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Full Length Research Paper

Malacological study of snail intermediate hosts of trematode parasites in Okitipupa Local Government Area, Ondo State, Nigeria

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Water bodies in specific sites were sampled for snail intermediate hosts of trematodes. Overall, a total of 949 snails were taken from the selected rivers with long handled scoop net, and in some areas with the aid of a pair of forceps. The sampled snails were placed in wide-mouthed universal bottles, loosely covered and taken to the laboratory for investigation. Examination was carried out by exposing groups of ten in beakers containing water to sunshine for 3 h, and this water was examined for cercariae. Snails that showed positivity were washed, re-exposed as earlier mentioned, and the water was checked for cercariae. Of all the snails found, only 5 (0.52%) *Lymnaea natalensis* and *Physa acuta* were positive with cercariae, while other species (*Potadoma*) were not infected. The present study reveals that *Lymnaea*, *Physa* and *Potadoma* species are common snails found in Okitipupa Local Government Area.

Key words: Trematodes, prevalence, freshwater molluscs, malacology.

INTRODUCTION

Trematode infections continue to persist as one of the most principal and widely diffused tropical diseases in Africa, especially among communities found around the coastal regions (Black et al., 2010; World Health Organisation, 2014).

The distribution and existence of the diseases in a community depends on the availability or absence of the particular parasite's snail intermediate host, for example, *Fasciola hepatica* and *Fasciola Gigantica* require the presence of the snail host, *Lymnaea species*; *Schistosoma haematobium* requires the presence of snail host *Bulinus*

species; *Schistosoma mansoni* requires *Biomphalaria species*; while the *Paragonimus westermani* requires the presence of *Segmentina species* (Bereket et al., 2017). This indicates that there is specificity in the intermediate host of the parasites (Adama and Hoker, 1997). Hence, the presence of a type of snail intermediate host in an area can account for the prevalence of a parasitic disease in the inhabitants of the community (Goll and Scott, 1979; Geleta et al., 2015). Most research on diseases caused by trematode parasites showed the level of rampant and threshold of

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infections in man and animal populations (Opisa et al., 2011). Knowledge of the presence of a type of snail in a locality could be used to check for local transmission which helps to clarify associations between disease point prevalence (Opisa et al., 2011; Sripa et al., 2016).

Recent malacology surveys provide information on the importance of integrations of snail observations with parasitology data from humans (Standley et al., 2011; Bereket et al., 2017). However, fragmentation of infection among man and animal populations in relation to snail survey makes it tough to indicate with certainty, the occupancy and spreading of trematode's snail host in the area (Opisa et al., 2011). This is further disconcerted by the fact that most people within endemic areas exhibit high gypsy (Standley et al., 2010) thus, complicating the pattern for locally acquired versus imported infections. Although chemotherapy plays a crucial role in reducing diseases and death rate due to trematode infections, the value and operational restraints impede its efficacy on a wider scale (Opisa et al., 2011).

Parallel preventive measures such as snail centered, whose alliance needs a complete perceptive of snail distribution seem credible. Also, the lastingness of trematode infections in man and animal hosts makes it tough to uncover the time and location of when transmissions really occur, without carrying out a snail for close observation. In as much as development of the larval stages of trematode parasites depends on snail host, their study provides important information on active transmission of foci. Therefore, the parasite and the intermediate host must be tackled with a view to breach the chain of circulation so as to get great achievement in regulating trematodes infections rates.

In this study, selected Rivers and Streams in Okitipupa Local Government Area, Ondo State, Nigeria was sampled for snail hosts of trematode parasites. Therefore, this study set out to:

- (1) Identify the presence of disease spreading snail vectors in the area.
- (2) Determine the prevalence of cercarial infection rates of snail intermediate hosts and
- (3) Determine snail's relative abundance

MATERIALS AND METHODS

Study area

The research was undertaken in Okitipupa Local Government Area, Ondo State, Nigeria, located between 6°25' and 6°25'N Latitude, and 4°35' and 4°50'E longitudes within the tropical rainforest zone of Nigeria. It covers a total land area of 636 sq km with total annual rainfall often exceeding 200 mm.

Aqua-contact exercises

Selection of sites were based on pre-field investigation as obvious water contact points, where people frequently go to fetch water,

wash clothes, bath, swim or play, and even where fishing activities takes place.

Aquatic snail sampling

Samplings of snails were conducted in June to December, 2014 in a five fresh water bodies (Igbodigo, Agbala, Ilutitun, Ikoya and Igbotako), where there was main human water contact. Most of the snails were found on the side of the leaves and water lilies. Snails were taken with long handled scoop net and a pair of forceps, where water was deep and picked with hand in gloves in some areas. The collection was randomly done for about 45 min per site. Rain boots and hand gloves were worn as precaution against infection by cercariae (Agbolade and Odaibo, 1996). The snails sampled were placed in a wide mouthed universal bottles containing water, loosely covered and taken to the laboratory.

Snail's identification

On arrival at the laboratory, the snails were separated and identified using the standard key by Brown and Kristensen (1993).

Cercariae shedding

Each species was placed in a separate container. Groups of 10 snails were placed in glass beaker containing about 100 ml of water, exposed to sunlight for 3 h to facilitate shedding of cercariae. Snails that were positive were washed clean, re-subjected as earlier mentioned, and the water was examined (Okoli and Owuala, 2001).

Physico-chemical characteristics

The physico-chemical parameters—Dissolved oxygen (DO), Alkalinity, conductivity and pH of water at each sampling site were measured using the modified Axide Winkler Method (Hach Chemical Company, 1997); while temperature was determined using a temperature probe.

Data analysis

Raw data were input into a Microsoft excel spread sheet, and descriptive statistics were used to summarize the data. The prevalence was calculated for all data as the number of infected individuals divided by number of individuals examined, and multiplied by 100 to express in percentage. Chi square test was used to assess the distribution of snail vectors with P-values <0.05 considered as statistically significant.

RESULTS

Dispersion and abundance of snail species

949 fresh water snail specimens were collected from 5 different water bodies within the same Local Government Area. From morphological point of view, 372 (39.35%) of the snails acquired were recognized as *Lymnaea natalensis*, 270 (28.5%) as *Physa acuta*, while 307 (32.3%) as *Potadoma spp* (Table 1). The dispersion of snails was crowded with less areas accounting for nearly

Table 1. Distribution of snail vectors among fresh water bodies in Okitipupa Local Government Area, Ondo State, June-December, 2014.

Water bodies	Snail species			Total Snail abundance
	<i>Lymnae natalensis</i>	<i>Physa acuta</i>	<i>Potadoma spp</i>	
Igbodigo	276 (108.18)	0(78.52)	0 (89.28)	276
Agbala	16 (232.45)	270(168.71)	307(191.83)	593
Ikoya	50(19.59)	0 (14.22)	0(16.17)	50
Ilutitun	0	0	0	0
Igbotako	30(11.75)	0 (8.53)	0 (9.70)	30
Total	372	270	307	949

$\chi^2 = 88.380$, $P = 0.05$.

Table 2. Prevalence of snail vectors among fresh water bodies in Okitipupa Local Government Area, Ondo State, June-December, 2014.

Water bodies	Parameter	Lymn	Phy	Pota	Overall
Igbodigo	Collected	276	-	-	276
	Infected	3	-	-	3
	% Infection	1.08	0.00	0.00	1.08
Agbala	Collected	16	270	309	593
	Infected	0	1	0	1
	% Infection	0.00	0.37	0.00	0.37
Ikoya	Collected	50	-	-	50
	Infected	1	-	-	1
	% Infection	2.0	0.00	0.00	2.0
Ilutitun	Collected	0	-	-	-
	Infected	0	-	-	-
	% Infection	0.00	0.00	0.00	0.00
Igbotako	Collected	30	-	-	30
	Infected	0	-	-	-
	% Infection	0.00	0.00	0.00	0.00
Overall	Collected	372	270	307	949
	Infected	4	1	0	5
	% Infection	1.08	0.37	0	0.526

Lymn: *Lymnae natalensis*, Phy: *Physa acuta*, Pota: *Potadoma*

all the snails. Overall, of the 5 water bodies surveyed, 1 did not yield any snail (Table 1). *Lymnae spp* were found at 4 out of 5 water bodies surveyed while *Physa spp* and *Potadoma spp* were found in only 1 water bodies (Table 1).

The number of snails' species found from each of the water bodies indicated that, Agbala River had the highest number of snail species, followed by Igbodigo River, Ikoya River and Igbotako River while Ilutitun River had no snail species (Table 1).

Snail infections

Interestingly, few snails found during the survey were found to emit cercariae. Of all the species sampled, 5 species (0.52%) of the snails were infected (Table 2).

Physico-chemical factors

Table 3 and 4 showed the mean value of physico-

Table 3. Occurrence of snails in relation to physico-chemical parameters of fresh water bodies in Okitipupa Local Government Area, Ondo State, June-December, 2014.

Fresh water bodies	Temp °C	pH (%)	Dissolved Oxygen $\mu\text{g/l}$	Conductivity Mg/l	Alkalinity	<i>Lymnae</i>	<i>Physa</i>	<i>Potadoma</i>
Igbodigo	28.8	6.0	10.0	2390	1.50	276	-	-
Agbala	29.1	5.9	11.0	2410	1.20	16	270	307
Ikoya	29.2	5.9	12.0	2380	1.60	50	-	-
Ilutitun	30.5	6.5	10.0	2370	0.90	-	-	-
Igbotako	31.4	5.8	13.0	2380	1.60	30	-	-
Total	-	-	-	-	-	372	270	307

Table 4. Mean height of snail species of fresh water bodies in Okitipupa Local Government Area, Ondo State, June-December, 2014.

Water bodies	<i>Lymnae natalensis</i> mean height (mm)	<i>Physa acuta</i> mean height (mm)	<i>Potadoma spp</i> mean height (mm)
Igbodigo	13	-	-
Agbala	12	21.5	9
Ikoya	11.5	-	-
Ilutitun	-	-	-
Igbotako	11.5	-	-
Total	12	21.5	9

chemical factors in water bodies. There were variations in physico-chemical parameters of the water bodies from months to months throughout the study period, while both conductivity and temperature values increased those of DO with a decreased in pH from June to December.

DISCUSSION

The study showed that water bodies have high abundance of aquatic snails. This study was able to identify three genera of aquatic gastropod molluscs: *Lymnaea*, *Physa* and *Potadoma*. The study showed that the prevalent species was *L. natalensis*, known for its potential as a host for *F. hepatica* in the tropics, which supported the findings of Emejulu et al. (1992). Examination of 949 snails showed that *L. natalensis* were the most abundant snails. The distribution of snails was restricted and crowded, with less areas accounting for nearly all of the snails, which was also a novel aiming to reveal the distribution and abundance of trematodes snail species in the communities.

Abundance and distribution of snails in relation to physico-chemical factors

Physico-chemical parameters that affect snail dispersion are frequently paying fewer attentions even though these can differ significantly from site to site and area to area,

even within short distances (Sharma et al., 2013). Of all the physico-chemical variables gauged in this study, water temperature turnout to be the essential determinant factors of snail abundance. The positive relationship between snail quantity and water temperature noticed in this study is in agreement with investigations from Uganda that, snail distributions were limited in the North and North-Eastern parts of the country with high temperatures (Stensgaard et al., 2006).

Of important was how broadly the pH values varied; snails were found in water bodies with pH ranging from 5.8 to more than 6.5. The lack of relationship between pH and snail quantity reported in this study has also been reported previously (Kahigi, 2000), indicating that pH may not be an important determinant factors of snail abundance, as is in the case with other freshwater organisms (Macan, 1974). But, on the other side, Levitz et al. (2013) have shown that a lower pH was associated with higher snail abundance. This discordance in findings on the association between pH and snail abundance remains to be clarified.

Implication of cercarial shedding in transmission

A considerable low numbers of snails in this study shed cercariae. Some trematodes cercariae are diurnal and are typically released during daylight hours, peaking around midday and dawn (Stelnauer et al., 2008). The emergence times correlates to times when their hypo-

thetical hosts are available in water for infection. But, this is not absolutely recent and the discoveries are in support with other studies from endemic areas, with high transmission found less or none of the snails collected shed any cercariae.

In a study by McClelland (1956), it was reported that although 90% of school children were infected with *S. haematobium*, there were challenges in finding infected snails. Somewhere, contrary to the high human prevalence of *S. haematobium* infection in Msambweni along with the Kenyan coast, the rate of snails shedding *S. haematobium* cercariae was only 1.2% (Kariuki et al., 2004). Still at the Kenyan coast, another study showed that cercarial shedding was either low (range = 0.14 to 3.4%) or altogether absent (Hamburger et al., 2004).

In the Lake Victoria basin in western Kenya, only 1.04% (236/22,641) of snails collected at various sites shed cercariae (Steinauer et al., 2008), while a current research in Sesse Islands of Lake Victoria, Uganda, revealed that none of the snails collected shed cercariae (Standley et al., 2010). Various reasons may be put forward for the absence or low numbers of snails shedding cercariae.

First, it has been explained that the percentage of infected snails may be very low or cercariae may be shed for only a limited period of time (McClelland, 1956). This confounded with the focal nature of trematodes diseases and the complexity of sampling ample areas, where snails distributed makes it tedious to precisely locate which site would contain most numbers of infected snails.

Second, snail population quantity, rates of infection and cercarial output are also under seasonal influence (Hamburger et al., 1998). Perhaps, it may not be optimal for snails to shed cercariae around the peak rainy season when there may be a decreased water contact activities associated with swimming and or domestic use.

Third, the very low proportion of infected snails in water bodies may also be due to enhanced dilution of human faecal matter, associated with mixing across a larger volume of water or perhaps, difficulties in miracidia locating snail hosts in an undulating aquatic environment has been suggested elsewhere (Levitz et al., 2013).

Fourth, cercarial release from field-collected snails may also be inhibited by a variety of contaminants and invertebrates harbored by the snails. Fifth, it has been suggested that field snails in heavily endemic areas are subjected to pulses of infection rather than to a continuous flow of miracidia (Sturrock et al., 1979).

Considering the fact that prepatent infection can last for several weeks with only a proportion of snails reaching the stage of cercarial shedding (Joubert et al., 1991), and that prepatent infection rates can be substantial, and exceed patent infection rates (Wool house and Chandiwana, 1989), it is also probable that majority of snails sampled in this study may have had prepatent infections.

Clarification of such prepatent infections may be done

using methods such as snail crushing in search of larvae or repeated shedding in the laboratory overtime, although such methods are unsuitable for accurate and large-scale monitoring. This may be necessary especially in light of the observation that as a method, cercarial emergence (which is routinely used) severely underestimates parasite prevalence (Curtis and Hubbard, 1990).

Although it is generally accepted that finding infected snails is the only confirmation of transmission of the disease, the study findings suggest that a cautious interpretation of transmission based on snail infection is necessary. Moreover, a single, brief exposure to cercariae-infested water is sufficient to effect transmission (Vercruyse et al., 1994), even where the number of shedding snails is low (Mubila and Rollinson, 2002).

Conclusion

Several snails' species were found in this study that act as intermediate hosts of different trematodes, which affect our livestock and birds. In this study, we identified the prevalence of trematodes cercariae on the basis of cercarial shedding, but this traditional method has several problems; first this method is laborious and time consuming, and second it does not give actual prevalence rate because this method depends upon cercarial shedding, whereas in the case of prepatent infection it gives a false result.

Due to these hindrances, parasitologists utilize molecular techniques for detection and characterization of parasites within their intermediate and final hosts. The development of a molecular approach for cercarial detection in infected snails is necessary and will be useful, such as polymerase chain reaction (PCR).

Therefore, it is recommended to use molecular techniques to diagnose the actual prevalence of snail's intermediate hosts infected with cercariae of different trematodes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Use of a homeopathic complex against *Haematobia irritans* infestation in dairy cattle, Paraná, Brazil

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Horn fly, *Haematobia irritans irritans* (Linnaeus), is an ectoparasite that feeds exclusively on the host's blood, preferably cattle, whose biological cycle occurs in its feces. This insect is scattered throughout Brazil in areas favorable to its development and also in other South American countries. The insect remains most of the day time on the animal. The adult fly can live 3 to 7 weeks. At high infestation rates, skin lesions may occur, predisposing the animal to bacterial infections. Biting activity is one of the harmful aspects of this fly; the painful bites cause irritation and stress in animals, impairing their development, milk production, reproductive activities, etc. Homeopathy is among some alternatives for parasite control aimed to seek means to control the major internal and external parasites in cattle, which are more efficient and less aggressive to animals and the environment. This study aimed to evaluate the efficacy of a homeopathic complex in 14 one-year-old Holstein heifers divided into two groups infested with *H. irritans* in a period of 60 days. There were statistically significant differences between groups in the number of horn flies on day 45 of the experiment, and the number of horn flies decreased in animals treated with the homeopathic complex. There were no significant differences between groups regarding hematocrit (%), total protein (g/dL), albumin (g/dL), and globulins (g/dL). The parasitic homeopathic complex proved to be effective against horn flies, with 64.7% reduction of these flies in animals at 45 days of experiment in treatment group animals.

Key words: Heifers, hematocrit, homeopathy, horn fly.

INTRODUCTION

The first records of horn fly (*Haematobia irritans irritans* (Linnaeus)) date back to 1830 in France, described by Linnaeus, later spreading throughout the rest of the world, especially where the cattle population was expanding, starting invasion in the Americas, first in the United States and then spreading across North America

and Central and South America (Brito et al., 2005).

In the mid-1970s, Brazil registers the entry of this parasite, first in the region of Roraima, spreading its habitat in other states, facilitated by climatic conditions (Brito et al., 2005). Climatic conditions in the tropics tend to favor the proliferation of insects for being exothermic

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and having their metabolism regulated by external environment. However, the interference of rainfall cannot be cited as a determining factor, and although the parasite's cycle depends on soil moisture, a significant increase in horn fly infestation according to rainfall is not observed, and relative air humidity seems to have greater influence on the cycle of this parasite. The ambient temperature conditions have a more precise relationship with *H. irritans* infestation, which proliferation is higher at higher temperatures (Almeida et al., 2010; Bianchin et al., 2002, 2006).

The cycle of development of the horn fly is relatively fast, being determined by the temperature, humidity and quality of the fecal mass, during the winter, the interval of generations can extend up to twenty or thirty days, whereas in the rainy season this interval can be reduced to eight or nine days (Honer et al., 1990).

Damages caused by horn fly are relevant to the current livestock scenario, resulting in decreased consumption, feed conversion, weight gain and increased stress, especially in cattle. Its action causes discomfort, not only due to its blood-sucking habit, but mainly for irritating animals. Damages caused by this parasite are considerable, and Bianchin et al. (2004) report loss of 10% in revenue, reaching 15 kg less of yield in slaughter houses. In Brazil, the damage attributed to the horn fly with respect to the national cattle herd was estimated at US\$ 2.56 billion in 2012 (Grisi et al., 2014).

Taking into account the economic aspects and animal welfare, the use of synthetic products becomes a viable solution at first, but it should be taken into account that these drugs can leave residues in animal origin products (Dell'Porto et al., 2012), and there is also the imminent danger of *H. irritans* to create resistance.

Producers guided by the demands of the population in the agricultural market seek healthier solutions to problems in the productive sector. Thus, the use and acceptance of products that do not leave residues in food increase in relation to chemical insecticides to combat ectoparasites (Pinto et al., 2005), stressing the importance of intensifying research on alternative products.

Homeopathy has proven to be an alternative remedy to improve animal production, by providing effective improvement from the burden of many diseases with a further advantage over insecticide use in addition to its cost tends to be lower than other treatments (Santos et al., 2006). The presence of resistance to insecticides in horn fly and bovine tick populations (*Rhipicephalus (Boophilus) microplus*) is currently observed, being necessary to seek new treatment alternatives for the control of these ectoparasites.

Furthermore, the use of homeopathic medicines do not leave residues in animal origin products, and this is added advantage in relation to products such as antibiotics, organophosphates, antiparasitic agents that leave residues in food and harm the health of end users.

Martins et al. (2007) point out that homeopathic products have no grace period, and their derivatives may be marketed normally.

This study aimed to evaluate the use of a homeopathic complex in controlling *H. irritans* infestation on dairy cattle.

MATERIALS AND METHODS

This experiment was conducted at the campus II of the Paranaense University (UNIPAR) with the aim of evaluating the efficacy of a homeopathic complex administered to one-year-old Holstein heifers to control horn fly infestation.

Animals

Fourteen, one-year-old Holstein heifers were used in this experiment and were divided into two groups: treatment group (TG) with 7 animals and control group (CG) with 7 other animals. Animals were distributed in two equal paddocks with covered bays, giant star grass (*Cynodon plectostachyus*) and water *ad-libitum*, feed and mineral salt. The experimental units were randomly divided. The animals' weight gain, visual count of the flies, hematocrit (%), plasma levels of total proteins (g/dL), albumins (g/dL) and globulins (g/dL) were evaluated in this experiment.

Product

The parasitic homeopathic complex was specifically designed for this study, which composition includes *Abrotanum* 10⁻¹², *Arsenicum album* 10⁻¹², *Calcarea carbónica* 10⁻¹², *R. (Boophilus) microplus* 10⁻¹², *Bunostomum* species 10⁻¹², *Damalinia ovis* 10⁻¹², *Dermatobia hominis* 10⁻¹², *Ferrum metallicum* 10⁻¹², *H. irritans* 10⁻¹², *Haemonchus* species, 10⁻¹², *Linognathus stenopsis* 10⁻¹², *Musca domestica* 10⁻¹², *Nematodirus* species 10⁻¹², *Oesophagostomum* species 10⁻¹², *Oestrus ovis* 10⁻¹², *Ostetagia ostertagi* 10⁻¹², *Sulfur* 10⁻¹², *Strongyloides* species 10⁻¹², *Trichostrongylus* species 10⁻¹², and *Triocharis* species 10⁻¹². Treatment group of animals received daily 20 g/animal/day of homeopathic complex for 60 days, while control group animals received 20 g/animal/day of calcium carbonate, the homeopathic complex vehicle for 60 days.

Weighing and collections

Every two weeks, visual counting of flies in the cervico-dorsal-lumbar region of animals was performed between 09:00 am and 10:00 am as suggested by Almeida et al. (2005), in which flies present from the neck to the hip of each animal were counted. Then, the animals were contained in individual trunk to collect blood samples by puncture of the tail vein for analysis of hematocrit, total protein, albumin and globulin; they were then individually weighed, and collections occurred every 30 days.

RESULTS

The use of the homeopathic complex to control *H. irritans* showed significant difference in relation to the control group at 45 days of the experiment (D45) ($p < 0.05$). The animal weight results (kg) and the number of horn flies in

Table 1. Mean \pm standard error of animal weight (kg) and number of horn flies in Holstein heifers receiving or not diet enriched with homeopathic complex on days 0, 15, 30, 45 and 60 of treatment (D0, D15, D30, D45 and D60, respectively).

Treatment	Weight (kg)	Number of horn flies
CG D0	211.85 \pm 8.75	35.86 \pm 7.70
TG D0	200.57 \pm 21.56	34.14 \pm 7.98
CG D15	204.14 \pm 17.08	20.14 \pm 7.09
TG D15	216.29 \pm 13.77	09.26 \pm 3.50
CG D30	215.00 \pm 16.05	23.49 \pm 7.55
TG D30	251.29 \pm 23.39	10.43 \pm 3.15
CG D45	219.00 \pm 21.94	19.57 \pm 4.01 ^a
TG D45	237.00 \pm 28.59	07.86 \pm 1.61 ^b
CG D60	210.57 \pm 10.69	25.85 \pm 7.47
TG D60	204.43 \pm 15.86	09.60 \pm 3.63

*Difference in letters mean statistical difference ($p < 0.05$) between groups.

animals are shown in Table 1.

In addition to hematophagous activity of *H. irritans*, it causes stress in animals, which in an attempt to get rid of them, the animals waste energy, reducing food and water intake, leading to weight loss. These factors may alter certain hematological parameters.

Table 2 shows the results of hematocrit, total protein, albumin and globulins of Holstein heifers treated with homeopathic complex and control group.

DISCUSSION

For greater productivity in cattle herds, animals should always be the closest as possible of their homeostasis, using few resources of their body energy. These losses in cattle performance directly affect economic parameters in the sector.

This study found significant difference in the number of flies on D45 (Table 1), and the decrease in the count of flies from D15 should be taken into account, and on D60, although no statistical difference was observed, the mean difference in the count of flies was 25.85 \pm 7.47 in CG and 09.60 \pm 3.63 in TG. In the final experiment's phase, a weight loss was observed in the animals of the two groups, this factor is due to the start of the winter period with the decrease in supply for the African star grass (*Cynodon plectostachyus*).

Pinto et al. (2005), using a homeopathic complex composed of biotherapies mixed in the mineral supplementation of Nelore heifers over 12 months, observed that the homeopathic core showed preventive effect on parasitism by *Dermatobia hominis* larvae. Salla et al. (2015) applying a homeopathic medicine topically in cattle, observed the effectiveness of the product against *H. irritans* for 30 days.

According to Signoretti et al. (2008), the use of homeopathic complex showed significant differences, decreasing the development of ticks in the larval and

adult stages; however, no significant differences were observed in the nymphal stage.

According to Marques et al. (2008), using pyrethroids, organophosphates, avermectin, phenyl pyrazoles, benzoylphenyl urea and homeopathic products, it was shown that all treatments were effective against horn fly in zebu-crossed or crossbred animals. However, one should take into consideration chemicals that may harm the African beetle cycle (*Digitonthophagos gazela*) that are in the feces, and these insects act as natural predators of flies, so the control of *Haematobia irritans* with 100% organic supplies is more environmentally interesting.

Signoretti et al. (2010) observed that with the continuous use of homeopathic products, there was no need for the use of chemicals to control ticks and horn flies, indicating the effectiveness of the use of homeopathic products.

According to Arenales (2002), homeopathic medicine does not promote the killing of flies, but the feces of animals undergoing treatment prevent continued insect cycle, not allowing the larval stages to become pupae, which demonstrates the effectiveness of treatment on D45 and the reduction of flies on D60.

Ferreira et al. (2014) used stable flies (*Stomoxys calcitrans*) and houseflies (*Musca domestica*) to produce a biotherapeutic at dilution 10⁻¹² and observed on the 8th day of experiment a 19% reduction of stable flies and 61% of houseflies and on the 15th day, there was a reduction of 20 and 53% for stable flies and houseflies, respectively. In the present work on the 15th day of treatment, there was a 29.2% decrease of horn flies in treatment group animals when compared with the control group animals.

There was no significant difference in the hematologic parameters (Table 2) between experimental and control groups, and the hematocrit values found are below the reference values (Table 3) in both groups and in all

Table 2. Mean \pm standard error in the analyses of hematocrit, total proteins, albumin and globulin of Holstein heifers receiving diets enriched or not with homeopathic complex on days 0, 30, 60 of treatment (D0, D30 and D60, respectively).

Treatment	Hematocrit (%)	Total proteins (g/dL)	Albumin (g/dL)	Globulins (g/dL)
TG D0	21.93 \pm 0.97	7.83 \pm 0.24	3.17 \pm 0.12	4.66 \pm 0.21
CG D0	22.20 \pm 0.69	8.17 \pm 0.20	3.35 \pm 0.06	4.76 \pm 0.24
CG D30	20.24 \pm 1.07	7.06 \pm 0.35	2.88 \pm 0.11	4.18 \pm 0.37
TG D30	21.36 \pm 1.17	7.13 \pm 0.25	2.86 \pm 0.16	4.25 \pm 0.14
CG D60	22.20 \pm 0.60	7.13 \pm 0.39	2.95 \pm 0.14	4.18 \pm 0.27
TG D60	22.33 \pm 0.80	6.93 \pm 0.17	2.93 \pm 0.08	4.00 \pm 0.15

*Not significant by analysis of variance.

Table 3. Reference values for bovine.

Analysis	Unit	Values
Hematocrit	%	24-46
Total proteins	g/dL	6.6-7.5
Albumin	g/dL	2.7-3.8
Globulin	g/dL	3.0-5.8

Source: Kaneko et al. (1997) and Jain (1993).

collections. The values obtained for total protein were slightly above the reference values in both groups at D0, becoming normal subsequently. Albumin and globulin values were within the reference values (Table 3). According to Pogliani and Birgel Jnr. (2007), animals raised under different environmental, climatic and management conditions can present variations of blood constituents. The values obtained for animals raised in a region cannot be considered without an adequate evaluation as a reference outside this region.

The use of homeopathic medicines can decrease the number of chemotherapeutic applications in animals and reduce the selection pressure of tick and fly strains still susceptible to conventional treatments, in addition to its allowed use in cattle kept in organic production system, acting on the welfare of animals and reducing stress (Honorato, 2006).

Conclusion

Oral supplementation with homeopathic complex under the experimental conditions evaluated reduced the number of horn flies at 45 days of experiment. The parasitic complex homeopathic proved to be effective against horn flies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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