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ORIGINAL ARTICLE

Parasites and Parasitic Diseases of Laboratory Animals in Plateau State Nigeria: The zoonotic implications

Olatunde B. Akanbi[1] · Shola David Ola-Fadunsin[2] · Sadiq Yahaya[3] · Rebecca Kaye[4] · Rebecca Shamaki[4]

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Abstract Parasitic agents in laboratory animals, are detrimental to the success of researches and can also infect personnel and researchers. This study is aimed at investigating the parasitic infections of laboratory animals maintained in animal houses of The National Veterinary Research Institute, Vom, Nigeria, as well as determining the zoonotic implications of these parasites. Two hundred and six laboratory animals (72 rabbits, 55 guinea pigs, 50 mice and 29 rats) were randomly sampled. Faecal samples and skin scrapings were collected and subjected to parasitological analyses. Pathological examinations were conducted on laboratory animals that had skin lesions. Sixteen different species comprising of 7 nematodes, 5 cestodes, 3 protozoans, and 1 mite were detected. *Eimeria* species (40/206; 19.42%; 95% CI = 14.44–25.25) was the most prevalent parasite, followed by *Syphacia muris* (26/206; 12.62%; 95% CI = 8.59–17.69). *Entamoeba caviae*, *Tritrichomonas caviae*, *Rodentolepis microstoma*, *Rodentolepis nana*, *Heterakis spumosa*, *Capillaria hepatica* and *Cysticercus fasciolaris* were the least prevalent with a 0.49% prevalence each. Three, four, five and six different species of parasites were detected in mice, guinea pigs, rats and rabbits respectively. The Chi-Square analysis revealed that the infection rate of parasites was significantly higher ($p = < 0.01$) in mice compared to rats, rabbits and guinea pigs. Of the Sixteen species of parasites detected, *Eimeria* species, *Syphacia muris*, *Rodentolepis diminuta*, *Rodentolepis microstoma*, *Rodentolepis nana*, and *Capillaria hepatica* are zoonotic. This study showed that 40.29% of the studied laboratory animals were infected with one parasite species or the other. The outcome of this study stresses the zoonotic implications of the parasites detected. We thereby advise researchers and handlers to take caution and apply utmost sanitary measures in the handling of laboratory animals so as to prevent themselves from being infected with these zoonotic parasites.

Keywords Laboratory animals · Nigeria · Parasites · Prevalence · Zoonosis

Introduction

Laboratory animals are animals used for biological studies in most academic and research institutions of the world. Laboratory animals include but are not limited to mice, rats, guinea pigs, rabbits, hamsters, and hare (Ademola and Ola-Fadunsin 2012). Laboratory animals have contributed significantly to the knowledge of biological structures and functions and are important tools in biological and medical researches, and training (Gudissa et al. 2011; Bassad et al. 2016). They are used for the diagnosis and studies of infective organisms, in the production of vaccines, sera and other biological substances of public health and veterinary importance, they are also used extensively in the safety evaluation of diverse therapeutic drugs, in comprehensive

✉ Shola David Ola-Fadunsin
olashodam2@yahoo.com

[1] Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Kwara State, Nigeria

[2] Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Kwara State, Nigeria

[3] Parasitology Unit, Central Diagnostic Laboratory, National Veterinary Research Institute, Vom, Plateau State, Nigeria

[4] Small Animal Experiment Unit, National Veterinary Research Institute, Vom, Plateau State, Nigeria

varieties of biological investigations and foods chemicals (Clark et al. 1997; Bassad et al. 2016).

There is a need for healthy laboratory animals, that are infection-free so that their purpose for research is not influenced by the infection(s) (Bicalho et al. 2007; Tanideh et al. 2010). Laboratory animals can become heavily parasitized both externally and internally, with a variety of organisms ranging from parasites, viruses, fungi, bacteria, and mycoplasmas. Infections and infestations with these organisms lead to loss of time, money, loss of quality in the affected laboratory animals and research effort (Griffiths 1971; Ademola and Ola-Fadunsin 2012; Bassad et al. 2016; Dolatkhah et al. 2017).

Parasites are of great concern to the wellbeing and use of laboratory animals, among other infectious organisms. They become a prime target for parasitic infections if appropriate management and preventive measures are not practised (Tanideh et al. 2010; Gudissa et al. 2011). Besides the high mortality caused by parasites in young animals, parasitic infections can also complicate research by inducing physiological, haematological, biochemical, pathological, and immunological alterations in the hosts, exaggerating or diminishing host susceptibility to experimental stress, inducing tissue damages, stimulating abnormal growth of tissues, competing with the host for nutrients, decreasing the volume of host's blood and body fluids and by mechanical interference (Aboel-Hadid and Gamal 2007; Dolatkhah et al. 2017). A good number of parasites that affects laboratory animals are zoonotic in nature, and these include *Aspicularia tetraptera, Eimeria* species, *Encephalitozoon cuniculi, Giardia* species, *Hymenolepis* (now *Rodentolepis*) *nana, R. diminuta, Physaloptera* species, *Polyplax* species, *Schistosoma* species, *Syphacia muris, S. obvelata, Taenia* species, *Trichomonas* species, lice, and mites, etc. (Huq et al. 1985; Tanideh et al. 2010; Taylor et al. 2016; Dolatkhah et al. 2017).

In view of the considerable adverse effects of parasitic infections on the health status and research usefulness of laboratory animals, and the possible transmission of zoonotic parasitic infections between laboratory animals and personnel or researchers, this study is therefore aimed to investigate the parasitic infections of laboratory animals maintained conventionally in the small animal houses of the National Veterinary Research Institute, Vom, Plateau State Nigeria, as well determining the zoonotic implications of these parasites.

Materials and methods

Study location

This study was conducted at the National Veterinary Research Institute (NVRI), Nigeria. The National Veterinary Research Institute, is located in Vom, Jos South Local Government Area of Plateau State. Plateau State covers a land mass of 27,147 square kilometers and is one of the largest states in Nigeria, and is almost centrally located between latitude 80° 24'N, and longitude 80° 32' and 100° 38' east of the Greenwich meridian (Fig. 1). The state has a high altitude ranging from approximately 1,200 to a peak of 1,829 m above sea level. Plateau State has an almost temperate climate with a mean annual rainfall of between 131.75 cm to 146 cm and a mean annual temperature of 16.3 °C to 28.1 °C. It records an average relative humidity of between 46.9% and 51.3% (Bolajoko et al. 2016; Agida et al. 2017; Karaye et al. 2018).

Study animals

A total of 206 laboratory animals comprising of 72 rabbits, 55 guinea pigs, 50 mice, and 29 rats were randomly sampled for this study. The laboratory animals were housed in groups, and they were sampled from the different laboratory animal houses in the institute.

Collection of samples

Freshly passed faecal samples were collected from each group of laboratory animals into clean and sterile sample bottles. Each laboratory animal was carefully and thoroughly examined for the presence of ectoparasite(s), and skin scrapings were taken from laboratory animals that had skin lesions. The collected samples were immediately transported to the parasitology unit of the Central Diagnostic Laboratory of the NVRI, Vom, Plateau State, for further parasitological and pathological analyses.

Parasitological processing

Faecal samples were analysed using the direct faecal smear and the simple flotation techniques. The direct faecal smear technique was carried out as described by Soulsby (1982). Briefly, faeces was emulsified in 1 or 2 drops of normal saline on a clean glass microscopic slide with an applicator. Afterwards, a drop of iodine was added to the mixture, then a coverslip was carefully placed over the suspension, ensuring that it is thin, uniform and clear. It was then examined under the microscope using the X10 or X40 objective of the microscope. The simple flotation technique

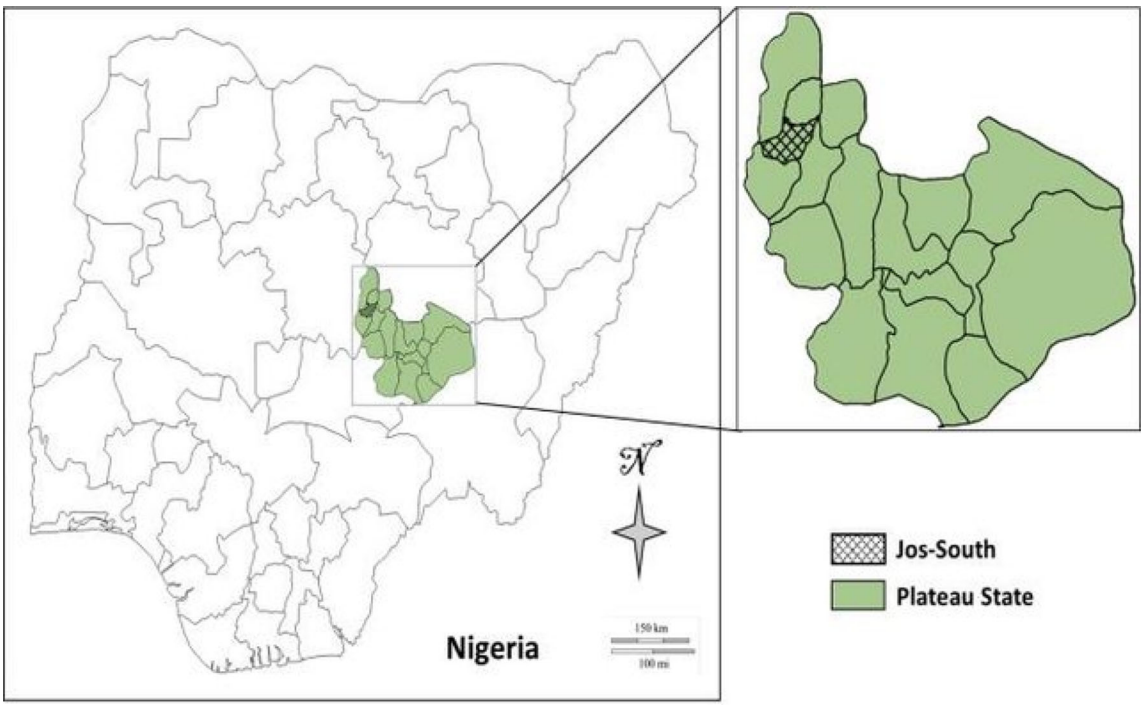

Fig. 1 Map of Nigeria showing the location of Plateau State. Insert map shows the location of Jos South Local Government Area in Plateau State (study location) (Agida et al. 2017)

was carried out as described by Taylor et al. (2016). Briefly, faecal samples were placed in universal bottles and mixed with little quantity of saturated sodium chloride (NaCl) solution. This mixture was then sieved into a test tube. Afterwards, the filtrate was filled to the brim (forming a meniscus) with more of the saturated NaCl solution and a clean coverslip was gently placed on top of the test tube whereby avoiding spillage. The coverslip was left for about 20 min; afterwards, the coverslip (having the harvested eggs) was lifted vertically from the test tube and placed on a clean glass slide for microscopic examination, using the X10 or X40 objective of the microscope.

Skin scrapings were analysed by dissolving it in 10% KOH, then it was viewed using the X10 or X40 objective of the microscope. Parasitological keys by Kassai (1999), Charles and Hendrix (2006), and Zajac and Conboy (2012) were used in the identification of the helminths, protozoans and mites detected in this study.

Gross and histopathological examination

Rats that had skin lesions were taken to the pathology unit of the Central Diagnostic Laboratory, NVRI Vom. The rats were humanely euthanized according to the guidelines of the Animal Welfare and Ethics Committee of the National Veterinary Research Institute, Vom, Nigeria. Skin, liver, and intestines with gross lesions were fixed in buffered formalin. Tissue cuts of 0.5 × 0.5 cm in diameter from the tissues were made with a scalpel blade, after which they were placed in an automatic tissue processor for processing, dehydrated in ethanol (70–100%), cleared in xylene, and embedded in paraffin. Five (5) -µm paraffin-wax sections of organs were dewaxed and stained with hematoxylin–eosin (H&E), mounted on charged microscope slides, and observed under a Carl Zeiss light microscope for histopathological changes as previously described by Kamani et al. (2013) or under a low and high-powered field of Carl Zeiss® Axio Imager A1 binocular microscope, and IC-3 mounted camera was used for photographing the microscopic lesion as described by Akanbi (2020).

Statistical analysis

Data were initially entered in Microsoft Excel version 2019 for the determination of prevalence (%) with their corresponding 95% confidence intervals (CI). The Statistical Package for Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, Illinois) was used for the statistical evaluation. Chi-Square (χ^2) test for discrete variables at 95% CI was used to determine the association between each

parasitic infection in relation to the different laboratory animals. Statistical significance was set at $p < 0.05$.

Results

Of the 206 laboratory animals sampled, 83 were infected with one parasite or the other (Fig. 2), representing 40.29% of the sampled population with a 95% CI of 33.75–47.10. In total, 16 parasites consisting of nematodes (7), cestodes (5), protozoans (3), and mites (1) were detected. *Eimeria* species (40/206; 19.42%; 95% CI = 14.44–25.25) was the most prevalent parasite species followed by *Syphacia muris* (26/206; 12.62%; 95% CI = 8.59–17.69). *Entamoeba caviae*, *Tritrichomonas caviae*, *Rodentolepis microstoma*, *Rodentolepis nana*, *Heterakis spumosa*, *Capillaria hepatica,* and *Cysticercus fasciolaris* were the least in occurrence with a 0.49% prevalence each (Fig. 3). *Eimeria* species and *Trichostrongylus retortaeformis* were the only parasites detected in two of the four laboratory animals studied.

Six different parasites species were detected in rabbits, with *Eimeria* species being the most prevalent (30.55%), while others recorded a prevalence of (2.78%) each (Table 1). In guinea pigs, 4 parasites were detected, with *Eimeria* species (32.73%; 95% CI = 21.34–45.89) being the most prevalent, while *Entamoeba caviae* and *Tritrichomonas caviae* were the least prevalent with (1.81%; 95% CI = 0.09–8.64) each (Table 2). *Syphacia muris* (26/50; 52.00%) was the most prevalent parasite amongst mice

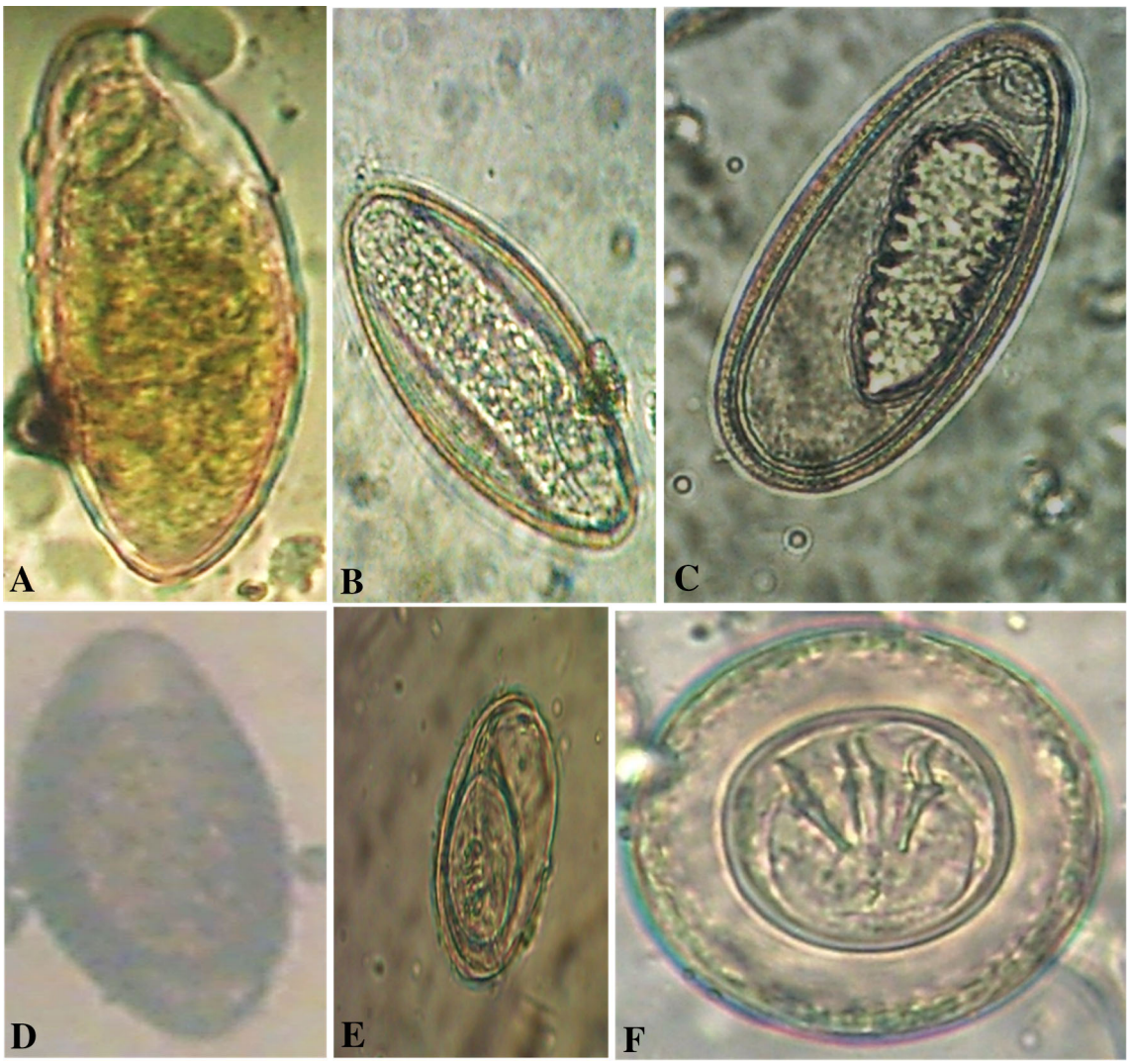

Fig. 2 Some parasites found in laboratory animals in Plateau State, Nigeria. A = *Trichostrongylus retortaeformis*; B = *Syphacia muris*; C = *Graphidium strigosum*; D = *Aspicularis tetraptera*; E = *Passalurus ambiguus* and F = *Rodentolepis diminuta* (X400 magnification)

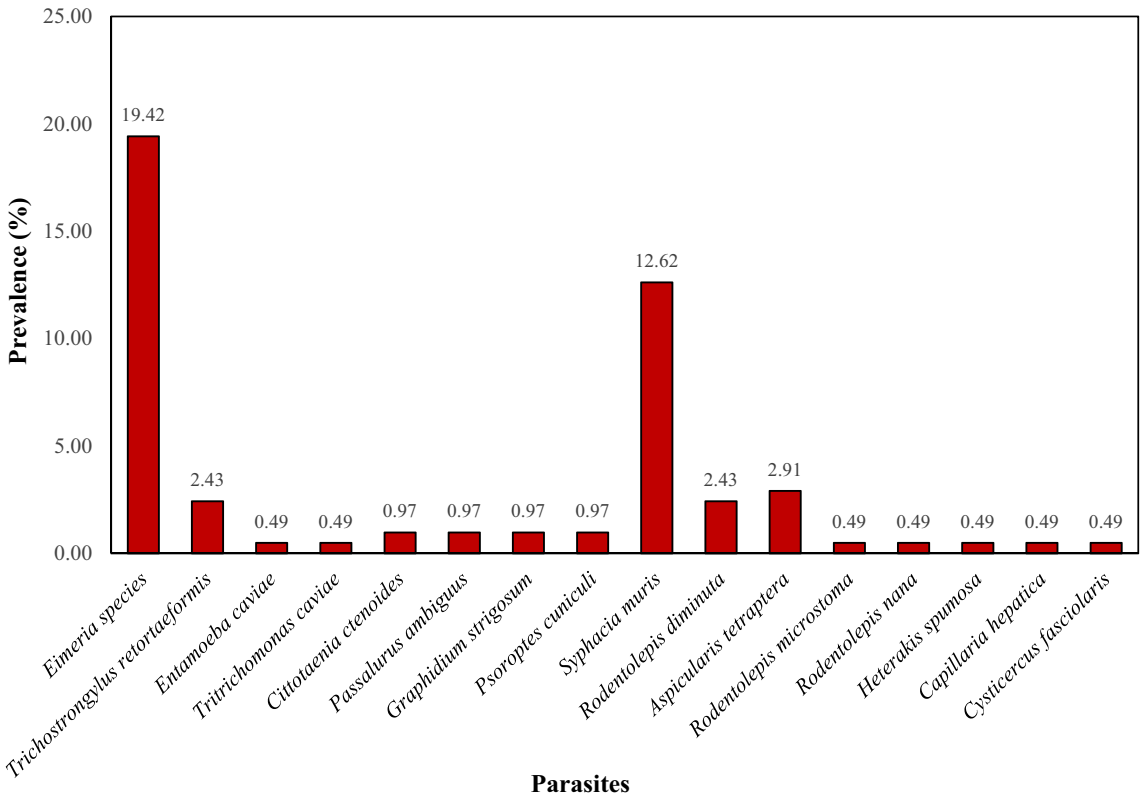

Fig. 3 Overall prevalence of parasites affecting laboratory animals (rabbits, guinea pigs, mice and rats) in Plateau State, Nigeria (n = 206)

Table 1 Prevalence of parasites affecting rabbits in Plateau State, Nigeria (n = 72)

Parasites	N	Prevalence (%)	95% CI
Eimeria species	22	30.55	20.76 – 41.89
Trichostrongylus retortaeformis	2	2.78	0.47 – 8.88
Cittotaenia ctenoides	2	2.78	0.47 – 8.88
Passalurus ambiguus	2	2.78	0.47 – 8.88
Graphidium strigosum	2	2.78	0.47 – 8.88
Psoroptes cuniculi	2	2.78	0.47 – 8.88

N = number of laboratory animals infected, CI = Confidence interval

Table 2 Prevalence of parasites infecting guinea pigs in Plateau State, Nigeria (n = 55)

Parasites	N	Prevalence (%)	95% CI
Eimeria species	18	32.73	21.34 – 45.89
Trichostrongylus retortaeformis	3	5.45	1.41 – 14.13
Entamoeba caviae	1	1.81	0.09 – 8.64
Tritrichomonas caviae	1	1.81	0.09 – 8.64

N = number of laboratory animals infected, CI = Confidence interval

followed by *Aspicularis tetraptera* (6/50; 12.00%), with *Rodentolepis diminuta* (5/50; 10.00%) being the least prevalent (Table 3). All the five parasites detected in rats had a single occurrence with a prevalence of 3.45% (95% CI = 0.17–15.85) (Table 4). The Chi-Square analysis showed that the infection rate of parasites was significantly higher ($p = < 0.01$) in mice (68.00%) compared to rats (10.34%), rabbits (36.11%), and guinea pigs (36.36%) (Table 5).

Grossly, rough skin coat, presence of parasitic vacuole, and rough intestinal mucosa were seen in two rats. Microscopic examination of skin, liver, and intestines revealed histopathological lesions. Within the liver, there was a single parasitic vacuole (cyst) on the right middle lobe (Fig. 4a). The liver section showed the presence of a parasitic cestode larvae in a cyst that resembles *Cysticercus fasciolaris* without any tissue reaction around the cyst (Fig. 4b). In another rat, the liver section contained several multifocal parasitic vacuoles with numerous *Capillaria hepatica* and two other vacuoles with intact cysts of *Cysticercus fasciolaris* (Fig. 4c), with severe diffuse chronic hepatitis and infiltration by mononuclear cells. Within the subcutaneous muscles, was a single parasite in the muscle of *Capillaria hepatica* infected rat (Fig. 4d).

Table 3 Prevalence of parasites infecting mice in Plateau State, Nigeria (n = 50)

Parasites	N	Prevalence (%)	95% CI
Syphacia muris	26	52.00	38.24 – 65.54
Rodentolepis diminuta	5	10.00	3.76 – 20.78
Aspicularis tetraptera	6	12.00	5.01 – 23.29

N = number of laboratory animals infected, CI = Confidence interval

Table 4 Prevalence of parasites infecting rats in Plateau State, Nigeria (n = 29)

Parasites	N	Prevalence (%)	95% CI
Rodentolepis microstoma	1	3.45	0.17 – 15.85
Rodentolepis nana	1	3.45	0.17 – 15.85
Heterakis spumosa	1	3.45	0.17 – 15.85
Capillaria hepatica	1	3.45	0.17 – 15.85
Cysticercus fasciolaris	1	3.45	0.17 – 15.85

N = number of laboratory animals infected, CI = Confidence interval

Table 5 Chi-Square analysis on parasites infection rate of different laboratory animals in Plateau State, Nigeria

Laboratory animals	n	Number positive (%)	χ^2	DF	p-value
Rabbits	72	26 (36.11)	27.64	3	$< 0.01^{\#}$
Guinea pigs	55	20 (36.36)			
Mice	50	34 (68.00)			
Rats	29	3 (10.34)			

n = Number of laboratory animals sampled

χ^2 = Chi-Square value

DF = Degrees of Freedom

$^{\#}$ = Significant at $p < 0.05$

Discussion

Of the Sixteen parasites species detected, *Eimeria* species, *Syphacia muris*, *Rodentolepis diminuta* (formerly *Hymenolepis diminuta*), *Rodentolepis microstoma* (formerly *Hymenolepis microstoma*), *Rodentolepis nana* (formerly *Hymenolepis nana*), and *Capillaria hepatica* are zoonotic (Tanideh et al. 2010; Taylor et al. 2016; Dolatkhah et al. 2017), thus stressing the need for researchers and handlers to take caution and apply utmost sanitary measures to prevent themselves from being infected.

The total prevalence of 40.29% observed in this study is lower than the 56.48% observed among laboratory animals in Ibadan, Nigeria (Ademola and Ola-Fadunsin 2012) and the 66.0% reported in Nasiriyah, Iraq (Bassad et al. 2016). Our reported prevalence is higher than the prevalence (37.62%) documented among laboratory animals in Addis Ababa, Ethiopia (Gudissa et al. 2011). These reports suggest that parasitic infections among laboratory animals is of cosmopolitan concern.

We reported sixteen different species, affecting laboratory animals in the study area. This number is higher than the five different species reported by Najafi et al. (2014) in Tehran, Iran, and the six reported by Tanideh et al. (2010) in Shiraz, Iran. Despite the fact that we detected a higher number than those documented in studies done in Iran, ours was lower than the seventeen different species reported among laboratory animals in Brazil (Gilioli et al. 2000). The disparity in the number of parasites affecting laboratory animals in these studies may be attributed to managemental, environmental and climatic factors. *Eimeria* species was the most prevalent parasite in this study. It was also the most prevalent parasite among guinea pigs and rabbits. Similarly, noticeable prevalence of *Eimeria* species has been reported among laboratory animals in Nigeria and other parts of the world (Gilioli et al. 2000; Gudissa et al. 2011; Ademola and Ola-Fadunsin 2012). This is not surprising as *Eimeria* species is believed to be ubiquitous in its distribution, being present wherever animals are raised (Ola-Fadunsin and Ademola 2013).

Syphacia muris was the most prevalent parasite found in mice. *Syphacia* species are known to infect mice and rats, and it has been documented to be the most prevalent helminth among mice and rats in previous studies (Gilioli et al. 2000; Aboel-Hadid and Gamal 2007; Tanideh et al. 2010). The high prevalence of *Syphacia muris* recorded among mice may be attributed to the fecundity of the parasite. *Syphacia muris* is a pinworm, and a gravid female can lay up to 50,000 eggs (Taylor et al. 2016).

Mice were most infected with parasites compared to rats, rabbits, and guinea pigs. This observation is in tandem with reports by Gudissa et al. (2011) and Najafi et al. (2014) who documented a higher prevalence of parasitism in mice compared to rats. Although, Hayunga (1991) documented a contrary report to our findings. The high prevalence of parasitism we recorded in mice may be attributed to the stocking density and husbandry practices of mice.

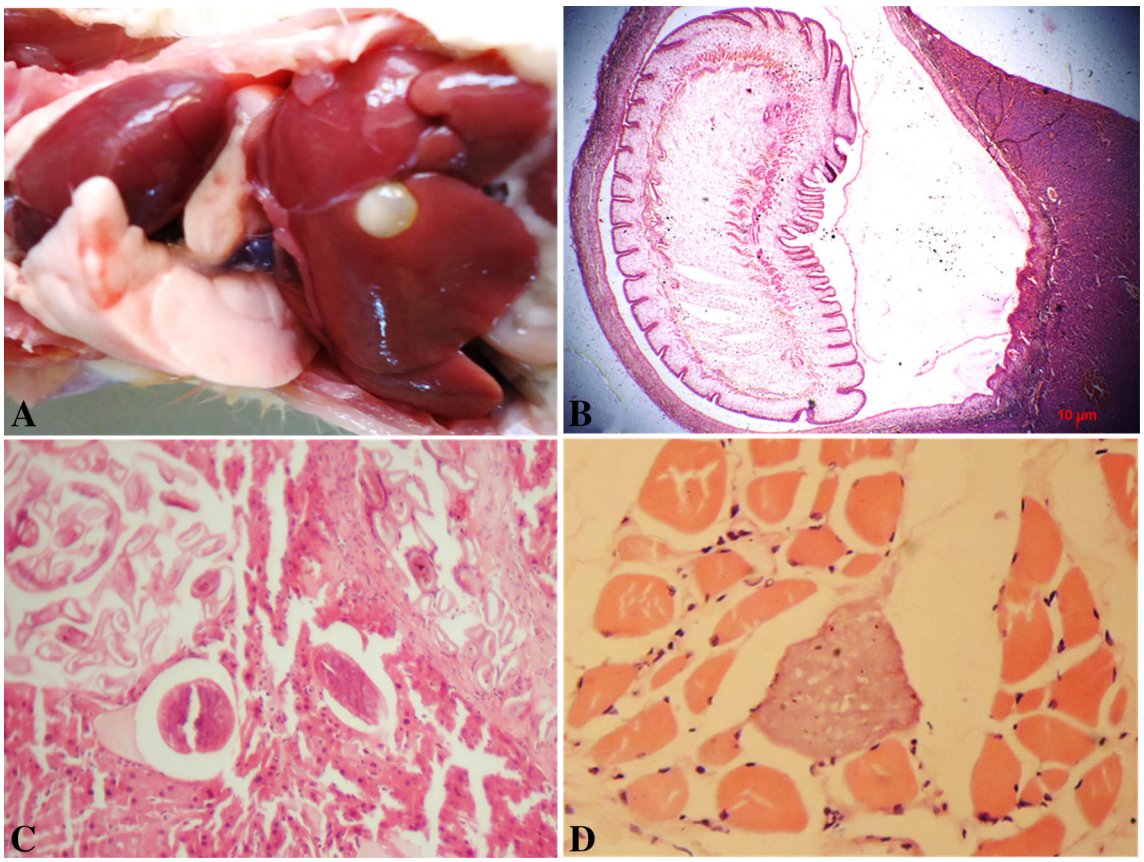

Fig. 4 Gross and histopathology of parasites in rats; 4a, the liver has a single parasitic vacuole (cyst of *Cysticercus fasciolaris*) on the right middle lobe. 4b, the liver section showed the presence of a parasitic cestode larvae in a cyst that resembles *Cysticercus fasciolaris*. 4c, the liver section contained several multifocal parasitic vacuoles with numerous *Capillaria hepatica* and two other vacuoles with unhatched cysts. 4d, the subcutaneous muscles contained a single parasite in the muscle identified as *Rodentolepis microstoma* same rat as 4c

Conclusion

About 41.00% of the sampled laboratory animals were infected with one parasite or the other. Sixteen different species of parasites were detected among the sampled laboratory animals, and these parasites cut across being nematodes, cestodes, protozoans, and mite, with nematodes being the most predominant. Mice were most infected with parasites compared to rats, rabbits, and guinea pigs. Of the sixteen parasites detected, six (*Eimeria* species, *Syphacia muris*, *Rodentolepis diminuta*, *Rodentolepis microstoma*, *Rodentolepis nana,* and *Capillaria hepatica*) are zoonotic. This outcome stresses the zoonotic implications of the parasites detected in our study. We thereby advise researchers and handlers to take caution and apply utmost sanitary measures in the handling of laboratory animals so as to prevent themselves from being infected with these zoonotic parasites.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This study was approved by the Animal Welfare and Ethics Committee of the National Veterinary Research Institute, Vom, Nigeria. All applicable international, national, and/or institutional guidelines for the collection of faecal samples and skin scrapings from laboratory animals were appropriately followed.

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