Molecular Identification of Poultry *Eimeria* species at Live Bird Markets in River State, Nigeria.

Agatha Ebicaelar Ojimelukwe[1]*, Gabriel Ogaba Agu[2] and Austin E. Abah[3].

[1]Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, PMB 5323 Port Harcourt, Rivers State, Nigeria.
[2]Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, PMB 5323 Port Harcourt, Rivers State, Nigeria.
[3]Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, PMB 5323 Port Harcourt, Rivers State, Nigeria.
*Corresponding author: Agatha Ebicaelar Ojimelukwe

Abstract: *Coccidiosis is considered as one the most economically important diseases of poultry and has tremendous socio-economic impact in Nigeria, where family incomes rely heavily on poultry farming. The molecular identification and prevalence of poultry Eimeria spp. in Rivers State was determined in this study. Live bird markets (LBM) in Port Harcourt city (the state capital) were targeted so as to obtain holistic data on Eimeria prevalence. Data on specific market characteristics were collected from 9 LBMs. Fecal samples were collected from bird cages and pooled per LBM. Eimeria oocysts were isolated and identified using oocysts morphology and species-specific multiplex PCR amplification using SCAR primers. Regression analysis were used to check for association between Eimeria spp. prevalence and specific market characteristics. The overall prevalence of poultry Eimeria was 77% (7 of 9). Four Eimeria species were confirmed with prevalence of E. tenella: 77%, E. necatrix: 55%, E. acervulina: 44% and E. mitis: 11%. Mixed Eimeria infections were common among LBMs sampled with an overall prevalence of 55%. Regression analysis revealed a positive association between market size and the prevalence of Eimeria (r = 0.38). The findings from this study has emphasized the importance of E. tenella, E. necatrix, and E. acervulina in the epidemiology of poultry coccidiosis in the state and should inform coccidiosis monitoring and surveillance programmes.*

Keywords: *Eimeria, Poultry, Live bird markets, Prevalence, Nigeria*

I. Introduction

Poultry coccidiosis is an enteric disease. It is caused by the Ampicomplexan parasite of the genus *Eimeria*. Seven species of *Eimeria* have been reported to cause disease in poultry. These include: *Eimeria acervulina, E. brunetti, E. maxima, E.tenella, E. praecox, E, mitis and E. necatrix* [1, 2]. *Eimeria* primarily targets the tissues of the intestinal epithelium which consequently results in a decline in growth and feed utilization in poultry [3, 4]. The disease is therefore associated with high production losses, high morbidity and mortality rates of above 50% [5, 6]. Coccidiosis is considered as one of the most challenging deterrent of poultry development [7] as birds with subclinical infection or that recover from coccidiosis may perform less optimally throughout their life [8,9]. Global losses due to poultry coccidiosis in terms of reduction in production efficiency and the cost of veterinary and prophylactic intervention is estimated at over £500M per year [10].

In Nigeria, poultry production is a very important economic activity. Chickens are particularly important in terms of their number (<90% of the poultry population in the country) and rate of investment [11]. With up to 64% of Nigerian households rearing backyard chickens [12], chickens are of substantial importance to not only the national economy but to the livelihood of the people especially low income earners. Nevertheless, the majority (over 60%) of the Nigerian poultry production systems are characterized by poor hygiene conditions and low biosecurity levels [13] factors that encourage diseases such as poultry coccidiosis to thrive.

Surveys from across Nigeria have reported the prevalence rate of poultry coccidiosis to be considerably high (between 14% and 69%) [14, 15, 16, 17, 18, 19, 20]. However, these surveys have largely focused on selected poultry farms of either native or exotic breeds of chickens. Only very few studies have identified the prevalence of the different species of poultry *Eimeria* in Nigeria [14, 18] and of these, none are from the southern region of the country.

Live bird markets (LBMs) are a traditional structure across Nigeria. They are places where live poultry are displayed in an open system for purchase by poultry consumers [21]. These LBMs are located across

---

Nigeria: within village markets in rural areas; and in major markets in towns and cities. They are important mixing points for birds of diverse breeds, age and origins. Bird sources include all sectors of the Nigerian poultry industry including the large scale commercial poultry holdings; small scale commercial poultry holdings; and scavenging village poultry, mixed together [21, 22].

The objective of this research therefore, is to determine the prevalence and molecular identification of poultry *Eimeria* species in Rivers state. In order to achieve this objective, our study area covered the major LBMs in Port Harcourt city.

II. Materials And Methods

2.1. Ethical clearance
The sampling protocols applied here were approved by the University of Port Harcourt Ethical Review Committee.

2.2. Study area
Rivers State is located in the south-south region of Nigeria (Fig 1). The state features a tropical wet climate with a lengthy and heavy rainy season and a very short dry season. Temperatures are relatively constant throughout the year with a mean daily minimum temperature of 21.2^0C – 23.3^0C and a mean maximum daily temperature of 28.7^0C -33.4^0C year round [23, 24]. This provides a conducive environment for the coccidian parasite to thrive year round [8].With an estimated human population of 6.7 million people, it is the sixth most populous state in Nigeria [25]. Poultry farming is practiced extensively by an estimated 64.4% of households in the region and there are several large to small scale commercial poultry holdings [12].
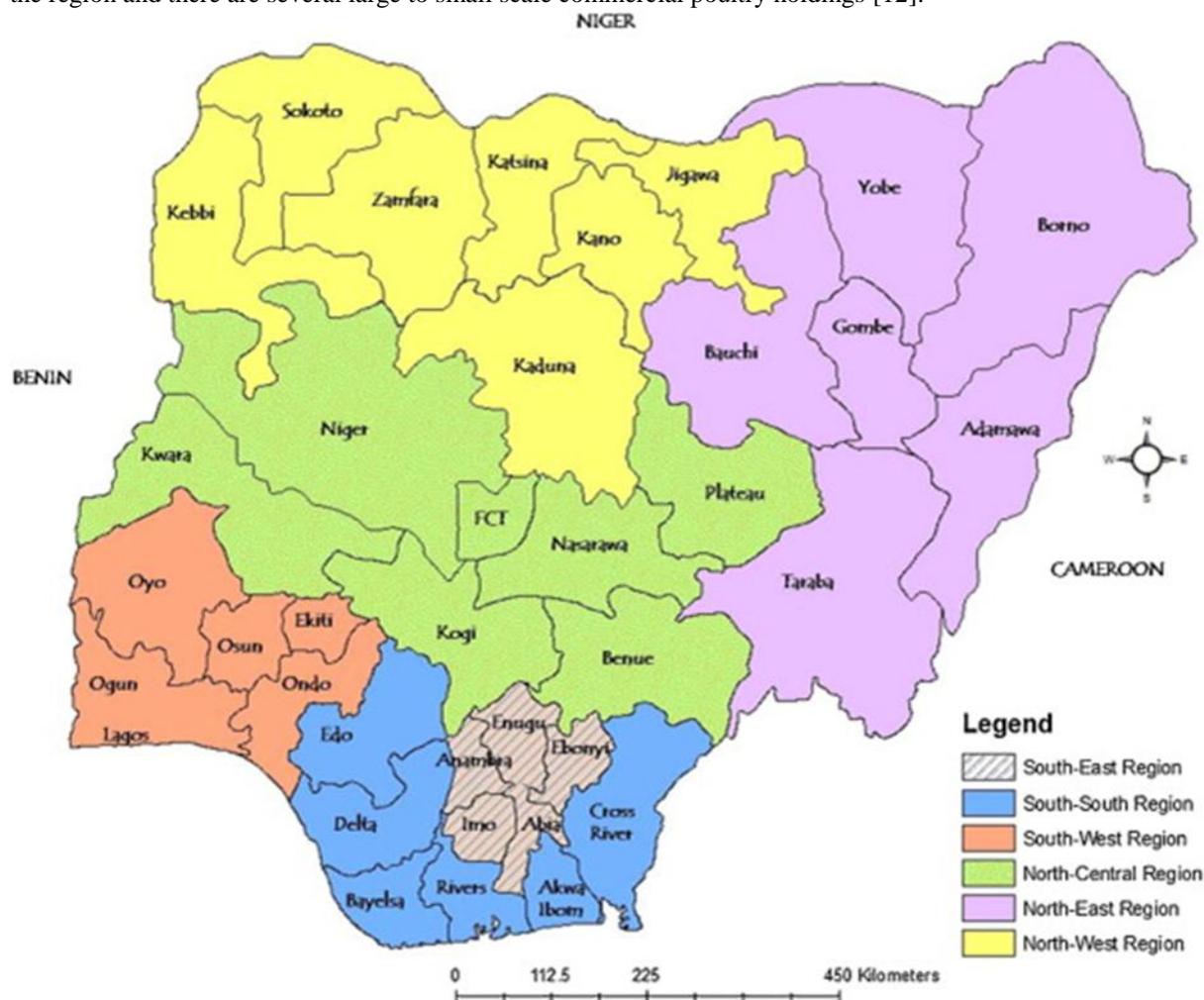
Figure 1 Map of Nigeria: The study area is in Rivers state in the south-south region of Nigeria [43]

2.3. Field study

From September to November 2017, field studies were conducted in 9 major LBMs in Port Harcourt metropolis. Port Harcourt is the capital of Rivers State and the third largest city in southern Nigeria. It is a diverse city located in the Niger Delta region and is economically significant as the centre of Nigeria's oil industry. LBMs in Port Harcourt city were selected purposively based on the fact that being a densely populated city, live poultry trade traffic would very likely flow into the city from different parts of the state including the rural, urban as well suburban areas. Markets were categorized as large, or small, based on the total number of poultry traders present at the day of sampling. Large markets are those with ten or more poultry traders. Small markets have one to nine poultry traders. Fig 2 is the map of Port Harcourt city showing the geographical distribution of LBMs surveyed.
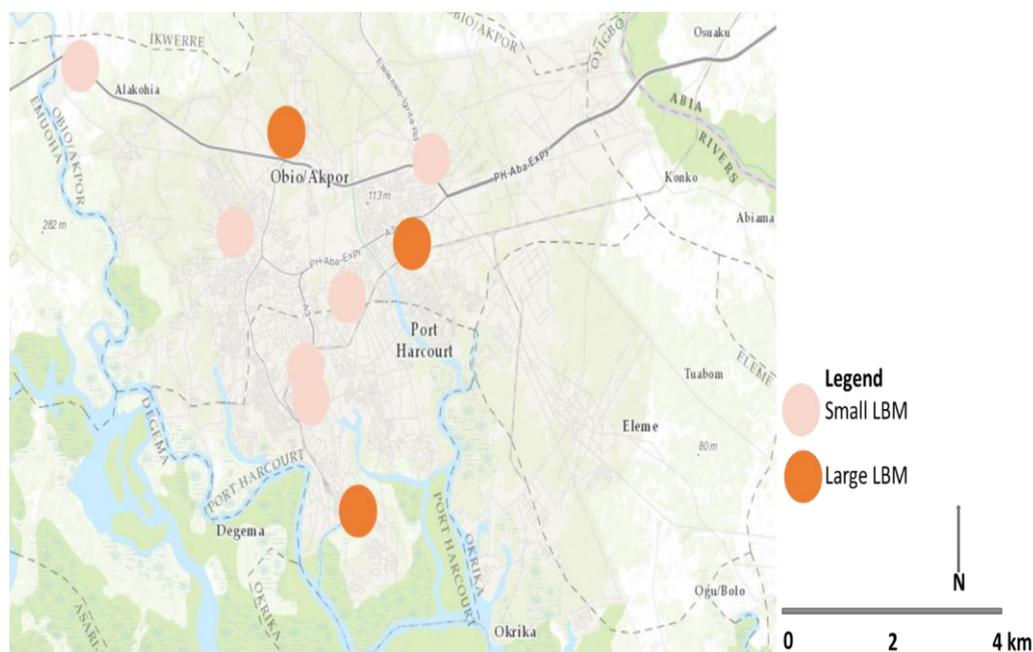
Figure 2. Geographical distribution of LBMs surveyed in Port Harcourt city. (Map generated using ArcGis and Microsoft power point)

2.4. Sample collection and processing

Five to seven fresh fecal samples were randomly collected from across each cage up to a maximum of twelve cages per market. The total number of: live poultry traders; cages present; and cages sampled per market, are shown in TABLE 1. Samples from each market were pooled into a bulk sample. The saturated sodium chloride floatation method (10 min at $1000 \times g$) was used to obtain oocysts. Oocysts were re-suspended in 2.5% potassium dichromate solution and allowed to sporulate at room temperature for seven days, with regular stirring.

2.5. Molecular and morphologic identification of oocysts

The presence of poultry *Eimeria* oocysts was confirmed based on their basic microscopic morphology [8]. The *Eimeria* species was further verified by a multiplex PCR assay. Six pairs of species-specific primers (TABLE 2) were used as previously described by [26]. Total genomic DNA extraction from oocysts of *Eimeria* species was done as follows: 20ml of each oocyst suspension, were centrifuged (750 g for 10 min) to pellet the oocysts. Each pellet was re-suspended in the minimum volume residual supernatant and transferred to a 1.5 ml screw top plastic tube containing glass beads (0.4-0.6 mm; Sigma, UK) and covered with sterile phosphate buffered saline (PBS; pH 8.0). The pelleted oocysts were then disrupted using a Mini Beadbeater-8, (Biospec Products Bartlesville, USA) for two minutes and total genomic DNA (gDNA) was isolated from the smashed oocysts homogenate using a Quick-DNA™ extraction kit (ZYMO RESEARCH) following the manufacturers protocol. The PCR amplification was based upon a 25µl volume consisting of 1µl genomic DNA template, 0.5µl of each primer, 12.5µl Taq DNA polymerase (Sigma, USA) and made up to 25µl with nuclease free water. The standardized cycling conditions consisted of initial denaturation: 96 ^{O}C for 5 minutes followed by 30 cycles of 95 ^{O}C denaturation for 1 minute, 59 ^{O}C annealing for 2 minutes; 72 ^{O}C extension for 1 minute, and a final extension: 72 ^{O}C for 7 minutes.

2.6. Statistical analysis

Data generated from the study were analyzed using descriptive statistical methods percentages and tabulation. Correlation analysis were used to check for association between market size and the prevalence of *Eimeria* spp.

III. Results

Seven out of nine LBMs surveyed were found to contain poultry *Eimeria* oocysts based on microscopy (Table 2) resulting in an overall prevalence of 77%. The results of the molecular identification showed that *E. tenella, E. necatrix, E. acervulina* and *E. mitis* are the predominant *Eimeria* species in our study location. *E. tenella* was present at 7 of 9 LBM (77%), *E. necatrix* at 5 of 9 (55%), *E. acervulina* at 4 of 9 (44%) and *E mitis* at 1 of 9 (11%) LBM. The primers used were specific enabling the discrimination of six *Eimeria* species. The amplified fragments presented different sizes: *E. acervulina* (811 bp), *E. tenella* (539 bp) *E. mitis* (310 bp) and *E. necatrix* (200 bp) (Figure 3). Mixed poultry *Eimeria* infections were found in 5 of 9 (55%) LBMs.

Eimeria was detected in 3 out of 3 large LBM and 4 out of 6 small LBM. Regression analysis revealed a positive correlation between market size and the prevalence of *Eimeria* species (r = 0.38), however the correlation was not significant.
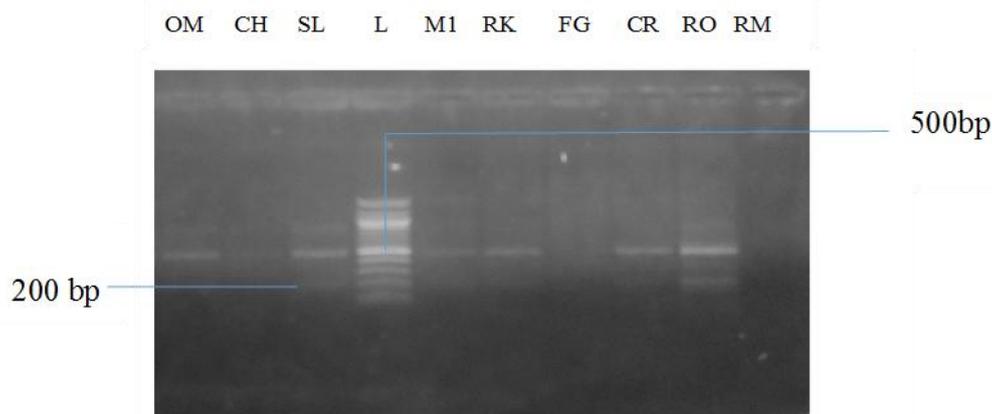
Figure 3. Agarose gel electrophoresis showing the amplified SCAR gene of the *Eimeria* spp.
Abbreviations: OM, Oil Mill; CH, Choba; SL, Slaughter; M1, Mile 1; RK, Rumuokuta; FG, Fruit garden; CR, Creek road; RO, Rumuokoro; RM, Rumuomasi; bp, base pairs

IV. Discussion

In this study, *Eimeria* oocysts from chicken fecal samples at LBMs were isolated and identified via two methods, the traditional morphologic technique; and by species specific multiplex PCR amplification of the SCAR rDNA region. An overall prevalence of 77% was recorded in our study by both methods. A prevalence of 77% is considerably high when compared with previous reports from surveys of poultry farms in this region of Nigeria which have been in the range of 15% - 29% [27]. While the high prevalence could be attributable to the conducive environment afforded the coccidian parasite by the region's climatic conditions [8], It also supports previous reports that the majority of LBMs and poultry farms in the region are characterized by unacceptably low levels of biosecurity and poor management practices [13, 12] factors which encourage the accumulation of the parasite [10]. This result also supports previous evidence that LBMs are ideal places for the targeted surveillance of poultry diseases owing to a higher probability of finding disease especially when pathogen circulation is low at the individual farm level [28, 29, 30, 31]. Nevertheless, our results are fairly in agreement with poultry *Eimeria* prevalence studies conducted across Nigeria where rates above 50% have been reported [32, 14, 19, 20].

No *Eimeria* oocysts were identified in 22% of LBMs surveyed. This perhaps reflects the small proportion of poultry farmers as well as traders who actively control poultry coccidiosis through proper poultry management practices at farms and LBMs [12]. The negative LBMs are Fruit-garden and Rumuomasi markets. These markets had one and four live poultry traders respectively. Our results showed a positive correlation between the number of poultry traders and the prevalence of *Eimeria* at LBMs. This is not surprising because fewer poultry traders suggest fewer bird sources and consequently a reduced chance of including an infected

bird. Though the correlation was not significant, probably due to our small sample size of nine markets, it is suggested that surveillance for infectious diseases at LBMs should focus on the large LBMs that have greater poultry traffic and consequently, a greater chance of finding disease.

Molecular identification of our isolates revealed that the most prevalent *Eimeria* species in Rivers state are *E. tenella* (77%),*E. necatrix* (55%) and *E. acervulina* (44%). The co-occurrence of multiple *Eimeria* species was also common. Our finding of the high prevalence of mixed *Eimeria* infections (55%) is in agreement with a related result (68%) found in northern Nigeria [18]. Our results therefore support evidence that the multiplex PCR amplification using SCAR primers is a sensitive method for the simultaneous discrimination of different species causing poultry coccidiosis [33]. The presence of *E. tenella* at all positive LBMs indicate that this species remains highly invasive amongst others and is the most economically important species causing poultry coccidiosis in Rivers state. Our results are in agreement with related studies in northern Nigeria [18], Ethiopia [34], India [35] and China [36, 37, 38] where *E. tenella* was reported as the most prevalent.

Table -1. Total number of poultry traders, total number of cages with birds for sale, and the number of cages sampled per LBM

Market	Number of traders	Number of cages with birds for sale	Number of cages sampled
Choba	8	16	12
Creek-Road	12	26	12
Fruit garden	1	2	2
Mile 1	9	15	10
Oil mill	5	8	8
Rumuomasi	4	9	9
Rumuokoro	10	26	12
Rumuokuta	2	4	4
Slaughter	15	34	12
TOTAL	**66**	**140**	**81**

Table-2. The primers used in multiplex PCR amplification

S/N	Species		Primer sequences 5' – 3'	Expected amplicon size (bp)
1	*E. acervulina*	F	AGTCAGCCACACAATAATGGCAAACATG	811
		R	AGTCAGCCACAGCGAAAGACGTATGTG	
2	*E. tenella*	F	CCGCCCAAACCAGGTGTCACG	539
		R	CCGCCCAAACATGCAAGATGGC	
3	*E. mitis*	F	AGTCAGCCACCAGTAGAGCCAATATTT	460
		R	AGTCAGCCACAAACAAATTCAAACTCTAC	
4	*E. maxima*	F	GGGTAACGCCAACTGCCGGGTATG	272
		R	AGCAAACCGTAAAGGCCGAAGTCCTAGA	
5	*E. necatrix*	F	TTCATTTCGCTTAACAATATTTGGCCTCA	200
		R	ACAACGCCTCATAACCCCAAGAAATTTTG	
6	*E. brunetti*	F	TGGTCGCAGAACCTACAGGGCTGT	626
		R	TGGTCGCAGACGTATATTAGGGGTCTG	

F: forward primer; R: reverse primer; bp: base pairs

Table -3 Morphologic and molecular identification of *Eimeria*spp from LBM

	Oocysts morphology	Multiplex PCR					
	Eimeria spp	*E. acervulina*	*E. mitis*	*E.necatrix*	*E. tenella*	*E.brunette*	*E. maxima*
Oil Mill	+	+	-	-	+	-	-
Choba	+	+	-	-	+	-	-
Slaughter	+	+	-	+	+	-	-
Mile 1	+	-	-	+	+	-	-
Rumuokuta	+	-	-	+	+	-	-
Fruit garden	-	-	-	-	-	-	-
Creek-road	+	-	-	+	+	-	-
Rumuokoro	+	+	+	+	+	-	-
Rumuomasi	-	-	-	-	-	-	-
TOTAL	7	4	1	5	7	0	0

E. tenella and *E. necatrix* are considered as species with very high potential to cause pathology in poultry birds [39, 40]. The detection of *Eimeria* oocysts in chicken fecal samples, is not often considered as a definitive diagnosis of poultry coccidiosis [34], but it is important to note that the circulation of high pathogenicity species such as *E. tenella* and *E. necatrix* can contribute to high morbidity and mortality in young chickens particularly under conditions where compounding factors such as malnutrition, poor sanitation and co-infection with other pathogens co-exist [1, 41]. This information is indeed crucial as such pathogenic species can compromise poultry productivity in the majority of Nigerian poultry farms which are characterized by unacceptably low sanitation and biosecurity levels [12]. Our study revealed *E. mitis*, to be the least abundant

species of *Eimeria* respectively. While *E. mitis* is not usually associated with severe disease in poultry, it has been reported to reduce feed efficiency in birds [39].

The present study is not without limitations. Firstly, our *Eimeria* oocysts were isolated from pooled fecal samples of live birds for sale at LBMs. Though our method seems suitable for the purpose of identifying the various poultry *Eimeria* species in or study location it must be noted that birds at LBMs are from multiple sources both within and possibly outside the state. Our prevalence result may therefore be slightly higher than the true situation in the Rivers state. Lastly, our PCR amplification was done using six species-specific primers including all species of poultry *Eimeria* with the exception of *E. praecox*. The species-specific primer of this species was excluded particularly due to some funding limitations at the time of the study. However, *E praecox* is considered as a species of minimal economic importance and minimal pathogenicity in poultry [42]. Based on these limitations, care should be taken when interpreting our results.

V. Conclusion

To the best of our knowledge this study is the first to identify the different species of *Eimeria* prevalent among poultry in Rivers State and the south-south region of Nigeria. Our molecular identification of *Eimeria*spp has highlighted the importance of *E. tenella*, *E. necatrix,* and *E. acervulina* in the epidemiology of poultry coccidiosis in the state. LBM are important places for the early detection and surveillance of infectious poultry diseases, however, such surveillance rounds should focus on large LBMs where the greater live poultry traffic will increase the chance of detecting disease. These results are important for the surveillance and control of poultry coccidiosis in the region.

Acknowledgements

References

[1]   McDougald, L.R. Coccidiosis. In Diseases of poultry. 11th edition. Edited by Saif YM, Barnes J, Glisson JR, Fadly AM, McDougald LR, Swayne DE. Ames, 2003 Iowa: Iowa State Press.
[2]   Blake, D.P. and Tomley, F.M. Securing poultry production from the ever-present *Eimeria* challenge *Trends in Parasitology*, *30*2014 http://dx.doi.org/10.1016/j.pt.2013.10.003
[3]   Levine, N. D. Taxonomy and life cycle of coccidia. In Biology of the coccidia, P. L. Long (ed.). University Park Press, Baltimore, Maryland 1982, p. 1-33.
[4]   Reid, W. M., Hines, T.K., Johnson, J., and Stino, K R. Coccidiosis: Effects of high environmental temperatures on anticoccidial protection. *Poultry Science55,* 1976, 1436– 1441.
[5]   Karaer, Z., Guven, E., Akcay, A., Kar, S., Nalbantoglu, S. and Cakmak, A. Prevalence of subclinical coccidiosis in broiler farms in Turkey. *Tropical Animal Health and Production*, *44(3),* 2012, 589-594.
[6]   Györke, A., Pop, L. and Cozma, V. Prevalence and distribution of *Eimeria* species in broiler chicken farms of different capacities, *Parasite, 20(1),* 2013, article 52.
[7]   Tewari, A.K. and Maharana, B.R. Control of poultry coccidiosis: changing trends.*Journal of Parasitic Diseases 35*(1), 2011, 10–17 DOI 10.1007/s12639-011-0034-7
[8]   Jordan, F.T.W. and Pattison, M. Poultry diseases, 3rd ed. Cambridge Univ. Press, 1998, 260-262.
[9]   Arabkhazaeli, F., Modrisanei, M., Nabian, S., Mansoori, B., Madani, A. Evaluating the resistance of *Eimeria* Spp. field isolates to anticoccidial drugs using three different indices. *Iranian Journal of Parasitology 8(2),* 2013, 234-241.
[10]   Shirley, M.W., Smith, A.L., Blake, D.P. Challenges in the successful control of avian coccidian. *Vaccine 25, 2007,*5540–5547
[11]   NBS and FMARD (National Bureau of Statistics and Federal Ministry of Agriculture And Rural Development) 2011. Collaborative study on national agriculture sample survey (NASS).
[12]   Uzochukwu-Obi, T., Olubukola, A. &Maina, G. A. Pro-poor HPAI risk reduction strategies in Nigeria—Background Paper,. Africa/Indonesia Team Working Paper, No. 5, IFPRI 2008.
[13]   (FAO) Food and Agricultural Organization of the United Nations. The Structure and importance of the commercial and village based poultry industry in Nigeria. Rome, 2008 pp. 44 -49
[14]   Muazu, A., Masdooq, A.A., Ngbede, J., Salihu, A.E., Haruna, G., Habu, A.K., Sati, M.N and Jamilu, H. 2008. Prevalence and identification of species of *Eimeria* causing coccidiosis in poultry within Vom, Plateau State, Nigeria. *International Journal of Poultry Science 7(9),* 2008, 917-918.
[15]   Nnadi, P.A. and George, S.O. A cross-sectional survey on parasites of chickens in selected villages in the sub-humid Zones of South- Eastern Nigeria. *Journal of Parasitology Research*, 2010, Article ID 141824, 1-6.
[16]   Agi, P.I., Nzeako, S.O., Imafidor, H.O., Saikpere, O.M. 2010.Prevalence of coccidiosis in free-range fowls in Obio-Akpor Local government area, Rivers State. *Nigerian Journal of Parasitology 31*(2) http://dx.doi.org/10.4314/njpar.v31i2.69466
[17]   Ukashatu, S., Magaji, A.S., Najamuddeen, G. and Saulawa, M.A. Survey of poultry diseases among exotic breeds of poultry in Katsina metropolis, Katsina state, Nigeria. *Scientific Journal of Zoology, 1(3)* 2012, 69-73.
[18]   Jatau, I.D., Sulaiman, N.H., Musa, I.W., Lawal, A.I., Okubanjo, O.O., Isah, I. and Magaji, Y. 2012. Prevalence of coccidia infection and preponderance *Eimeria* species in free range indigenous and intensively managed exotic chickens during hot-wet season, in Zaria, Nigeria. *Asian Journal of Poultry Science, 6,* 2012: 79-88.
[19]   Olanrewaju, C.A. and Agbor, R.Y. 2014. Prevalence of coccidiosis among poultry birds slaughtered at Gwagwalada main market, Abuja, FCT, Nigeria. *The International Journal of Engineering and Science, 3(1),* 2014, 41-45.
[20]   Lawal, J.R., Jajere, S.M., Ibrahim, U.I., Geidam, Y.A., Gulani, I.A., Musa, G., Ibekwe, B.U. Prevalence of coccidiosis among village and exotic breed of chickens in Maiduguri, Nigeria, *Veterinary World, 9(6),*2016, 653-659.

[21]   AICP. (Animal Health) Avian Influenza Control and Human Pandemic Preparedness and Response Project: The Development of Live Bird Markets in Nigeria.2008 PP 1 - 77

[22]   Paola, P., Abimiku, J.E. and Emeka-Okolie, W.Assessment of the Nigerian poultry market chain to improve biosecurity by Food and Agricultural Organization. 2008, PP 1 – 60.

[23]   NOAA (National Oceanic and Atmospheric Administration). Port Harcourt climate normal 1961 – 1990. Available at ftp://ftp.atdd.noaa.gov/pub/GCOS/WMO-Normals/TABLES/REG_I/NI/65250.TXT Retrieved 28th August 2017

[24]   WMO (World Meteorological Organization). World weather information services – Port Harcourt. Available at: worldweather.wmo.int/en/city.html?cityid=325 Retrieved on 28th August 2017.

[25]   (NPC) National Population Commission 2006. Port Harcourt Priority tables. Available at: http://population.gov.ng/core-activities/surveys/datasets/2006-phc-priority-tables/Retrieved on October 10 2017

[26]   Fernandez, S., Pagotto, A.H., Furtado, M.M., Katsuyama, A.M., Madeira, A.M., Gruber, A.A. A multiplex PCR assay for the simultaneous detection and discrimination of the seven *Eimeria* species that infect domestic fowl. *Parasitology Research*, 127, 2003, 317 - 325

[27]   Mohammed, B.R and Sunday, O.S. 2015.An overview of the prevalence of avian coccidiosis in poultry production and its economic importance in Nigeria. *Veterinary Research International 3(3),* 2015, 35-45.

[28]   Wang M, Di B, Zhou DH, Zheng BJ, Jing H, Lin YP, Liu YF, Wu XW, Qin PZ, Wang YL. Food markets with live birds as source of avian influenza *Emerging infectious diseases*, *12(11),* 2006, 1773-1775

[29]   Bulaga, L.L. Garber, L., Senne, D.A., Myers, T.J., Good, R., Wainwright, S., Trock, S., and Suarez, D.L. Epidemiologic and surveillance studies on avian influenza in live-bird markets in New York and New Jersey, 2001*Avian Diseases 47(3),* 2003: 996-1001

[30]   Samaan, G., Indriani, R., Carrasco, R.L., Lokuge, K., Cook, A.R., Kelly, M.P., Adjid, R. 2012. Prioritizing live bird markets at risk of avian influenza H5N1 virus contamination for intervention: A simple tool for low resource settings. *Preventive Veterinary Medicine107*, 2012, 280-285.

[31]   Molia, S., Boly, A.I., Duboz, R., Coulibaly, B., Guitian, J., Grosbois, V., Fournié, G., Pfeiffer, D.U. 2016. Live bird markets characterization and trading network analysis in Mali: Implications for the surveillance and control of avian influenza and Newcastle disease. *ActaTropica 155,* 2016, 77-88.

[32]   Adene, D.F. and Oluloye, O.B. Coccidiosis of poultry. The biology, diagnosis, treatment and control. In: D. F. Adene, Poultry health and production-principles and practices. Sterling- Horden Publishers (Nig.) Ltd. Ibadan 2004.pp 129- 164.

[33]   Venkateswara Rao, P., Raman, M., DhinakarRaj, G., Abdul Basith, S., Gomathinayagam, S. Multiplex PCR assay using SCAR primers to detect Eimeria spp. in chicken. *Journal of Parasitic Disease, 37(1),* 2013, 110 – 113

[34]   Luu, L., Bettridge, J., Christley, R.M., Melese, K., Blake, D., Dessie, T., Wigley, P., Desta, T.T., Hanotte, O., Kaiser, P., Terfa, Z.G., Collins, M., Lynch, S.E., 2013. Prevalence and molecular characterization of *Eimeria* species in Ethiopian village chickens. *BMC Veterinary Research 9*, 2013, 208.

[35]   Prakashbabu, C.B., Thenmozhi, V., Limon, G., Kundu, K., Kumar, S., Garg, R., Clark, E.L., Rao, A.S.R.Srinivasa, Raj, D.G., Raman, M., Banerjee, P.S., Tomley, F.M., Guitian, J., Blake, D.P.*Eimeria* species occurrence varies between geographic regions and poultry production systems and may influence parasite genetic diversity. *Veterinary Parasitology* 2016 http://dx.doi.org/10.1016/j.vetpar.2016.12.003

[36]   Clark E.L., Tomley, F.M., Blake, D.P. Are *Eimeria* genetically diverse, and does it matter? *Trends in Parasitology*, *33(3),* 2016, 231-241.

[37]   Lan, L.H., Sun, B.B., Zuo, B.X.Z., Chen, X.Q. and Du, A.F. Prevalence and drug resistance of avian *Eimeria* species in broiler chicken farms of Zhejiang province, China. *Poultry Science*,*96,* 2017, 2104–2109 http://dx.doi.org/10.3382/ps/pew499

[38]   Tan, L., Li, Y., Yang, X., Ke, Q., Lei, W., Mughai, M.N., Fang, R., Zhou, Y Shen, B., Zhao, J. Genetic diversity and drug sensitivity studies on *Eimeriatenella* field isolates from Hubei Province of China. *Parasites and Vectors*, *10*, 2017, 137 – 147.

[39]   Conway, D.P., Sasai, K., Gaafar, S.M., Smothers, C.D. *E. tenella*, and *E. maxima* on plasma constituents, packed cell volume, lesion scores, and performance in chickens. *Avian Diseases37,* 1993, 118–123.

[40]   Blake, D.P., Clark, E.L., Macdonald, S.E., Thenmozhi, V., Kundu, K., Garg, R., Jatau, I.D., Ayoade, S., Kawahara, F., Moftah, A., Reid, A.J., Adebambo, A.O., Alvarez Zapata, R., Srinivasa Rao, A.S., Thangaraj, K., Banerjee, P.S., Dhinakar-Raj, G., Raman, M., Tomley, F.M.. Population, genetic, and antigenic diversity of the apicomplexan Eimeria tenella and their relevance to vaccine development. *Proceedings of the National Academy of Science USA* 112, 2015, E5343-5350.

[41]   Williams, R.B., Marshall, R.N., Pages, M., Dardi, M., delCacho, E., 2009. Pathogenesis of *Eimeria praecox* in chickens: virulence of field strains compared with laboratory strains of *E. praecox* and *Eimeria acervulina*. *Avian Pathology38,* 2009, 359-366.

[42]   Conway, D.P. and Mckenzie, M.E. 2007. Poultry coccidiosis diagnosis and testing procedures. 3rd Edition Iowa: Blackwell publishing.

[43]   Ekong, P.S., Ducheyne, E., Carpenter, T.E., Owolodun, O.A., Oladokun, A.T., Lombin,L.H., Berkvens, D., 2012. Spatio-temporal epidemiology of highly pathogenic avian influenza (H5N1) outbreaks in Nigeria, 2006–2008. Prev. Vet. Med. 103,170–177.