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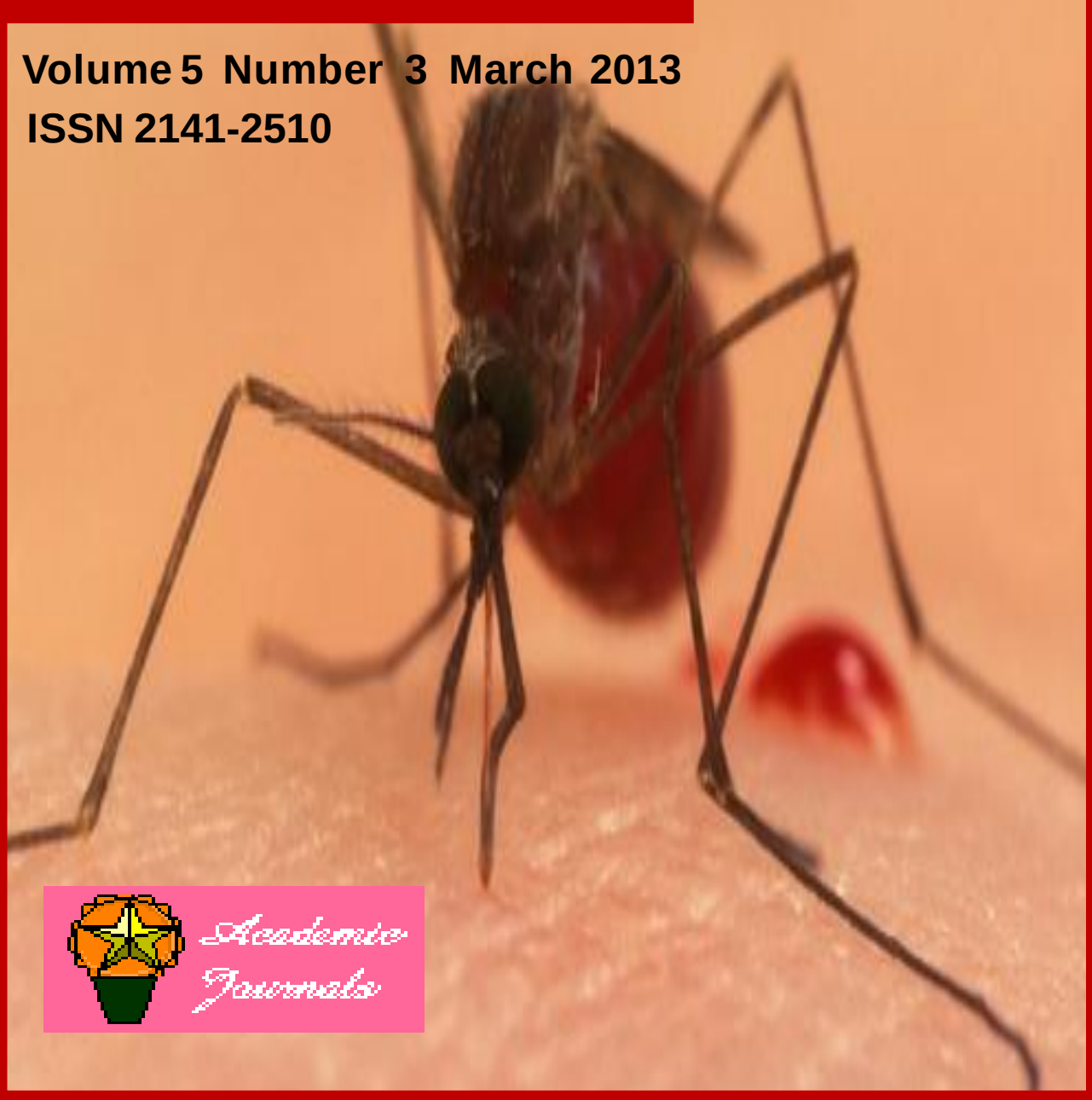

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Table of Content: Volume 5 Number 3 March 2013

ARTICLES

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Full Length Research Paper

Morphological diagnosis and occurrence of *Blastocystis* spp. obtained from the stool samples of domestic bird species commercialized in municipal markets

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***Blastocystis hominis* is a gastrointestinal tract parasite whose pathogenic role, and zoonotic potential remains unclear. Several microorganisms similar to *B. hominis* have been reported in a variety of non-human hosts, and described as *Blastocystis* spp. The study aimed to verify the occurrence of *Blastocystis* spp. in three species of poultry of two municipal markets, by observing the microorganisms' forms in stool samples stained with Giemsa. A total of 214 birds, distributed into two markets, were studied. In each of the markets were observed, 35 ducks (*Anas platyrhynchos*), 35 Japanese quail (*Coturnix japonica*) and 35 chicks (*Gallus gallus*). In market A, 8 (22.9%) chicks, 15 (42.9%) quails and 13 (37.1%) and ducks were diagnosed with *Blastocystis* spp.; in market B, 15 (42.9%) chicks, 2 (5.56%) quails, and 21 (55.3%) ducks. A significant difference was observed only between quails from the two markets but no statistical difference was observed when all infected birds in the two markets were compared. In stool samples positive with *Blastocystis* spp. and stained with Giemsa were observed forms as vacuolar, granular, amoeboid, and cystic, and some types of reproduction, such as binary fission, plasmotomy and budding. Both markets had unhygienic conditions of animal facilities, favoring the infection among them.**

Key words: Poultry, pleomorphic organism, gastrointestinal parasite, giemsa, microscopy, unhygienic.

INTRODUCTION

The first description of the genus *Blastocystis* was given by Alexeieff (1911), and currently the correct diagnosis of the microorganism is still a challenge, especially due to its uncertain pathogenicity. In addition, *Blastocystis* is considered a pleomorphic organism that can be confused with many structures in fecal samples without staining. *Blastocystis* spp. obtained from the feces of humans and animals have been reported as morphologically similar, although some authors have described distinct differences between those isolates (Singh et al., 1996; Stenzel et al., 1994, 1997). However, to differentiate one isolate from another, morphology cannot be used as the single criterion. In the 1970s and 1980s, the studies of Charles Zierdt caught the attention of biologists and clinicians (Tan et al., 2002), and from there, many other studies were performed, mainly focusing on the morphology of *B. hominis* (Stenzel and Boreham, 1996). *Blastocystis* spp. is the most common microorganism in the gastrointestinal tract of humans in various parts of the world (Stenzel and Boreham, 1996). It is also found in a wide variety of animal hosts (Boreham and Stenzel, 1993) and is considered to be a pathogen with high

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zoonotic potential (Tan, 2004). New species of *Blastocystis* have been proposed for non-human hosts (Belova and Kostenko, 1990; Belova, 1992; Teow et al., 1991), but these species have not been accepted among researchers because most isolates of *Blastocystis* are morphologically indistinguishable by observation under optical and electron microscopy (Noël et al., 2005; Suresh and Smith, 2004). Nevertheless, until there is further confirmation using molecular data, *B. hominis* is defined as a parasite isolated from humans, and *Blastocystis* spp. is defined as a parasite obtained from other animals (Noël et al., 2005).

The diagnosis of *Blastocystis* is usually carried out by the examination of fecal samples under an optical microscope (Zierdt, 1991; Garcia and Bruckner, 1993), followed by other diagnostic methods, such as the molecular characterization of subtypes (Forsell et al., 2012). For diagnosis by light microscopy, fecal smears can be stained to observe the various forms of *Blastocystis* spp. with various dyes, such as the trichrome stain (Garcia and Bruckner, 1993), iron hematoxylin (Guimarães and Sogayar, 1993), Giemsa (Dawes et al., 1990), gram (Zierdt, 1991) and wright (Vannatta et al., 1985) stains. Because there is no indication of a method that is more efficient, and difficulties in identifying the various forms of the microorganism in fecal material, these dyes seem to be the procedure of choice for the diagnosis of *Blastocystis* spp. as they show some of its internal structures and morphological characteristics.

Many isolates of *Blastocystis* that have been obtained from diverse hosts have shown some similarities (Tanizaki et al., 2005). These authors observed that isolates from chickens, quails and geese were able to infect chickens, demonstrating the existence of cross infection between bird species. In addition, a non-infected chicken got infected with *Blastocystis* after being raised with infected ones, emphasizing that the transmission of *Blastocystis* may occur easily between the same species or between different species of birds. Tanizaki et al. (2005) observed that isolates obtained from chicken, quails and geese were able to infect chickens, and demonstrated the existence of cross infection among bird species. Besides, a non-infected chicken got infected after being raised with infected ones, indicating that transmission of *Blastocystis* may easily happen among birds from the same or different species, showing a lack of host specificity.

Abe et al. (2002) conducted a research with zoo animals and reported a great prevalence of *Blastocystis* infection in pheasant and ducks. In another study, the *Blastocystis* cystic forms were diagnosed to infect chickens (Stenzel et al., 1997). The experimental infection of chickens and mice using four subtypes isolated from humans confirmed that some subtypes could infect chickens and rats, indicating the zoonotic potential of *Blastocystis.* These results also suggested that these hosts may be adequate animal models for the study of this microorganism (Iguchi et al., 2007).

The aim of the present study was to verify the occurrence of *Blastocystis* spp. by morphological diagnosis in stool samples stained with Giemsa that were obtained from ducks (*A. platyrhynchos*), quail (*C. japonica*) and chickens (*Gallus gallus*), which were naturally infected and commercialized in markets in the state of Rio de Janeiro, Brazil.

MATERIALS AND METHODS

The current study was conducted with a total of 214 live domestic birds commercialized in two municipal markets, named A and B, in Rio de Janeiro City, Brazil. A total of 71 Japanese quails, 73 ducks and 70 chickens were acquired from these two markets. It was analyzed, with 105 birds (35 ducks, 35 quails and 35 chickens) from market A and 109 birds (38 ducks, 36 quails and 35 chickens) from market B. The animals were marketed at approximately one week of age. Both markets had similar characteristics and were approximately 110 km apart from each other. The animals of each market had different origins and suppliers. The birds were housed in metal cages that were placed on shelves and had a high population density, favoring the stress of the animals. Food and water were provided in the cages, but the containers were full of feces due to the high density of the birds.

In each market, the birds were randomly selected, immediately transported and housed in individual cages where they were provided with food and water. Individual fecal samples were analyzed daily. For each bird, 4 g of fecal samples were weighed and homogenized in 5 ml of sodium chloride 0.9%, and a small aliquot of this material was observed between a slide and a cover slip by microscopy to diagnose the presence of *Blastocystis* spp. Then, the positive fecal smears were fixed with methanol and stained with Giemsa to observe the detailed morphology of the microorganism. The forms of *Blastocystis* spp. observed by microscopy after staining were analyzed according to the shape and size of the microorganism, the number of nuclei, and the presence or absence of a vacuole. Fisher's exact test was used in the statistical analysis of the results, using program Epinfo's Statcalc (Dean et al., 2002).

RESULTS AND DISCUSSION

Occurrence of natural infection of *Blastocystis* spp. in poultry

In a total of 214 birds, 22.9% (8/35) of the chickens from market A presented *Blastocystis* spp. in the feces, while 42.9% (15/35) from market B were positive. Although market B had a higher number of positive chickens, no significant difference ($p = 0.74$) was observed between the two markets. With respect to the ducks, 37.14% (13/35) from market A presented *Blastocystis* spp. in the feces, while 55.26% (21/38) from market B were positive. As with the chickens, despite the higher number of positive animals found in market B, there was no significant difference ($p = 0.121$) between the two markets. With respect to the quails, 42.85% (15/35) from market A presented *Blastocystis* spp. in the feces, while only 5.56% (2/36) from market B were positive. In this

Table 1. Occurrence of *Blastocystis* in chickens (*G. gallus*), ducks (*A. platyrhynchos*) and Japanese quails (*C. japonica*) obtained from two municipal markets in Rio de Janeiro, Brazil.

Host	Market	Examined animals/Infected	Positive animals (%)	Positive animals (%) (N = 214)
Chicks	A	35/8	8/22.90	23/10.70
	B	35/15	15/42.90	
Ducks	A	35/13	13/37.14	34/15.90
	B	38/21	21/355.26	
Japanese quails	A	35/15	15/42.85	17/7.90
	B	36/2	2/5.56	
Total	A and B	214/74	74	74/34.60

case, a significant difference was observed between the two markets ($p < 0.001$) (Table 1).

The rate of *Blastocystis* spp. naturally infecting the chickens in the current study was similar to the one observed by Stenzel et al. (1997) who reported a natural infection rate of 31%. In the same study, they observed that infected chickens were held in cages that were stocked with two or three per cage, in contrast with the present study, where the birds were originally held in cages with a high population density. In another study, Lee and Stenzel (1999) observed the prevalence of *Blastocystis* in 227 domestic chickens from 5 commercial farms and found a high rate of infection that was approximately 95% in four of the five farms studied, which is a high percentage in comparison with the observed current study.

Regarding the ducks of the current study, the rates observed in the two municipal markets are very similar to that observed by Abe et al. (2002), in which the infection rate was 56% in ducks from the Zoo of Osaka City, in Japan. From the 214 birds investigated in markets A and B, 74 (34.6%) birds presented *Blastocystis* spp. in the feces, of which 15.9% (34/74) were ducks, 10.7% (23/74) were chickens, and 7.9% (17/74) were quails (Table 1). The results showed no significant difference between the infection of ducks and chickens or between chickens and quails; however, there was a difference between ducks and quails. These results suggest that ducks may be more susceptible to *Blastocystis* spp. infection than chickens and quails. Importantly, chickens and quails belong to the Order Galliformes, while ducks belong to the Order Anseriformes. Because they belong to different Orders, ducks may also have a different immune response from that of chickens and quails.

With respect to the birds positive with *Blastocystis* spp. infection, 36 (34.3%) were from market A, and 38 (34.9%) were from market B. These results indicate that there is no significant difference ($p = 0.92$) between the two municipal markets in the total number of birds with *Blastocystis* spp. infection. Thus, the markets likely did not have a great influence on the infection rates of these poultry because the hygiene and sanitary conditions of both markets, A and B, were very similar.

The observation of clinical signs, such as lethargy, appetite loss and fatigue, in poultry may suggest gastrointestinal infections, among them the *Blastocystis* spp. infection. One way to prevent such an infection would be the separation of the poultry after the observation of the first clinical signs, thus reducing the spread of the microorganism. The clinical signs mentioned above were previously reported by Stenzel and Boraham (1996) and Moe et al. (1997) in birds infected with *Blastocystis* spp. Therefore, avoiding the acquisition of symptomatic birds or separating the symptomatic from the asymptomatic birds would circumvent the dissemination of the infectious forms of this organism.

The consistency of the feces eliminated by the birds during the collection was another important aspect that was observed. Most of the fecal samples positive with *Blastocystis* spp. had an abnormal aspect; they were diarrheic and sometimes passed out blood colored with mucus feces. There was no correlation between the feces aspect and the occurrence of *Blastocystis* infection. In agreement, Tan (2004) observed that describing the clinical signs of *Blastocystis* infection as a real cause of disease is difficult. In contrast, several authors reported the occurrence of pasty feces and diarrhea in infections caused by *Blastocystis*. Quilez et al. (1995) observed pasty feces in infected pigs, Stenzel and Boreham (1996) described diarrhea in infected humans, and Moe et al. (1997) reported pasty feces in infected lab rats.

Lee and Stenzel (1999) investigated a property with high-quality hygiene and sanitary conditions, and none of the birds studied were positive for *Blastocystis*. They investigated the conditions of the (properly cleaned) floors, utensils and equipment, the removal of feces, and the cleanliness of the food and water containers. Their study indicated that good hygiene and sanitary conditions were possible inhibitors of environmental contamination

Table 2. Morphometric data of some of the forms of *Blastocystis* spp. observed in stool samples stained with Giemsa that were obtained from poultry sold at local markets.

Characteristic	Form			
	Vacuolar (n = 100)	Granular (n = 100)	Amoeboid (n = 17)	Cystic (n=200)
Maximum size (µm)	20.3±4.2	16.9±4.1	45.5	5.5
Minimum size (µm)	18.3±3.9	15.3±3.9	13.4	2.1
Number of nuclei	1 and 7	1 and 4	1 and 4	1 and 4
Index morphometrics	1.11±0.02	1.11±0.03	-	1.4±0.03

and of the fecal-oral transmission of *Blastocystis* infection among chickens. Therefore an adequate management of the birds could contribute to better health maintenance of the animals.

Diagnosis of *Blastocystis* spp. observed in stool samples stained with giemsa

Four forms of *Blastocystis* spp. were observed in stool samples stained with giemsa: vacuolar, granular, amoeboid and cystic. These forms have been reported by several authors, especially in studies related to *B. hominis*. With respect to the *Blastocystis* spp. isolated from animals, there are few descriptions of the morphological characteristics of the microorganism diagnosed in feces. The most common form observed was vacuolar, rounded and containing a central body resembling a large vacuole that occupies approximately 90% of the cell, with a thin layer of peripheral cytoplasm. In the cytoplasm, nuclei can be observed in a peripheral location, with up to seven nuclei per cell and an average of two nuclei in cells arranged at the opposite poles. Images of the form mentioned above from a fecal smear stained with Giemsa are shown in Figure 1; A and B.

The morphometric data presented in Table 2 are consistent with those described by Lee and Stenzel (1999) which were obtained from domestic chickens. The measurements of the vacuolar forms of *Blastocystis* spp. were quite varied, with a minimum measurement of 10.9 µm and a maximum of 32.1 µm.

The granular form of *Blastocystis* spp. was very similar to the vacuolar one, with multiple central granules in the vacuole. Dunn et al. (1989) proposed that these structures could be similar to myelin inclusions, small vesicles, crystalline granules and lipid droplets. As found in the vacuolar forms, more than one nucleus was observed in its restricted cytoplasm. Up to four nuclei, with an average of two per cell, were observed. Images of the granular form in stool smears stained with giemsa are shown in Figure 1C to F.

In the granular form, the *Blastocystis* spp. were slightly smaller than the vacuolar forms (Table 2) but similar to those reported by Stenzel and Boreham (1996). The diameter of the cells ranged from 9.0 to 28.3 µm. The granular form showed a different quantity of granules in their interior, which was noted because of the change in the intensity of staining when observed under microscopy. This observation may be due to the affinity of the dye used in this study. Similar to the vacuolar form, the

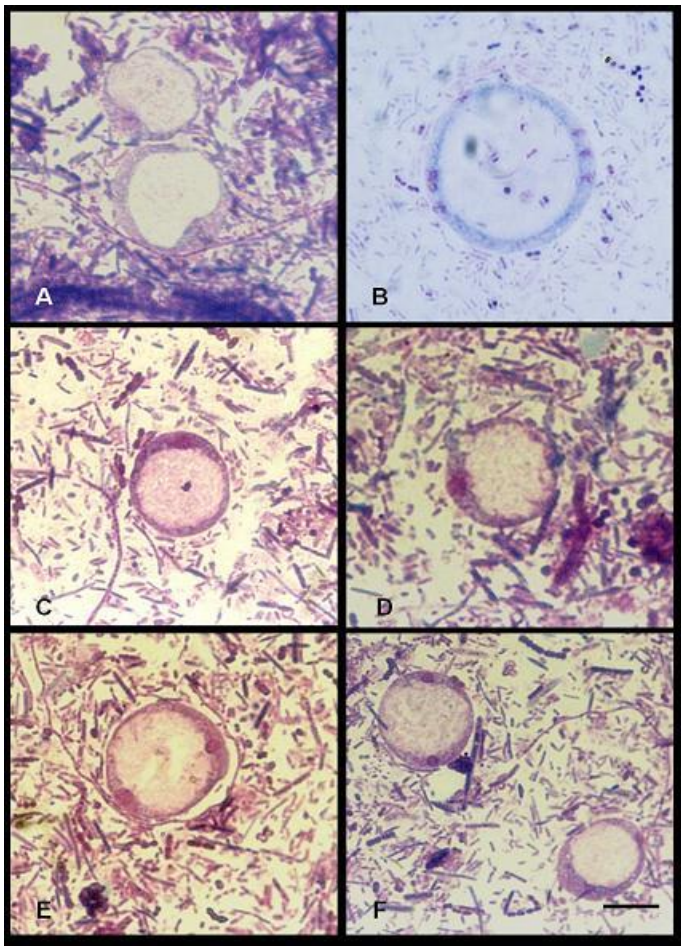

Figure 1. Forms of *Blastocystis* spp. obtained from the giemsa-stained fecal samples from poultry. Vacuolar form: A: central vacuole (→); B: seven nuclei in the cytoplasm. Granular form: C and D: some cytoplasmic inclusions, and one single nucleus; E and F: multiple nuclei (— = 10 µm).

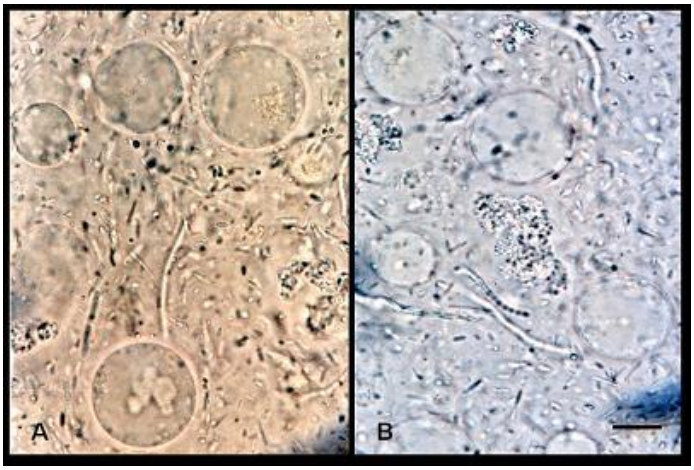

Figure 2. Forms of *Blastocystis* spp. obtained in the stool samples from poultry homogenized with sodium chloride 0.9%. A: bright field; B: phase contrast (— = 10 µm).

granular forms were also present in various samples. In addition, both were commonly observed in recently eliminated fecal samples and could be observed without staining (Figure 2; A and B).

In this study, the amoeboid form was found in small amounts in the stained fecal smears. In samples without staining, however, its observation was difficult. These forms presented irregular borders, often resembling extensions such as "pseudopods", and lacked a central vacuole (Figure 3; A and B). The measurements of the amoeboid forms of *Blastocystis* spp. are given in Table 2. The average size was 22 µm, and the measurements ranged from 13.4 to 45.5 µm. These values are similar to those found by Tan and Suresh (2006), who observed measurements ranging from 5 to 50 µm.

The morphological characteristics of the *Blastocytis* spp. ameboid forms are not in agreement with some reports. Dunn et al. (1989) reported cells of 2.6 to 7.8 µm in diameter that were irregularly shaped, had no central vacuole and had a structure similar to an extended pseudopod. In contrast, Tan and Zierdt (1973) reported oval amoeboid cells containing one or two long pseudopods and a large central vacuole. The cystic forms of the Blastocystis spp. observed in giemsa-stained fecal samples were in general, arranged in groups and rarely individually. The cysts, when grouped, were surrounded by a membrane or by a membrane trace. Stenzel et al. (1997), after observing the membrane of the cysts obtained from the feces of domestic chickens, using transmission electron microscopy, concluded that the membrane is composed of a fibrillar layer. In addition, Zaman et al. (1999) observed that the cysts might be surrounded by a fibrillar layer that could be intact or fragmented. Stenzel et al. (1997) also observed that each cyst might contain one to four nuclei. Nevertheless, in this study, the cysts were characterized as rounded or ovoid,

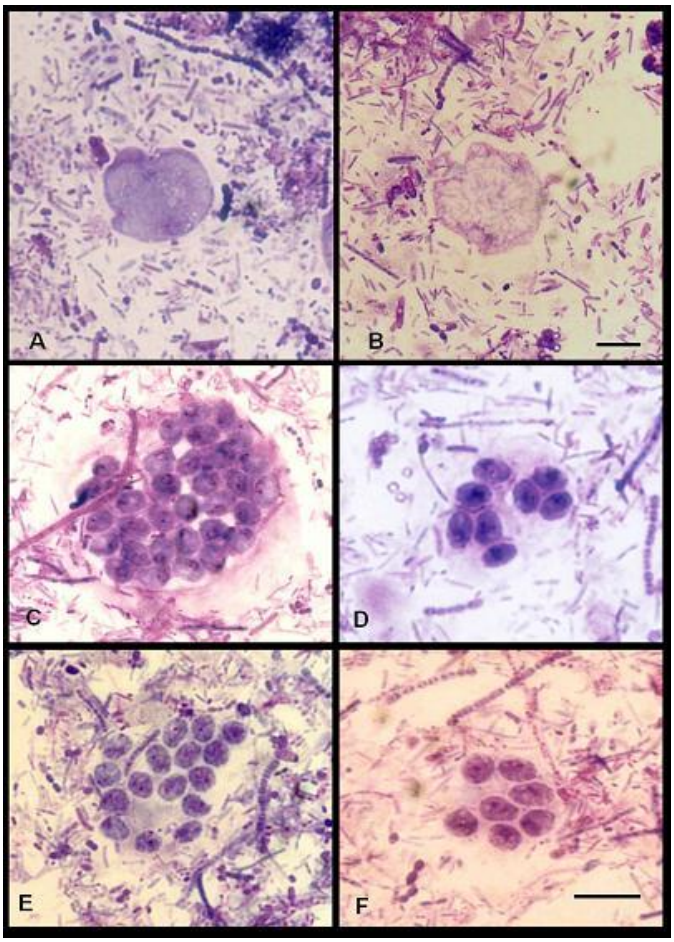

Figure 3. Forms of *Blastocystis* spp. obtained from the giemsa-stained fecal samples of poultry. Amoeboid form: A and B: projections from the cytoplasm similar to a pseudopod; cells with nuclei cyst: C: a clear fibrillar layer is observed covering the nucleated cysts; D, E and F: nucleated cysts with a trace fibrillar layer (— = 10 µm).

with one or two internal nuclei (Figure 3; C to F). The measurements of the cystic form of *Blastocystis* ranged from 2.1 to 5.5 µm (Table 2), agreeing with the measurements described by Stenzel and Boreham (1996). Zaman et al. (1999) found daughter cells within *Blastocystis* cysts, and some of them could be observed in the vacuoles. The rupture of these cysts resulted in the emergence of these daughter cells. In the present study, internal structures similar to those described by Zaman et al. (1999) were observed inside the cystic form, with one or two nuclei and a structure similar to a vacuole (Figure 4; A and B). This variation in the number of nuclei within the cysts was most likely related to the stage of maturity. Some of the cystic forms had daughter cells that were more clearly detailed when the cysts were disrupted (Figure 4C). In this study, three types of reproduction of the *Blastocystis* spp. could be observed: binary division, plasmotomy and budding. In addition, Zhang et al. (2007) described two more types of reproduction: endodyogeny

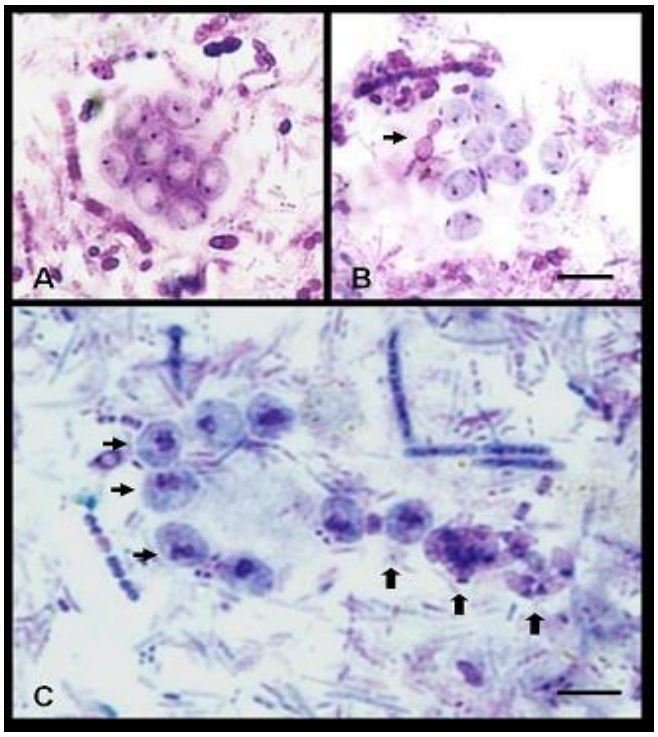

Figure 4. Cystic form of *Blastocystis* spp. observed in the giemsa-stained poultry fecal samples. A and B: presence of one or two nuclei inside and the vacuoles; B (→): budding yeast; C: daughter cells observed after the cyst break (— = 10 µm).

and schizogony. The most common reproductive form found in this study was binary division, which is characterized by the partition of the cytoplasm of the mother cell and results in two daughter cells with an equal size and shape (Figure 5; A to C). In binary division, the cytoplasm of the cell is elongated, and after the elongation of the cytoplasm, cell is compressed toward its center until it breaks, resulting in two daughter cells.

In this study, using fresh and stained fecal samples, plasmotomy was considered a rare observation. Zhang et al. (2007) were able to observe this type of reproduction only in *in vitro* culture. Plasmotomy is characterized by the extension of the cytoplasmic membrane and cell surface, by which the cell increases its size. The daughter cells are formed from the extension or expansion of the cytoplasmic surface of the mother cell (Figure 5D). The other type of reproduction found in this study using giemsa-stained fecal samples was budding, in which the mother cell forms a new daughter cell from its side. Sometimes this mother cell may give rise to two or even three daughter cells, which are always smaller than the original cell (Figure 5; E and F).

The different forms of *Blastocystis* spp. mentioned above were diagnosed in the three species of poultry used in the present study.

More studies in other host species should be conducted for further information on the morphology and identification of the various forms of *Blastocystis* spp. in stool samples to better elucidate its biological cycle and to establish the control and prophylaxis of the microorganism.

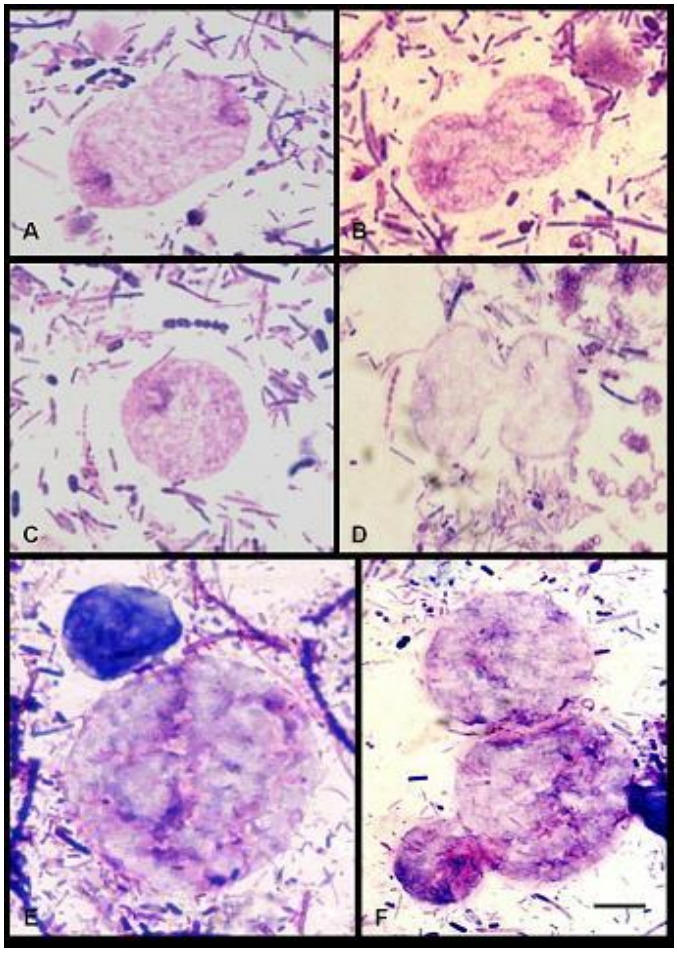

Figure 5. Types of reproduction of *Blastocystis* spp. obtained from the giemsa-stained fecal samples of poultry. Binary fission: A: beginning of the binary fission, with cell elongation and the nuclei in opposite poles; B: cell in cytoplasmic division; C: a cell possibly originating from binary fission; Plasmotomy: D: extension of the cytoplasmic membrane that will originate another cell; Budding: E: growth of one side of the cell, originating a new and smaller cell; F: several daughter cells of various sizes originating from a bigger mother cell (— = 10 µm).

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Full Length Research Paper

Mode of anticoccidial drug utilization and their financial cost in Benin litter-based exotic layer rearing system

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Anticoccidial drug mode of utilization and their financial cost in Benin litter-based commercial layer system were investigated on 81 layer farms with a total of 120 poultry flocks consisting of 81 adult and 39 young bird flocks. The collected data include bird age category, coccidiosis control program, the anticoccidial molecules, theirs financial prices and their mode of administration. Most of the farmers adopted the preventive mode of coccidiosis control. Six anticoccidial molecules were identified as regularly used by farmers to control coccidiosis, administrated through drinking water. Amprolium is the most prevalent anticoccidial molecule used for young birds (84.6%), followed in a very less extent by sulfadimidine (15.3%) which is the most used anticoccidial molecule in adult birds (74% of the flocks). The entire sulfa-based drugs in use in the production system accounted for 88.8%. Poultry producers in Benin spend 137 FCFA or 0.30 $ per layer chicken in litter-based system to control coccidiosis with the use of anticoccidial drug during the bird lifespan.

Key words: Coccidiosis, sulfa-based drugs, amprolium, sulfadimidine, prevention.

INTRODUCTION

Coccidiosis in bird is a gastrointestinal parasitic disease caused by *Eimeria* genus, with the most pathogenic species including *Eimeria tenella, Eimeria acervulina, Eimeria maxima* and *Eimeria brunetti*. The economical incidence of the disease is world widely estimated at 2.3 billion € with 70% of the lost ascribed to the unapparent subclinical form of the disease that depress considerably weight gain and feed conversion ratio (Sørensen et al., 2006). Coccidiosis treatment and prevention measures are based on the use of anticoccidial drugs or chemoprophylaxie and the use of live vaccines. There are two groups of anticoccidial drugs known to be in use: ionophorous antibiotics known as ionophores and synthetically produced drugs known as chemicals (De Gussem, 2005). Polyether ionophores such as monensin, lasalocid, salinomycin, narasin and maduramycin act through general mechanism of altering ion transport and disrupting osmotic balance of the parasite and chemicals such as amprolium, clodipol, halofuginone decoquinate act against the parasite metabolism (Badran and Lukešová, 2006). Chapman (1994) reported that some degree of resistance to all anticoccidial drugs, including ionophores has developed. The use of drugs in rotational basis upon consecutive flocks (McDougald, 2003) or in a shuttle or dual drug using program reported by Williams, (1998) and recommended by Chapman et al. (2005), is purportedly directed against the establishment of drug-resistant parasite strain that can jeopardize the

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effectiveness of bird coccidiosis control. The drugs are also associated to drug-resistant live vaccine to augment the efficacy of the vaccine, by controlling the side effect of vaccine parasite replication phase, in the intestinal epithelial cells. The recombinant vaccine developed lately consisting of coccidian antigens and many otherimmunogenic molecules (micronemes and cytokines) proved to be very efficient (Lee et al., 2009; Tewari et al., 2010; Berezin et al., 2010). Layer chicken intensively reared for table egg production in Benin is a growing activity and the current lack of information about coccidiosis control in this bird population estimated at 13,690,940 heads (FAO, 2011) can in the future jeopardize its development.

This study reports for the first time, the anticoccidial products used to control coccidiosis and their financial cost in Benin litter-based high stocking density exotic layer rearing system.

MATERIALS AND METHODS

Field survey and data collection

This study is an observational cross-sectional study with anticoccidial molecules, their mode of utilization and their financial cost as the main investigation outcomes. The sample size was obtained by considering the theoretical coccidiosis prevalence in litter-based layer rearing system, which is 31% (Lunden et al., 2010). A minimum of 20% as null proportion and an alternative proportion of 40% were considered, with 90% of power and under 0.05 significant levels. The power procedure in Statistical Analysis System (SAS; version 9.2) with Z test applied to the aforementioned estimates, gave an optimal sample size of 42 laying hen farms included in this study. The sampling method used the weighted technique and the random selection of the surveyed farms with a total of 81 farms consisting of 120 flocks (39 young bird flocks and 81 adult flocks) enrolled in the survey. The survey was carried out in the Northern, Central and Southern regions of Benin (6° 28′ N 2° 36′ E; 114 763 km^2). The collected data include bird age category, coccidiosis control program (preventive or curative mode of control), the anticoccidial molecules used by the farmers, theirs financial prices and their mode of administration.

Statistical analyses

Frequency procedure in SAS (version 9.2) was used to estimate and compare proportions. General Linear Model (GLM) was applied to the period interval between two coccidiosis treatments (estimation of mean value ± standard error and comparison with F test).

Economical analyses

The financial cost of anticoccidial drugs used to control coccidiosis in one layer chicken from day 0 to the end of its laying period (18 months) was accessed as follow: anticoccidial drug financial cost = (anticoccidial drug cost in preventive mode + anticoccidial drug cost in curative mode in young bird form 0 to 4 months of age) + (anticoccidial drug cost in preventive mode + anticoccidial drug cost in curative mode in adult bird from 4 to 18 months of age).

RESULTS

Mode of anticoccidial drugs utilization

Table 1 shows that an overwhelming majority of young layer chicken flocks from 0 to 4 months of age were submitted to the preventive mode of coccidiosis control ($p < 0.05$), while in adult layer flocks from 4 to 18 months of age, the proportions of farms which adopted the preventive or the curative mode of coccidiosis control were more or less the same ($p > 0.05$). The frequency of drug administration recorded in the preventive mode of coccidiosis control was twice that observed in the curative mode of control ($p < 0.05$).

Anticoccidial molecules used by producers

Two anticoccidial molecules were found to be at great utility in the production system, among the 6 anticoccidial molecules identified as regularly used by farmers to control coccidiosis (Figure 1) and all of them were administrated through drinking water.

Amprolium is the most prevalent anticoccidial molecule used for young layer chicken from 0 to 4 months of age (84.6%) followed in a very less extent by sulfadimidine (15.3%) which is the most used anticoccidial molecule to control the disease in adult birds in a proportion of 74%. Amprolium is not at all used for laying hen coccidiosis control in the surveyed farms. The entire sulfa-based drugs that were used in adult layer flocks account for 88.8% of the total anticoccidial drugs identified in adult layer flocks.

Financial cost of drugs used in one layer chicken from day 0 to 18 months of age

In one treatment phase, an average of 0.57 and 0.37 g of anticoccidial drugs were administered in drinking water for the account of each young (Table 2) and adult layer chicken (Table 3) respectively. These quantities were given in an average interval period of 30.7 days in prevention model and in an average interval period of 60.8 days in curative model (Table 1) during the lifespan of the bird.

The financial cost of anticoccidial drug was 136.9 FCFA or 0.30 $. In other words, poultry producers in Benin have to spend approximately about 137 FCFA in minimum to control coccidiosis in one layer chicken from chick age (Day 0) to the end of its laying period (18 months).

DISCUSSION

Preventive medication against coccidiosis is the most observed disease control model in exotic layer chicken in Benin. According to McDougald (2003), early emphasis in chemotherapy was centered on the treatment of outbreaks

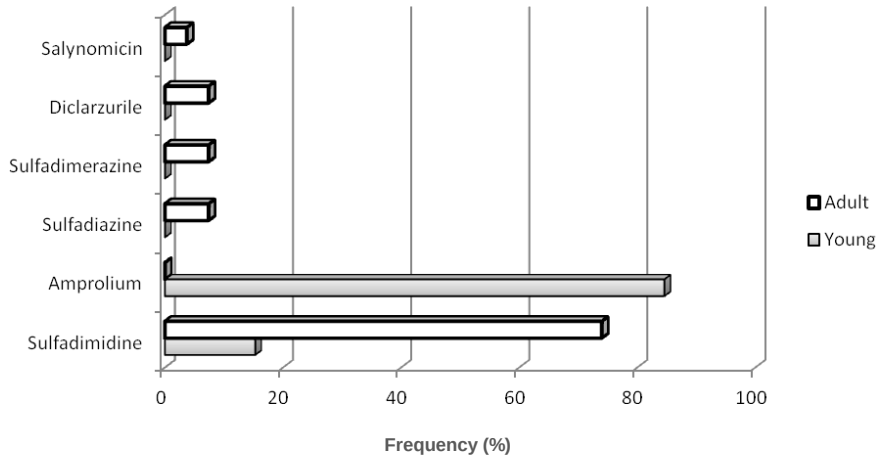

Figure 1. Frequency (%) of the identified anticoccidial molecules.

Table 1. Mode of anticoccidial drugs utilization.

Mode of control	Preventive mode	Curative mode
Young layer flocks (%)	92.3	7.6
Adult layer flocks (%)	48.1	51.8
Interval of ATC administrations (day)	$30.7^{a} \pm 2.1$	$60.8^{b} \pm 2.7$

ATC: Anticoccidian; M: mean; SE: standard error. Values in the same line that do not share the same superscript are significantly different, $p < 0.05$.

Table 2. Cost of drug per young layer in one treatment phase (C1).

Anticoccidian	Drug price/g (FCFA)	%	Drug quantity (g)	Cost (FFCA)	Cost ($)
Amprolium	19.5	84.6	0.53	10.33	0.02
Sulfadimidine	20.5	15.4	0.04	0.82	0.002
Total (C1)		100	0.57	11.15	0.024

%: Proportion of flock that use the different anticoccidial drugs; FFCA: West African French speaking country currency; $: US Dollar; g: gram.

Table 3. Cost of the drug per adult layer in one treatment phase (C2)

Anticoccidian	Drug price/g (FCFA)	%	Drug quantity (g)	Cost (FFCA)	Cost ($)
Sulfa-based drugs	20.5	88.8	0.32	6.56	0.016
Diclazuril	16.05	7.4	0.027	0.43	0.0009
Salynomicin	21	3.8	0.01	0.21	0.0004
Total (C2)		100	0.37	7.2	0.017

%: Proportion of flock that use the different anticoccidial drugs; FFCA: West African French speaking country currency; $: US Dollar; g: gram.

outbreaks with sulfonamides or other compounds after signs of infection were apparent; but, soon the concept of preventive medication emerged with the realization that most of the damage is done once signs of coccidiosis are widespread in a flock. Coccidiosis prevention also called chemoprophylaxis is by far the most used control measure in broiler chicken sector, where 95% of the farms prevent coccidiosis from day 0 to slaughter with anticoccidial drugs as feed additive (Chapman et al., 2005). The findings revealed that in adult layer birds, a slight majority of poultry producers adopted the curative mode of controlling coccidiosis. The main reason could

be the economical incidence of coccidiosis prevention that could oblige poultry producers to wait for the disease in the flock, before having recourse to anticoccidial drugs. As shown by the current study, the anticoccidial drug administration frequency in preventive mode is higher than that in curative model of coccidiosis control.

Anticoccidial drugs administered in drinking water are the only means of coccidiosis prevention and treatment encountered in Benin commercial layer chicken. Among the 6 anticoccidial drugs known by poultry farmers which are used in some extent, only two of these compounds were importantly utilized by the latter. The first anti-coccidial product is a chemical compound: amprolium, exclusively used in young birds at the starting rearing period, up to the pullet stage. This drug act by impeding the metabolism of the parasite (Badran and Lukešová, 2006), especially the absorption of thiamine (McDougald, 2003) and has no drug withdrawal period restriction (Feed Additive Compendium, 1989). The second most important anticoccidial drug is sulfadimidine, massively used in adult bird. It competes for metabolism of the parasite folic acid (McDougald, 2003). This control model used by two different anticoccidial drugs during the bird production cycle is a sort of shuttle or dual drugs using program applied in broiler poultry production industry, reported by Williams (1998) and recommended by Chapman et al. (2005). This preferential use of two chemical: amprolium and sulfadimidine could be due to their proved efficacy and their lower inducing coccidial drug-resistance potential expression or merely the ineffectiveness of the other marketed anticoccidial products. The amprolium drug has no withdrawal period restriction. But on the contrary, sulfadimidine, a sulfa-based drug, used to prevent or cure coccidiosis in a continual egg-producing bird, irrespective of it withdrawal period restriction of about 5 to 10 days (Feed additive Compendium, 1989) represents a great jeopardy for public health. Anticoccidial drug residues might be present in eggs.

Producers spend 137 FCFA per chicken, tantamount to 0.30 $ per chicken to control coccidiosis with the use of anticoccidial drug from the starting day 0 to the end of the laying period (18 months) in Benin exotic layer chicken production system. This is certainly the much heavier economical incidence in diseases control financial cost in this system and it is in line with several previous reports on the expensiveness of coccidiosis control cost in broiler or exotic layer flocks (Williams, 1999; McDougald, 2003; Sørensen et al., 2006).

These findings revealed an intense use of anticoccidial drugs, principally amprolium and sulfa-based drugs (sulfadimidine) with consequently some drug residue presence in eggs (Cannavan et al., 2000; Mortier et al., 2005; Danaher et al., 2008) in the administration period and the 10 days period following administration period, prejudicial to consumer health.

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Full Length Research Paper

Laboratory feeding of *Bulinus truncatus* and *Bulinus globosus* with *Tridax procumbens* leaves

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Suitability of *Tridax procumbens* leaves in laboratory feeding of *Bulinus truncatus* and *Bulinus globosus* was assessed in comparison with *Lactuca sativa* between September and October, 2011. The snails were collected from Eri-lope stream in Ago-Iwoye, while *T. procumbens* were collected from the Mini Campus of the Olabisi Onabanjo University, Ago-Iwoye, Ijebu North, Southwestern Nigeria. For *B. truncatus*, fresh, sun-dried and oven-dried *T. procumbens* were used, while only fresh *T. procumbens* were used for *B. globosus*. The mean percentage survivals of *B. truncatus* fed with fresh, sun-dried and oven-dried *T. procumbens* compared with those of the corresponding control snails showed no significant difference (χ^2 = 0.51, 1.85, and 2.21, respectively). *B. truncatus* fed with fresh *T. procumbens* had the highest mean live-weight percentage increase (46.4%) as compared to those fed with sun-dried and oven-dried (χ^2 = 45.65). The mean percentage survival of *B. globosus* fed with fresh *T. procumbens* (79.2%) was similar with that of the control (84.6%) (χ^2 = 0.18). The percentage increase in mean live-weight of *B. globosus* fed with fresh *T. procumbens* (24.0%) was lower as compared to the control (χ^2 = 5.34). This study showed that feeding the studied snails using *T. procumbens* may be profitable in Ijebu North, Southwestern Nigeria.

Key words: *Bulinus truncatus*, *Bulinus globosus*, laboratory feeding, *Tridax procumbens*.

INTRODUCTION

All species of *Bulinus*, including *Bulinus truncatus* and *Bulinus globosus*, are freshwater gastropod snails of the family Planorbidae. *Bulinus* species are widespread in Africa, having been reported from regions such as Northwest Africa, Ethiopia, Sudan, Egypt, Tanzania and Nigeria (Brown, 1994; Chitsulo et al., 2000). *B. globosus*, *B. truncatus* and some other *Bulinus* spp. are established vectors of *Schistosoma haematobium* (Ukoli, 1984; WHO, 1993). Therefore, much attention has been consistently devoted towards understanding the biology of the aforementioned vectors and other vector snails towards sustainable control of human schistosomiasis in tropical Africa including Ijebu North, South-western Nigeria (Adewunmi et al., 1991; Agbolade et al., 2004; Madsen and Stauffer, 2011).

Tridax procumbens is a dicotyledonous plant in the Daisy family and is a commonly widespread weed and pest plant in the tropical, sub-tropical and mild temperate regions of the world (http://en.wikipedia.org/wiki/Tridax_procumbens).

Nevertheless, some studies have revealed some usefulness of *T. procumbens*. It has been reported that the plant is rich in nutritionally valuable substances such as protein, fat, carbohydrate, fibre, sodium, potassium, calcium, carotenoids and flavonoids (Jude et al., 2009). Moreover, it is known that the leaves of *T. procumbens*

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are eaten by *Archachatina marginata* (Odaibo, 1997), and a recent study showed that domestication of *Lanistes libycus* (an edible freshwater apple snail) using the leaves of *T. procumbens* could be a profitable venture (Agbolade et al., 2010).

In view of the public health significance of *B. truncatus* and *B. globosus*, it is often necessary to culture them in the laboratory for research purposes. Unfortunately, *Lactuca sativa* (lettuce) conventionally used to feed freshwater snails seems scarce and expensive in Ijebu North area, Southwestern Nigeria. This study was therefore designed to assess the suitability of the leaves of *T. procumbens* in the laboratory feeding of *B. truncatus* and *B. globosus*.

MATERIALS AND METHODS

Samples' source and collection methods

B. truncatus and *B. globosus* specimens were collected from Erilope stream in Ago-Iwoye. Ago-Iwoye (6° 56' 55" N, 3° 54' 40" E) is the location town of the main Campus of the Olabisi Onabanjo University, and it is located in Ijebu North area of Ogun State, Southwestern Nigeria. The snails were collected by picking them from among submerged and emergent plants, fallen leaves and twigs in the water body. The specimens were transported to the laboratory in a well-aerated container. *T. procumbens* plants were hand-collected from the Mini Campus of the Olabisi Onabanjo University, Ago-Iwoye. *L. sativa* (lettuce) was purchased from a market in Lagos, Nigeria. Snails' collection and culturing were carried out between September and October, 2011.

Culturing method

In the laboratory, the specimens were identified and sorted into species with reference to Brown and Kristensen (1993). Specimens of each species were divided into size classes after initial shell length measurement. For *B. truncatus*, the snails were later divided into three categories according to their diet, placed in bowls labelled fresh, sun-dried, and oven-dried. Each category had three replicates (A, B, C), and one control. Each of the bowls was lined with transparent nylon to make easy the collection of eggs. Dechlorinated water was added to each culture bowl. 20 g of fresh and 10 g of dried *T. procumbens* leaves (for trial bowls) and *L. sativa* (for control bowls) were used in feeding *B. truncatus* snails as appropriate. For *B. globosus*, only fresh *T. procumbens* leaves (for trial bowls) and *L. sativa* (for control bowls) were used. There were four trial replicates (A, B, C, D) and one control.

For both species of *Bulinus*, each of the trial and control bowls was covered with polythene mosquito net held in place with rubber band to prevent snails from escaping. Changing of water and feeding were done every other day. Any mortality was recorded and the specimen was removed immediately on detection. Shell length measurement of the snails was done once a fortnight. The mean live-weight of the snails in each bowl was measured using weighing balance on day 0 (initial weight) and subsequently fortnightly. The final shell length and live-weight measurements were taken at the end of the 7th week.

Statistical analysis

Percentage increase in live-weight and shell length were calculated by comparing the mean values on day 0 with the corresponding mean values at the end of the 7th week using the following formulae:

Live-weight increase (%) = (Final live-weight – Initial live-weight / Initial live-weight) × 100

Shell length increase (%) = (Final length – Initial length / Initial length) × 100

Chi-square (χ^2) was used to compare percentages of survival and increase in live-weight and shell length. For mean live-weight and shell length, the replicates with the least values of percentage increase were used as benchmarks for comparison with the control values.

RESULTS

The survival, live-weight and shell length of *B. truncatus* fed with fresh, sun-dried and oven-dried *T. procumbens* leaves are summarised in Table 1. At the end of the 7th week, the percentages of surviving control snails fed with fresh (84.6%), sun-dried (84.6%) and oven-dried (85.7%) *L. sativa* were not significantly different ($\chi^2 = 0.01$, df = 2, $P > 0.05$). The mean percentage survivals of *B. truncatus* fed with fresh (75.6%), sun-dried (67.8%) and oven-dried (67.3%) *T. procumbens* were not significantly different ($\chi^2 = 0.62$, df = 2, $P > 0.05$). The mean percentage survivals of snails fed with fresh, sun-dried and oven-dried *T. procumbens* when compared with those of the corresponding control snails (fresh = 84.6%, sun-dried = 84.6%, oven-dried = 85.7%) showed no statistically significant difference (χ^2 = 0.51, 1.85, and 2.21, respectively; df = 1, $P > 0.05$ for each category).

The percentage increase in the mean live-weight of *B. truncatus* specimens fed with fresh (70.0%) *L. sativa* was significantly the highest when compared with those fed with sun-dried (42.9%) and oven-dried (42.6%) *L. sativa* ($\chi^2 = 9.55$, df = 2, $P < 0.01$). Similarly, mean live-weight percentage increase of *B. truncatus* fed with fresh *T. procumbens* (46.4%) was significantly the highest when compared with those fed with sun-dried (14.6%) and oven-dried (3.7%) *T. procumbens* ($\chi^2 = 45.65$, df = 2, $P < 0.001$). The percentage increases in the mean live-weight of *B. truncatus* fed with fresh, sun-dried and oven-dried *T. procumbens* were significantly lower than those of the corresponding control snails ($\chi^2 = 4.78$, $P < 0.05$; $\chi^2 = 6.96$, $P < 0.01$; $\chi^2 = 16.34$, $P < 0.001$, respectively; df = 1 for each category).

The percentage increases in the mean shell length of *B. truncatus* fed with fresh (8.8%), sun-dried (5.4%) and oven-dried (1.2%) *L. sativa* were not significantly different ($\chi^2 = 5.65$, df = 2, $P > 0.05$). Likewise, the percentage increases in mean shell length of *B. truncatus* fed with fresh (5.9%), sun-dried (1.8%) and oven-dried (0.8%) *T. procumbens* leaves were not significantly different ($\chi^2 = 5.16$, df = 2, $P > 0.05$). In addition, comparison of feeding with fresh, sun-dried and oven-dried *L. sativa* (control) and *T. procumbens* (trial) showed no significant differences

Table 1. Survival, live-weight and shell length of *B. truncatus* fed with fresh, sun-dried and oven-dried *T. procumbens* leaves.

Week	Replicate	Fresh			Sun-dried			Oven-dried		
		No. (%) surviving	Mean live-weight (g)	Mean shell length (mm)	No. (%) surviving	Mean live-weight (g)	Mean shell length (mm)	No. (%) surviving	Mean live-weight (g)	Mean shell length (mm)
0	A	24 (100)	0.29	3.50	23 (100)	0.41	5.80	20 (100)	0.51	12.00
	B	26 (100)	0.30	3.40	23 (100)	0.42	5.60	23 (100)	0.54	11.90
	C	24 (100)	0.28	3.50	25 (100)	0.41	6.00	27 (100)	0.55	11.90
	Control	26 (100)	0.30	3.40	26 (100)	0.42	5.60	21 (100)	0.54	12.08
2	A	22 (91.7)	0.33	3.55	21 (91.3)	0.43	5.80	18 (90.0)	0.52	12.01
	B	24 (92.3)	0.34	3.45	21 (91.3)	0.44	5.63	21 (91.3)	0.55	11.93
	C	22 (91.7)	0.31	3.58	22 (88.0)	0.43	6.05	24 (88.9)	0.56	11.93
	Control	25 (96.2)	0.35	3.48	25 (96.2)	0.47	5.68	20 (95.2)	0.60	12.08
4	A	20 (83.3)	0.37	3.60	19 (82.6)	0.45	5.80	16 (80.0)	0.53	12.05
	B	22 (91.7)	0.37	3.50	19 (82.6)	0.46	5.65	19 (82.6)	0.55	11.95
	C	20 (83.3)	0.35	3.65	19 (76.0)	0.44	6.10	21 (77.8)	0.57	11.95
	Control	24 (92.3)	0.41	3.55	24 (92.3)	0.51	5.75	19 (90.5)	0.66	12.15
6	A	18 (75.0)	0.40	3.65	17 (73.9)	0.47	5.80	15 (75.0)	0.53	12.08
	B	21 (80.8)	0.41	3.55	18 (78.3)	0.47	5.68	18 (78.3)	0.56	11.98
	C	19 (79.2)	0.38	3.65	16 (64.0)	0.46	6.15	19 (70.4)	0.57	11.98
	Control	23 (88.5)	0.46	3.63	23 (88.5)	0.56	5.83	18 (85.7)	0.71	12.23
7	A	17 (70.8)	0.44	3.70	16 (69.6)	0.49	5.80	14 (70.0)	0.54	12.10
	B	20 (76.9)	0.44	3.60	17 (73.9)	0.49	5.70	15 (65.2)	0.56	12.00
	C	19 (79.2)	0.41	3.80	15 (60.0)	0.47	6.20	18 (66.7)	0.58	12.00
	Control	22 (84.6)	0.51	3.70	22 (84.6)	0.60	5.90	18 (85.7)	0.77	12.23

in mean shell length percentage increases of *B. truncatus* (χ^2 = 0.57, 1.80, 0.08, respectively; df = 1, P > 0.05 for each category). Table 2 summarises the survival, live-weight and shell length of *B. globosus* fed with fresh *T. procumbens* leaves. The mean percentage survival of *B. globosus* fed with fresh *T. procumbens* (79.2%) was not significantly different from that of control (84.6%) (χ^2 = 0.18, df = 1, P > 0.05). The percentage increase in mean live-weight of *B. globosus* fed with fresh *T. procumbens* (24.0%) was statistically lower as compared to the control (42.9%) (χ^2 = 5.34, df = 1, P < 0.05). However, the percentage increase in the mean shell length of *B. globosus* fed with fresh *T. procumbens* (2.35%) was not significantly different from that of the control (4.71%) (χ^2 = 0.79, df = 1, P > 0.05).

DISCUSSION

The results of this study show clearly that *Bulinus* spp. can be cultured in the laboratory using the free available *T. procumbens* instead of the

Table 2. Survival, live-weight and shell length of *B. globosus* fed with fresh *T. procumbens* leaves.

Week	Replicate	No. (%) surviving	Mean live-weight (g)	Mean shell length (mm)
0	A	25(100)	0.49	8.70
	B	25(100)	0.47	8.90
	C	25(100)	0.50	8.80
	D	26(100)	0.49	8.50
	Control	26(100)	0.49	8.50
2	A	24(96.0)	0.53	8.80
	B	23(92.0)	0.52	8.96
	C	23(92.0)	0.55	8.88
	D	24(92.3)	0.54	8.55
	Control	25(96.2)	0.54	8.60
4	A	23(92.0)	0.57	8.90
	B	21(84.0)	0.57	9.05
	C	21(84.0)	0.61	8.95
	D	22(84.6)	0.59	8.60
	Control	24(92.3)	0.60	8.70
6	A	22(88.0)	0.61	9.00
	B	20(80.0)	0.62	9.13
	C	20(80.0)	0.66	9.03
	D	21(80.8)	0.64	8.65
	Control	23(88.5)	0.65	8.80
7	A	21(84.0)	0.65	9.10
	B	19(76.0)	0.62	9.20
	C	19(76.0)	0.66	9.10
	D	21(80.8)	0.64	8.70
	Control	22(84.6)	0.70	8.90

expensive *L. sativa* with similar results in terms of percentage survival. This may give a sigh of relief for freshwater snails' researchers who may thereby expend their often meagre finance with higher frugality.

The observed laboratory survival of *B. truncatus* and *B. globosus* exclusively on *T. procumbens* leaves diet in this study is the first of its kind from Ijebu North area, Southwestern Nigeria. Literature shows, for instance, that the leaves of *T. procumbens* are consumed by *A. marginata* (Odaibo, 1997). Similarly, the leaves of this plant have earlier been found to better support *L. libycus*, in comparison with some other common dicotyledonous plants, in laboratory cultures (Agbolade et al., 2010). Survival of *B. truncatus* and *B. globosus* fed with *T. procumbens* corroborates the established nutritional values of the plant (Jude et al., 2009). In this study, *B. truncatus* seems to survive most on fresh *T. procumbens* leaves as opposed to *L. libycus* which prefers dried *T. procumbens* leaves. Although, in this study, *B. globosus* was fed only on fresh *T. procumbens* leaves; previously unreported observations suggest that fresh leaves of *T. procumbens* are likely to yield the best result on the survival of this species.

The percentage of shell growth shows that fresh *T. procumbens* leaves yielded relatively the highest increase as compared to dried ones, although the difference is not statistically significant. From the results of this study, it seems drying diminishes the dietary composition of *T. procumbens* leaves, particularly when done in the oven. The difference in the nutritional values of fresh *T. procumbens* leaves and dried ones seems magnified by the recorded percentage of increase in live-weight of *B. truncatus* when fed with fresh leaves. This seems to show that oven-dried leaves are poorer nutritionally. A previous study revealed depreciation of live-weight of *L. libycus* fed with dried *T. procumbens* (Agbolade et al., 2010). Like argued in an earlier paper, drying *T. procumbens* leaves might cause the destruction of many essential nutrients (such vitamins and proteins) needed by the water snails.

By and large, the results of this study have shown that laboratory culturing of *B. truncatus* and *B. globosus* using *T. procumbens* leaves may constitute a great forward step to water snails' researchers in Ijebu North, South-western Nigeria, using this free and easily available plant from the environment. However, for optimal performance the snails should be fed with fresh *T. procumbens*. Nevertheless, further studies (particularly parallel studies) are required to assess the reproductive potentials of *Bulinus* snails cultured using *T. procumbens* leaves.

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