevalence, body condition score and risk factors of bovine fasciolosis in South Africa. Vet Anim Sci. 2017;4:1–7. <https://doi.org/10.1016/j.vas.2017.06.001> PMID: 32734037
17. Mpisana Z, Jaja IF, Byaruhanga C, Marufu MC. Body condition scores, fluke intensity, liver pathology, and carcass quality of different dairy cattle genotypes infected with *Fasciola* species at high throughput abattoirs in South Africa. Parasitol Res. 2022;121(6):1671–82. <https://doi.org/10.1007/s00436-022-07504-9> PMID: 35366098
18. Dp A, Mb C, Cc B, Dm D, Os PJ, Br A. Distribution and factors associated with *Fasciola hepatica* infection in cattle in the south of Espírito Santo State, Brazil. Journal of Venomous Animals and Toxins including Tropical Diseases. 2011;17:271–6.
19. Rashid M, Rashid MI, Akbar H, Ahmad L, Hassan MA, Ashraf K, et al. A systematic review on modelling approaches for economic losses studies caused by parasites and their associated diseases in cattle. Parasitology. 2019;146(2):129–41. <https://doi.org/10.1017/S0031182018001282> PMID: 30068403
20. Ngcamphalala PI, Malatji MP, Mukaratirwa S. Geography and ecology of invasive *Pseudosuccinea columella* (Gastropoda: Lymnaeidae) and implications in the transmission of *Fasciola* species (Digenea: Fasciolidae) - A review. J Helminthol. 2022;96:e1. <https://doi.org/10.1017/S0022149X21000717> PMID: 34991739
21. Nukeri S, Malatji MP, Sithole MI, Ngcamphalala PI, Nyagura I, Tembe D. Infection rates of *Fasciola* spp. in cattle slaughtered at 13 abattoirs in six of nine provinces of South Africa. Food and Waterborne Parasitology. 2025:e00260.
22. Olaogun SC. Knowledge, practices, and epidemiology of bovine fasciolosis in smallholder farming areas of South Africa: University of Pretoria (South Africa). 2022.
23. Black J, Ntusi N, Stead P, Mayosi B, Mendelson M. Human fascioliasis in South Africa. S Afr Med J. 2013;103(9):658–9. <https://doi.org/10.7196/samj.7184> PMID: 24300687
24. Brown DS. Freshwater snails of Africa and their medical importance. CRC Press; 1994.
25. Appleton CC. Freshwater Molluscs of Southern Africa: With a Chapter on Bilharzia and Its Snail Hosts. University of KwaZulu Natal Press; 1996.
26. Moazeni M, Ahmadi A, Mootabi Alavi A. A new method for laboratory rearing of *Galba truncatula*, the intermediate host of *Fasciola hepatica*. Vet Parasitol. 2018;253:12–5. <https://doi.org/10.1016/j.vetpar.2018.02.008> PMID: 29604994
27. de Souza CP, Magalhaes KG, Passos LKJ, Pereira dos Santos GC, Ribeiro F, Katz N. Aspects of the maintenance of the life cycle of *Fasciola hepatica* in *Lymnaea columella* in Minas Gerais, Brazil. Mem Inst Oswaldo Cruz. 2002;97(3):407–10. <https://doi.org/10.1590/s0074-02762002000300023> PMID: 12048573
28. Someren VDV. The Habitats and Tolerance Ranges of *Lymnaea (Radix) caillaudi*, the Intermediate Snail Host of Liver Fluke in East Africa. The Journal of Animal Ecology. 1946;15(2):170. <https://doi.org/10.2307/1557>
29. Harrison A, Shiff C. Factors influencing the distribution of some species of aquatic snails. South African Journal of Science. 1966;62(8):253.