Scientific African 8 (2020) e00469

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Prevalence of bovine fasciolosis from the Bolgatanga abattoir, Ghana

Francis Addy[a,*], Kwame Gyan[a], Enoch Arhin[a], Marion Wassermann[b]

[a] Department of Biotechnology, Faculty of Agriculture, University for Development Studies, P. O. Box TL 1882, Tamale, Ghana
[b] Parasitology Unit, Institute of Zoology, University of Hohenheim, Emil-Wolff-Str. 34 70599 Stuttgart, Germany

ARTICLE INFO

Keywords:
Fasciola
Fasciolosis
Prevalence
Cattle
Bolgatanga
Ghana

ABSTRACT

Fasciolosis is a neglected tropical foodborne trematodiasis caused by *Fasciola hepatica* and *Fasciola gigantica*. It is a widely distributed infection in livestock in Africa but its disease situation is less understood in many countries such as Ghana. In the present study, a cross-sectional survey was conducted at the Bolgatanga abattoir, in the Upper East Region of Ghana, to determine the prevalence and distribution of bovine fasciolosis and the *Fasciola* species involved. A total of 263 cattle were screened at slaughter and isolated *Fasciola* flukes were molecularly identified to species level by PCR-RFLP of the 28SrRNA gene using Ava II endonuclease. Fasciolosis prevalence was 10.27% across all age categories, and female and male animals were affected alike. *Fasciola* species differentiation revealed *Fasciola gigantica* in all cases. This study confirms the occurrence of *F. gigantica* and its predominance in Ghana Upper East Region and provides basal data for further investigations into the prevalence of *Fasciola* spp. in other parts of Ghana and neighbouring countries.

Introduction

Fasciolosis is a cosmopolitan foodborne parasitic disease in humans and animals. It is one of the seven high priority food/water borne trematodiases classified by WHO. It is estimated that 2.4 – 17 million people are affected globally [1] and the disease causes about 90,000 DALYs per year [2]. In livestock, its impact is mainly due to loss in meat and milk production, fertility, and draught power of affected animals [3]. Acute fasciolosis outbreaks in sheep with considerable morbidity and mortality are also known [4]. Reliable figures about the annual financial losses due to liver fluke infection in livestock are not available, but will surely be in the double- or triple-digit billion each year making fasciolosis a major socio-economic problem [5].

Fasciolosis is caused by the liver flukes *Fasciola hepatica* and *Fasciola gigantica* that are transmitted between mammalian hosts such as buffalo, cattle, sheep and other wild ruminants (or humans) as definitive hosts, and freshwater lymnaeid snails as intermediate hosts. In definite hosts, adult flukes reside in the bile ducts and the eggs are released into the environment through host faeces. Provided they come into contact with water, the unembryonated eggs develop further into miracidia,

* Corresponding author.
E-mail address: faddy@uds.edu.gh (F. Addy).

https://doi.org/10.1016/j.sciaf.2020.e00469

which hatch and infect aquatic snail as intermediate hosts. In the snail host the parasite eventually develops into cercariae before exiting to encyst on aquatic vegetation to form metacercariae. The cycle is complete when suitable definitive host ingest the infectious metacercariae on contaminated food substances or in water.

Fasciola spp. have a complex reproduction system: adult flukes are hermaphrodites and reproduce sexually by self-fertilization or cross-fertilization with other individuals in same vicinity in the bile duct of definitive host, whereas in the intermediate host, the trematode multiplies asexually. Health complications of fasciolosis arise not only from the migratory acute phase but also from obstruction of the bile duct caused by fluke development, crowding and cholangitis [6] which may result in severe morbidity and mortality in high endemic regions. *Fasciola hepatica* is widely distributed in the temperate regions, thus Europe, America and Australia, whereas *F. gigantica* is commonly found in the tropics and sub-tropics in Asia and Africa. The two species overlap in occurrence in some countries in Africa such as in Egypt and Zimbabwe where conditions enable their sympatric transmissions [14,17,20]. Fasciolosis is widely distributed in Africa, with varied prevalence values across endemic countries and within a country. In many parts of Africa, the disease situation of fasciolosis is not well understood.

In western Africa, accounts of livestock fasciolosis are available from almost all countries but several of these accounts could not conclusively determine the *Fasciola* spp. involved. In Ghana for instance, earlier reports indicate *Fasciola hepatica* or simply *Fasciola* spp. [7,8] but molecular characterisation of Ghanaian isolates by McGarry et al. [9] and Addy et al. [10] indicate the presence of *F. gigantica.* Our previous work was based on an opportunistic samples from Bolgatanga and Wa slaughterhouses in Ghana Upper East and Upper West Regions, respectively, in which the extent of endemicity of fasciolosis in the locality could not be determined [10]. The present study was therefore undertaken to determine the fasciolosis situation in terms of prevalence, distribution and identification of the *Fasciola* species in cattle in Ghana's Upper East Region.

Materials and methods

Study area

In 2018, a survey was conducted from September to December at the Bolgatanga slaughterhouse to investigate prevalence of fasciolosis in cattle. Bolgatanga is the capital town of the Upper East Region (top right-corner of Ghana) and the Bolgatanga Municipality. The slaughterhouse is the major one in the Region and animals brought for slaughter originate from various localities and neighbouring Burkina Faso. During the survey, animals slaughtered were said to have come from communities in and around Bongo (74), Bolgatanga (33), Garu (18), Navrongo (61), Paga (9), Tongo (44), Zebilla (11) and the border region of the neighbouring Burkina Faso (13).

Sample collection

Data on age (estimation based on dentition), sex and origin (according to middlemen present) of cattle were taken before slaughter. After slaughter, carcasses were traced to inspect the liver for flukes. Meat inspection was conducted as routine practice by veterinary staff manning the slaughterhouse. Liver was inspected visually accompanied by 2–3 incisions to reveal major bile ducts and then squeezed to spill flukes. A total of 263 livers were inspected and isolated flukes were individually preserved in 70% ethanol and subsequently transported to the Faculty of Agriculture Laboratory Complex (Nyankpala campus) of the University for Development Studies, Tamale, for molecular analysis.

Polymerase chain reaction of 28S rRNA gene

DNA of ethanol fixed *Fasciola* isolates was obtained by lysing in NaOH as described by Addy et al. [10]. In brief, small tissue pieces from the extreme posterior of adult flukes were lysed in 30 µl 0.03 M NaOH at 99 °C for 30 min. Supernatant of lysate was used directly as DNA template in the polymerase chain reaction.

Polymerase chain reaction (PCR) was conducted in a 25 µl reaction mixture consisting of 10 pmol of each primer, 20 mM Tris–HCl (pH 8.9 @ 25 °C), 22 mM NH_4Cl, 22 mM KCl, 1.8 mM $MgCl_2$, 0.2 mM dNTPs, 5% Glycerol, 0.06% IGEPAL® CA-630, 0.05% Tween® 20, 0.3125 U OneTaq® DNA Polymerase (New England BioLabs Inc) and 2 µl of crude lysate. The 28S rRNA gene (618 bp) was amplified using the primer pairs forward 5′-ACGTGATTACCCGCTGAACT-3′ and reverse 5′-CTGAGAAAGTGCACTGACAAG-3 [11]'. Reaction was cycled under the following thermal conditions: initial denaturation at 95 °C for 5 min. followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and elongation at 72 °C for 60 s, followed by final elongation at 72 °C for 5 min. Amplicons were viewed on 1.5% agarose gel stained with ethidium bromide.

Restriction fragment length polymorphism of 28S rRNA

The 28S rRNA gene amplicons were digested with Ava II endonuclease (New England BioLabs Inc.) following the manufacturer's protocol. Digestion was carried out in 50 μl reaction mixture that composed of 4 μl PCR amplicon, 5 μl buffer (provided with the enzyme), 1 μl Ava II endonuclease and 40 μl nuclease-free H_2O. Mixture was incubated at 37 °C for 3 h and enzyme inactivated at 80 °C for 20 min. Resultant restricted fragments were separated on 2% agarose gel stained with Ethidium bromide.

Table 1
Prevalence of fasciolosis in cattle, Chi-square and Odds Ratio of infection between male and female and young and old animals.

		n	Prevalence (%)	Odds Ratio (OR)	Chi-square (x^2)	p-value
Sex	Male	127	12/127 (9.45)	0.84	0.18	0.67
	Female	136	15/136 (11.03)			
Age category	Young animals (1–3 years)	57	8/57 (14.04)	0.62	1.12	0.29
	Old animals (≥4 years)	206	19/206 (9.22)			
	Total	263	27/263 (10.27)			

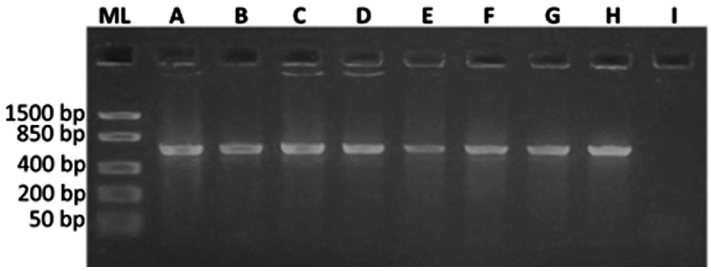

Fig. 1. Polymerase chain reaction (PCR) amplification of the 28S rRNA (618 bp) of *Fasciola* isolates. Lane ML: FastRuler® low range molecular ladder, lane A-F: representative *Fasciola* isolates from the present study, lane G & H: reference *Fasciola hepatica* and *Fasciola gigantica* isolates respectively as positive controls, lane I: negative control (Nuclease free water).

Data analysis

Statistical analysis was carried out using IBM SPSS statistics 20. Fasciolosis prevalence was determined as number of individual cattle infected with fluke per total number of animals screened. Chi-square test (x^2) and Odds Ratio of male to female exposure/risk to fasciolosis infection were also determined.

Results

Prevalence of bovine fasciolosis

A total of 263 cattle aged one year and above were inspected at necropsy for liver fluke infection (fasciolosis), out of which 27 animals were found harbouring the trematode, representing a prevalence of 10.27% (27/263) (Table 1). Females and male animals were infected alike ($x^2 = 0.18$, $p = 0.67$), as well as old and young animals ($x^2 = 1.12$, $p = 0.29$). On a whole, fasciolosis infected cattle harboured 1 – 15 flukes, out of which older animals harboured on average, twice as much as younger infected animals (1.88 vs. 4.26). No difference in prevalence was observed amongst the different communities where the animals came from and none of the animals (13) that came from border region of neighbouring Burkina Faso was found infected.

Identity of fasciola isolates

Using the *Fasciola* genus primer pairs indicated in section 2.3, the 28S rRNA gene (618 bp) of 60 fluke specimens subjected to PCR were successfully amplified (Fig. 1). Upon digestion of the PCR amplicons with Ava II endonuclease, all isolates were identified as *F. gigantica* (Fig. 2). The digestion produced identifiable double banding pattern of 322 bp and 269 bp in length as was the case of the referenced *F. gigantica.* The referenced *F. hepatica* also showed the characteristic 529 bp band after the digestion. For both species used, the 27 bp of *F. hepatica* and *F. gigantica* was hardly visible, likewise the 62 bp of *F. hepatica,* but these shorter fragments are not required for species differentiation [11].

Discussion

Fasciolosis is endemic in whole of Africa but understanding of the disease situation is incomplete since data is available from only few countries. Our current understanding of fasciolosis on the continent is largely based on studies from northern and southern Africa [13–24], meanwhile isolated accounts from eastern, central and western Africa show the trematodiasis to be an important food-borne parasitic disease in these regions too [25–27]. Three recent studies from southern Ghana reported fasciolosis prevalence of 2.0% in humans and up to 51.1% in cattle [7,8,28]. Unfortunately, the causative species were not determined in these studies. The only molecularly identified Ghanaian *Fasciola* specimens were not part of an epidemiological study investigating the prevalence. McGarry et al. [9] used Ghanaian samples as positive control for diagnostic methods and Addy et al. [10] examined 19 fluke isolates taken by opportunistic means to confirm the presence of *Fasciola*

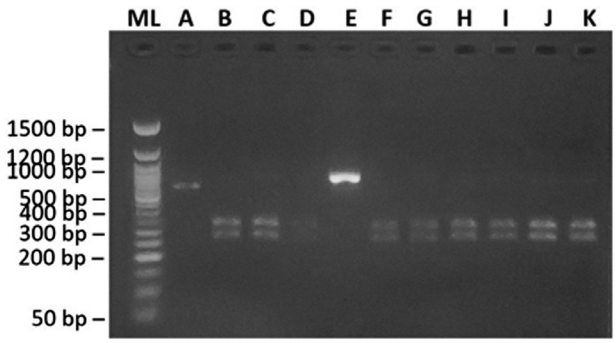

Fig. 2. Restricted fragment length polymorphism (RFLP) of 618 bp long 28S rRNA gene amplicons after digestion with Ava II endonuclease. Lane ML: 50 bp DNA ladder RTU (GeneDirex Inc, UK), lane A and B: amplicons of previously characterized *F. hepatica* and *F. gigantica* as positive controls [10,12], lanes C, D, F - K: *Fasciola* isolates from the present study, lane E: control (undigested PCR amplicon).

sp., identified the species and analysed the genetic variability. In both studies the isolates were characterized to be *Fasciola gigantica* [9,10]. The current work is based on the previous study and combines the identification of the causative species and the determination of the prevalence in the Upper East Region in northern Ghana.

In the present study a prevalence of bovine fasciolosis of 10.27% could be detected. This survey confirms our previous data [10] and has shown fasciolosis to be endemic in Ghana's Upper East Region. In the 1960s, Odei reported 8.60% in cattle from a slaughterhouse survey in Wa and other localities in the then Upper Region of Ghana [29]. Over almost five decades (Odei [29] and present study), fasciolosis is still persistent in northern Ghana. We attribute this persistence to undisturbed transmission systems where there are several dams for dry season vegetable cultivation, animal rearing and household usage [30,31]. As at 2008, the Upper East Region had 278 small dams and dugouts [31] which may serve as sources/sites where livestock can get infected. Mollusk control exercise such as the use of flamethrower will be needed around dams, dugouts and other standing water sources to disrupt the life cycle and curb the transmission of fasciolosis. None of the 13 animals from neighbouring Burkina Faso included here had fasciolosis but the number of animals is too small to generalize. Since Burkina Faso is endemic to fasciolosis [21], it will be beneficial to examine more of such animals influx from its border regions to Ghana to know how much of the trematode is introduced.

Bovine fasciolosis incidence is known across Ghana but prevalence values vary from place to place. Prevalence in the present study (northern Ghana) is far below what was reported from the Greater Accra Region of Ghana (southern Ghana) that could reach 51% [8]. Factors responsible for this difference in prevalence could not be readily deduced. The fundamental differences between the two studies were postmortem liver inspection vs. coprological examination [8], dry season vs. wet season [8] and guinea savannah vs. coastal savannah [8]. How these differences may impact the difference in prevalence records needs further investigation. The present prevalence record of 10.27% is however likely to underestimate the trematode infection in cattle since only normal meat inspection procedures were followed. As reported by Phiri et al. [32], detail (purposive) inspection of liver for flukes may reveal higher fasciolosis infection than would be recorded under regular meat inspection procedure. Elsewhere, cattle management systems, land cover and climatic factors are known to have effects on trematodiasis infection [33,34]. It will be necessary to study the distribution of fasciolosis in the different ecological zones following its endemicity from north to south of Ghana to map out the disease foci and transmission hotspots for effective controls strategies.

All isolates characterized by RFLP in the present study reflect *F. gigantica* infections. This is a confirmation of our initial report of the tropical *Fasciola* species in Ghana's northern regions [10]. West Africa generally is thought to be endemic mainly to *F. gigantica*, as such its predominance in Ghana so far is not surprising [9,10]. Meanwhile earlier accounts of the trematode in Ghana were based on examination of faecal matter of hosts where authors reported it as *Fasciola* spp. [8,28] or to be *F. hepatica* [7]. It is very likely that these accounts of *Fasciola* were in fact *F. gigantica* due to the reported absence of *Galba tranculata* snail (intermediate host for *F. hepatica*) in West Africa [1]. Yet, the new account of *F. gigantica* and *F. hepatica* co-occurrence in Nigeria reveals aspect of the parasites distribution in the subregion that was previously unknown [35]. Molecular characterisation of more isolates from different endemic areas is therefore needed to verify whether *Fasciola gigantica* is really the only liver fluke species in Ghana and to determine its distribution and prevalence.

The present study has confirmed the persistence of fasciolosis in Ghana's Upper East Region caused by *Fasciola gigantica.* This certainly shed some light on the disease situation in livestock in northern Ghana but comprehensive understanding of the situation of fasciolosis will need data on other aspects such as its disease burden, infection in humans, transmission risk factors and evolutionary pattern of the *Fasciola* parasites.

Funding

This research did not receive any grant.

Declaration of Competing Interest

None.

CRediT authorship contribution statement

Francis Addy: Conceptualization, Formal analysis, Validation, Writing - original draft. **Kwame Gyan:** Investigation, Formal analysis. **Enoch Arhin:** Investigation, Formal analysis. **Marion Wassermann:** Resources, Writing - review & editing.

Acknowledgements

Authors wish to thank the Upper East Regional Veterinary Department for allowing for the survey as well as the veterinary and sanitation officers and butchers at the Bolgatanga slaughterhouse for the cooperation during the field survey.

References

[1] S. Mas-Coma, M.A. Valero, M.D. Bargues, Fasciola, Lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control, Adv. Parasitol. 69 (2009) 41–146.
[2] P.R. Torgerson, B. Devleesschauwer, N. Praet, N. Speybroeck, A.L. Willingham, F. Kasuga, M.B. Rokni, X.N. Zhou, E.M. Fèvre, B. Sripa, N. Gargouri, T. Fürst, C.M. Budke, H. Carabin, M.D. Kirk, F.J. Angulo, A. Havelaar, N. de Silva, World health organization estimates of the global and regional disease burden of 11 foodborne parasitic diseases, 2010: a data synthesis, PLoS Med. 12 (2015) 1–22.
[3] M.K. Khan, M.S. Sajid, H. Riaz, N.E. Ahmad, L. He, M. Shahzad, A. Hussain, M.N. Khan, Z. Iqbal, J. Zhao, The global burden of fasciolosis in domestic animals with an outlook on the contribution of new approaches for diagnosis and control, Parasitol. Res. 112 (2013) 2421–2430.
[4] A. Bosco, L. Rinaldi, V. Musella, A. Amadesi, Outbreak of acute fasciolosis in sheep farms in a Mediterranean area arising as a possible consequence of climate change, Geospat. Health 9 (2015) 319–324.
[5] P.R. Torgerson, One world health : socioeconomic burden and parasitic disease control priorities, Vet. Parasitol. 195 (2013) 223–232.
[6] S. Mas-Coma, M.D. Bargues, M.A. Valero, Fascioliasis and other plant-borne trematode zoonoses, Int. J. Parasitol. 35 (2005) 1255–1278.
[7] K.O. Duedu, E. Peprah, I. Anim-Baidoo, P.F. Ayeh-Kumi, Prevalence of intestinal parasites and association with malnutrition at a Ghanaian orphanage, Hum. Parasit. Dis. 7 (2015) 5–9.
[8] S.A. Squire, H. Amafu-Dey, J. Beyuo, Epidemiology of gastrointestinal parasites of cattle from selected locations in Southern Ghana, Livest Res. Rural Dev. 25 (2013) 1–13.
[9] J.W. McGarry, P.L. Ortiz, J.E. Hodgkinson, I. Goreish, D.J.L. Williams, PCR-based differentiation of Fasciola species (Trematoda: fasciolidae), using primers based on RAPD-derived sequences, Ann. Trop. Med. Parasitol. 101 (2007) 415–421.
[10] F. Addy, T. Romig, M. Wassermann, Genetic characterisation of Fasciola gigantica from Ghana, Vet. Parasitol. Reg. Stud. Rep. 14 (2018) 106–110.
[11] A. Marcilla, M.D. Bargues, S. Mas-Coma, A PCR-RFLP assay for the distinction between Fasciola hepatica and Fasciola gigantica, Mol. Cell Probes 16 (2002) 327–333.
[12] S. Aghayan, H. Gevorgian, D. Ebi, H.A. Atoyan, F. Addy, U. Mackenstedt, T. Romig, M. Wassermann, Fasciola spp . in Armenia : genetic diversity in a global context, Vet. Parasitol. 268 (2019) 21–31.
[13] S. Amer, Y. Dar, M. Ichikawa, Y. Fukuda, C. Tada, T. Itagaki, Y. Nakai, Identification of Fasciola species isolated from Egypt based on sequence analysis of genomic (ITS1 and ITS2) and mitochondrial (NDI and COI) gene markers, Parasitol. Int. 60 (2011) 5–12.
[14] S. Amer, A. Elkhatam, S. Zidan, Y. Feng, L. Xiao, Identity of Fasciola spp in sheep in Egypt, Parasit. Vectors 9 (2016) 1–8.
[15] A.M. Phiri, A. Chota, J.B. Muma, M. Munyeme, C.S. Sikasunge, Helminth parasites of the Kafue lechwe antelope (Kobus leche kafuensis): a potential source of infection to domestic animals in the Kafue wetlands of Zambia, J. Helminthol. 85 (2011) 20–27.
[16] J. Yabe, I.K. Phiri, A.M. Phiri, M. Chembensofu, P. Dorny, J. Vercruysse, Concurrent infections of fasciola, schistosoma and amphistomum SPP. In cattle from Kafue and Zambezi river basins of Zambia, J. Helminthol. 82 (2008) 373–376.
[17] Y. Dar, S. Amer, A. Mercier, B. Courtioux, G. Dreyfuss, Molecular identification of Fasciola SPP. (Digenea: fasciolidae) in Egypt, Parasite 19 (2011) 177–182.
[18] S. Farjallah, D. Sanna, N. Amor, Mehel B Ben, M.C. Piras, P. Merella, M. Casu, M. Curini-Galletti, K. Said, G. Garippa, Genetic characterization of Fasciola hepatica from Tunisia and algeria based on mitochondrial and nuclear DNA sequences, Parasitol. Res. 105 (2009) 1617–1621.
[19] M. Fawzi, A. El-Sahn, H.F. Ibrahim, A.I. Shehata, Vegetable-transmitted parasites among inhabitants of El-Prince, Alexandria and its relation to housewives' knowledge and practices, J. Egypt Public Health Assoc. 79 (2004) 13–29.
[20] V.T. Mucheka, J.M. Lamb, D.M. Pfukenyi, S. Mukaratirwa, DNA sequence analyses reveal co-occurrence of novel haplotypes of Fasciola gigantica with F . hepatica in South Africa and Zimbabwe, Vet. Parasitol. 214 (2015) 144–151.
[21] V. Periago M, M.A. Valero, M. Panova, S. Mas-Coma, Phenotypic comparison of allopatric populations of Fasciola hepatica and Fasciola gigantica from European and African bovines using a computer image analysis system (CIAS), Parasitol. Res. 99 (2006) 368–378.
[22] M.V. Periago, M.A. Valero, M. El Sayed, K. Ashrafi, A. El Wakeel, M.Y. Mohamed, M. Desquesnes, F. Curtale, S. Mas-coma, First phenotypic description of Fasciola hepatica / Fasciola gigantica intermediate forms from the human endemic area of the Nile Delta, Egypt, Infect. Genet. Evol. 8 (2008) 51–58.
[23] A.M. Phiri, I.K. Phiri, C.S. Sikasunge, J. Monrad, Prevalence of fasciolosis in Zambian cattle observed at selected abattoirs with emphasis on age, sex and origin, J. Vet. Med. Ser. B 52 (2005) 414–416.
[24] A.M. Phiri, I.K. Phiri, J. Monrad, Prevalence of amphistomiasis and its association with Fasciola gigantica infections in Zambian cattle from communal grazing areas, J. Helminthol. 80 (2006) 65–68.
[25] H. Ali, L. Ai, H.Q. Song, S. Ali, R.Q. Lin, B. Seyni, G. Issa, X.Q. Zhu, Genetic characterisation of Fasciola samples from different host species and geographical localities revealed the existence of F . hepatica and F . Gigantica in Niger, Parasitol. Res. 102 (2008) 1021–1024.
[26] N. Amor, A. Halajian, S. Farjallah, P. Merella, K. Said, B. Ben, Molecular characterization of Fasciola SPP. from the endemic area of northern Iran based on nuclear ribosomal DNA sequences, Exp Parasitol 128 (2011) 196–204.
[27] M. Ichikawa-Seki, M. Tokashiki, M.N. Opara, G. Iroh, K. Hayashi, U.M. Kumar, T. Itagaki, Molecular characterization and phylogenetic analysis of Fasciola gigantica from Nigeria, Parasitol. Int. 66 (2017) 893–897.
[28] B. Janneh, Dissertation, University of Ghana, 2016.
[29] M.A. Odei, A note on dicrocoeliasis and Fasciola gigantica infection in livestock in Northern Ghana, with a record of spurious and of genuine Dicrocoelium hospes infections in man, Ann. Trop. Med. Parasitol. 2 (1966) 215–218.
[30] A.B. Kpieta, P.B. Laari, Small-scale dams water quality and the possible health risk to users of the water in the Upper West Region of Ghana, Eur. Sci. J. 10 (2014) 249–270.
[31] Namara R.E., Horowitz L., Nyamadi B., Barry B. (2011) Irrigation development in Ghana: past experiences, emerging opportunities, and future directions. GSSP working paper #26.
[32] A.M. Phiri, I.K. Phiri, C.S. Sikasunge, M. Chembensofu, J. Monrad, Comparative fluke burden and pathology in condemned and non-condemned cattle livers from selected abattoirs in Zambia, Onderstepoort J. Vet. Res. 73 (2006) 275–281.

[33] S. Affroze, N. Begum, M.S. Islam, S.A. Rony, M.A. Islam, M.M.H. Mondal, Risk factors and gross pathology of bovine liver fluke risk factors and gross pathology of bovine liver fluke infection at Netrokona District, Bangladesh, J. Anim. Sci. Adv. 3 (2013) 83–90.
[34] A.K.M.A. Rahman, S.K.S. Islam, H. Talukder, K. Hassan, N.K. Dhand, M.P. Ward, Fascioliasis risk factors and space-time clusters in domestic ruminants in Bangladesh, Parasit. Vectors 10 (2017) 29–31.
[35] V.C.G. de Agüero, J. Luka, J. Gandasegui, E. Valderas-García, O.J. Ajanusi, N.P. Chiezey, M. Martínez-Valladares, Fasciola hepatica and Fasciola gigantica coexistence in domestic ruminants in Nigeria: application of a PCR-based tool, Trop. Anim. Health Pro. (2020), doi:10.1007/s11250-020-02333-3.