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PRELIMINARY OBSERVATION ON THE EFFICACY OF LIVACOX VACCINE AGAINST COCCIDIOSIS IN LAYING BIRDS IN A BACKYARD POULTRY FARM IN JOS, PLATEAU STATE, NIGERIA

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ABSTRACT

Poultry coccidiosis caused by *Eimeria spp*. remains a major impediment to poultry farmers causing high economic losses in Nigeria and other parts of the world. It is a protozoal parasitic disease that affects the gut of birds resulting in high morbidity and mortality. Live attenuated vaccines have been developed to confer immunity against coccidiosis in birds. This preliminary study investigates the efficacy of Livacox vaccine against coccidiosis in 1,930 pullets in a backyard poultry farm in Bukuru, Jos South Local Government, Plateau State, Nigeria between April and August 2014. The investigated birds were grouped into two broad categories as vaccinated (950) and unvaccinated (980) based on the farmer's pattern of rearing. The vaccinated group received single oral dose of livacox vaccine in water, while the unvaccinated group received water only. The observation of clinical signs in sub-clinically sick birds, litter faecal appearance pattern and post mortem lesions in dead birds were monitored and ranked according to the severity. The post mortem gross lesion scores of dead pullets were also determined. Additionally, a review of farm records was carried out to monitor the mortality trend. There was 50% morbidity and 0.13% mortality in the control group. Remarkably, the Livacox vaccinated group showed shorter term morbidity and litter faecal appearance pattern with highly reduced mortality. The mean gross lesion scores was statistically significantly different between the vaccinated and unvaccinated groups ($P<0.05$). The result indicated that the administration of single oral dose of Livacox vaccination at an early stage in a pullet's life considerably protect against coccidiosis.

INTRODUCTION

Coccidiosis is an intestinal disease caused by single-celled microscopic protozoal parasites of the *Eimeria spp* belonging to the phylum Apicomplexa. It is an important disease that affects different species of animals including poultry and humans (Calnek *et al.*, 1997). Coccidiosis is highly prevalent among intensively managed poultry farms in Nigeria, affecting both broilers and pullets, resulting to high flock mortality, lower productivity and severe economic losses (Maikasuwa and Jabo, 2011). It is common in farms where the litter management and biosafety are compromised. Infected birds continue to shed oocysts in litter resulting to infection of other flock members, where the given conditions are favourable for sporulation (Etuk *et al.*, 2004).

Nine species of *Eimeria spp*. are known to cause poultry coccidiosis with different levels

of virulence and pathogenicity. They include *E. brunetti, E. maxima, E. necatrix, E. tenella, E. acervulina, E. mitis, E. mivati, E. praecox* and *E. hagani.* The pathogenicity of *Eimeria spp.* in poultry have been described as dependant on the age of birds, density of birds per house, dose and viability of ingested oocysts and virulence of the different strains (Calnek *et al.*, 1997; Bachaya *et al.,* 2012; Blake and Tomley, 2014). Apart from the litter management and biosafety, the season of the year, particularly ambient temperature and humidity have great influence on the occurrence of coccidiosis. Given the tropical temperature and the high humid conditions in the rainy season, the occurrence of coccidiosis in birds is higher. Furthermore, Nematollahi *et al.* (2009) classified the nine species by their pathogenicity, *E. brunetti, E. maxima, E. necatrix* and *E. tenella* as highly pathogenic, *E. acervulina, E. mitis* and *E. mivati* are rather less pathogenic, while *E. praecox* and *E. hagani* are regarded as the least pathogenic. Six *Eimeria* spp. are considered the most common incriminants in poultry coccidiosis. These include *E. brunetti, E. maxima, E. necatrix, E. tenella, E. acervulina* and *E. mitis* (Nematollahi *et al.,* 2009). Muazu *et al.* (2010) found four *Eimeria spp*. namely *E. tenella, E. maxima, E. necatrix* and *E. acervulina* to be important in causing poultry coccidiosis in Vom, Plateau State. The different *Eimeria spp.* have predilection sites in different sections of the chicken gut, where lesions are produced. As a result coccidiosis in poultry has been described as caecal or intestinal coccidiosis depending on the *Eimeria spp*. and site where lesions are found (Calnek *et al.,* 1997). The common clinical signs of coccidiosis in pullets and layers include ruffled feathers, droopiness and listlessness, bloody and diarrhoeal faeces, weight loss, high mortality and low egg production (Conway and McKenzie, 2007).

Coccidiosis causes high economic losses in poultry worldwide and such losses have been estimated to reach $3 billion per annum (Blake and Tomley, 2014). Although the poultry industry is one of the most developed and capitalized agricultural sector in Nigeria, it has large numbers of medium and small scale farmers who raised birds in their backyard farms especially around major cities and state capitals (Etuk *et al.,* 2004; Musa *et al.,* 2010). Furthermore, the industry is a major source of animal protein in form of meat and eggs, manure, income and employment. The backyard farmers depend on commercial farms for the supply of day old chicks (DOCs) and feeds. This intricate relationship of the various production systems has heightened the significance of poultry farming in sustaining livelihoods. Additionally, the low start off capital, high profitability, quick economic returns, high energy and protein conversion ratio, and easiness for most farmers to understand the production and management principles involved has made the industry attractive to most small scale farmers in Nigeria (Nnadi and George, 2010; Maikasuwa and Jabo, 2011). The bulk of poultry meat and eggs consumed in Nigeria have been produced largely by small holder poultry farmers (Etuk *et al.,* 2004; Musa *et al.,* 2010).

With the proliferation of small-scale poultry farms in Jos, Plateau State, Nigeria, coccidiosis has become one of the most economically significant parasitic diseases of poultry (Jatau *et al.,* 2012; Olarewanju and Agbor, 2014). It is a major cause of poor performance with consequent reduction in productivity. It is incriminated for the cause of high morbidity and mortality in poultry farms. A high mortality of 50% was reported in a commercial poultry broiler breeder farm in Zaria (Musa *et al.,* 2010) and a prevalence of 12% in epidemiological studies was also reported by Adene and Oluleye (2004); Muazu *et al.* (2010) had a prevalence of 36.7% in adult birds and 52.9% in younger birds in Vom, Plateau State, Nigeria. Thus, poultry farmers have spared no effort into looking for more effective ways to control poultry coccidiosis apart from using coccidiostats. The emergence of live attenuated coccidiosis vaccine seems to be paving way for farmers to better manage and control coccidiosis in poultry. Previous

investigation on the use of live attenuated vaccines in south western part of Nigeria has been documented (Oladoja and Olusanya, 2007). However, the efficacy of the vaccine against coccidiosis has not been investigated in Nigeria. This preliminary study in a backyard poultry farm in Jos, Plateau State is being carried out to investigate the efficacy of the vaccine.

MATERIALS AND METHODS

Study Area

The vaccine trial was carried out in a backyard poultry farm in Bukuru, Jos South Local Government Area of Plateau State, Nigeria. Bukuru, a suburb of Jos is located between latitude 9^o 48'N and longitude 8^o 52'East of the Equator and at an elevation of 1,238 meters above sea level (Encyclopaedia Britannica, 2012). It has a semi-temperate climate (temperature: 18$\pm$ 6^oC, rainfall: 1099mm, relative humidity: 55 – 58%) that provides favourable weather for poultry farming and the production of other farm animals in general. The farmers in Bukuru practise peri-urban farming in order to take advantage of the demand for poultry products in Jos, the Plateau State capital.

Study Population

This preliminary study is based on observational study of live birds including a review of the poultry farm records kept by the farmer between February and September 2014. A total of 1,930 pullets aged 6 months used for the study were raised in intensively managed backyard poultry deep litter system. The birds were arranged in two batches of 950 birds as vaccinated group and a second 980 as unvaccinated or placebo group.

Sample Size

The sample size was determined by the use of the formular as presented by the International Fund for Agricultural Development (IFAD).

$$n = \frac{t^2 \, x \, p(1-p)}{m^2}$$

where $\boldsymbol{n}$ = minimum sample size

$\boldsymbol{t}$ = confidence interval at 95% (standard value of 1.96)

$\boldsymbol{p}$ = the estimated prevalence of coccidiosis in the area, which is 50.33%

$\boldsymbol{m}$ = margin of error at 5% (standard value of 0.05)

An average prevalence of 50.33% was used in the determination of the sample size based on studies carried out by Muazu *et al*. (2008), Musa *et al*. (2010) and Olanrewaju and Agbor (2014). The sample size was calculated as 20 pullets. The analysis of litter faecal pattern from both groups involved the collection and visual examination and scoring of 20 bloody faecal materials and post mortem examination was carried out on 40 dead pullets drawn from both vaccinated and the unvaccinated groups. While the two backyard poultry houses were monitored for occurrence of coccidiosis, mortality trends were analysed for both vaccinated and the unvaccinated pullets.

Vaccine

The anticoccidial vaccine used was Livacox vaccine manufactured by Biopharm, Prague, Czech Republic. It is a live attenuated vaccine consisting of live sporulated oocysts which induce cell mediated immunity.

Vaccine Administration

One batch of the pullets described above were subjected to vaccine trials using the Livacox vaccine by administering a single dose of Livacox vaccine orally in drinking water at the dosage of 10ml/bird at the age of 7 days of life as recommended by Biopharm. The second batch was given water only.

Monitoring for the Presence of Coccidiosis

A. Observation of birds for Clinical Signs:

Each set of birds were kept under observation for 16 weeks as from the 7th day post vaccination during which the occurrence of coccidiosis was monitored as from February – September 2014. The birds were observed for presence of clinical signs such as ruffled feathers/ droopiness/ weakness, poor feeding (quantity of left over feeds in feeders) and presence of diarrheal vents.
The litter faecal patterns were also monitored for the presence of spots of diarrhoeal faeces, franks blood faeces, mucoid-bloody faeces or rust-like faeces through visual scoring of bloody faeces. Furthermore, post mortem examinations of the gut of dead birds were carried out to identify lesions of coccidiosis.
Post mortem observations were also made especially in dead birds.

B. Clinical signs in the Vaccinated birds:

The vaccinated birds also developed clear signs of coccidiosis such as ruffled feathers/ droopiness/ weakness, poor feeding (quantity of left over feeds in feeders) and presence of diarrheal vents. Morbidity and mortality was also noticed. The vaccinated group was not given any anticoccidial drug treatment despite the obvious signs of coccidiosis after the administration of the vaccine. However, the farmer gave vitamin/mineral supplementation and anticoccidial treatment to the unvaccinated group by the 9th and 11th week post vaccination.

Analysis of data

Data generated were analysed using descriptive statistics (Tables, simple mean, graphics) of the Statistical Package for Social Sciences (SPSS 21).

Determination of Vaccine Efficacy

1. The presence or absence of clinical signs was rated through visual scoring of the amount and duration of blood faecal material in litter in line with the description by Dogo *et al*. (2007).

2. This was also determined by the post mortem examination of the different parts of the bird's intestines (upper, mid, lower and caeca). The gross lesions observed were ranked on a 4 point scale of 0 – no gross lesions and 4 – extremely severe gross lesions based on the classification by Johnson and Reid (1970).

RESULTS

The following observations were noted in the vaccinated and unvaccinated birds.

Clinical signs

The vaccinated group showed clinical signs of coccidiosis after 7 days post vaccination. The birds while under observation had high morbidity of 85% with a mortality of 0.03% within the period of 16 weeks from February to September 2014. Sick birds had ruffled feathers with dull and weak appearance. Contrast to this, the unvaccinated/placebo group showed a more prolonged morbidity of 50% with similar clinical signs as the vaccinated group and a higher mortality of 0.13%. Additionally, the unvaccinated group showed general malaise and depression with 10% of them culled and kept in a holding pen. .
A graphic analysis (Fig. 1) of the mortality trends from farm records showed an undulating curve for the unvaccinated group with spikes of mortality by the 8th, 28th and 73rd day indicating higher mortality pattern within the period of sixteen weeks. The mortality trend for the vaccinated group showed a single lower spike of mortality by the 7th day post vaccination and the curve was more levelled than that of the unvaccinated group within the duration of the 16 weeks.

Litter Faecal Pattern

The litter bloody faecal pattern visual scores in vaccinated and unvaccinated groups are presented in Table 1. Several spots of brownish diarrhoea and bloody faeces were observed in the litter of both vaccinated and unvaccinated groups and were visually scored using the method advanced by Dogo *et al*. (2007). The unvaccinated group showed faeces with high bloody material (20 pluses) over 8 weeks period of observation when compared with the vaccinated group (8 pluses). The observation of the presence of bloody faeces in litter was of longer duration in the unvaccinated group than the vaccinated. However, the faeces became normal after treatment with anticoccidial drug.

Post Mortem Lesions

During post mortem examination of dead birds, varying degrees of gross lesions were observed and scored on a scale of 0 (no gross lesion) to 4 (extremely severe gross lesion) as described by Jonhnson and Reid (1970). Some of the typical gross lesions include ballooning of the caecum, presence of frank blood in the intestines, haemorrhages, necrosis and purulent lesions (Fig 2).

The significant (2-tailed) value of 0.002 is less than 0.05, indicating that there is a statistically high significant difference between the mean gross lesion scores for the vaccinated and the unvaccinated group.

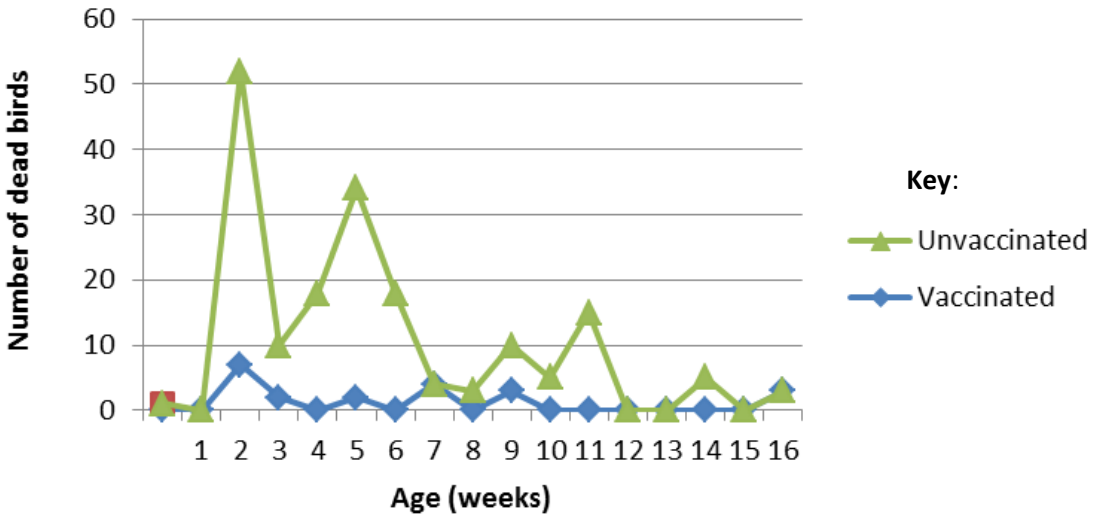

Fig. 1: Comparison of weekly mortality between vaccinated and unvaccinated groups

Table 1: Assessment of degree of bloody faeces post vaccination

Period (wks)	1	2	3	4	5	6	7	8
Vaccinated	+	+++	++	+	+	-	-	-
Unvaccinated	-	++++	+++	++	++++	+++	++	++

Notes: Normal faeces (-); 25% blood in faecal material (+); 26–50% blood in faecal material (++); 51-75% blood in faecal material (+++); >75% blood in faecal material (++++)

Table 2: Total number of dead pullets examined during post mortem and severity of gross lesion Observed

	Number Vaccinated	Number Unvaccinated	Score	Description
	0	0	0	No lesion
	3	2	1	Mild gross lesion
	9	3	2	Moderate gross lesion
	8	11	3	Severe gross lesion
	0	4	4	Extremely severe gross lesion
Total	20	20		

Table 3: Gross lesion score for the Vaccinated and the unvaccinated groups

	no gross lesion	mild gross lesion	moderate gross lesion	severe gross lesion	extremely severe gross lesion	**Total Scores**	**Mean gross lesion scores**
Vaccinated	**0**	**0**	**26**	**21**	**0**	**47**	**2.35**
Unvaccinated	**0**	**0**	**6**	**39**	**16**	**61**	**3.05**

DISCUSSION

This study has demonstrated that vaccination of pullets with life attenuated vaccines such as Livacox vaccine confers protection against coccidiosis. This was evidenced by lower mortality trend, lower visual scores of bloody faeces in litter and reduced mean gross lesion scores in the vaccinated pullets. Hence the observations in this study strengthen existing reports (Kitandu *et al*., 2005; Conway and Mckenzie, 2007; Akande *et al*., 2012) on the use of life attenuated vaccines for the protection of pullets. These reports indicated that vaccination of pullets with life attenuated vaccines confers life immunity against coccidiosis. The report by Akande *et al*. (2012) showed that the vaccine was protective to varying degrees against heamatological challenge and thereby demonstrates the importance of coccidial vaccines.

The lower morbidity and higher mortality with the unvaccinated group as observed in our study was expected because peaks in vaccinated birds in the first two weeks was due to the birds adjusting to the vaccination unlike in the same period for the unvaccinated group. This could be ascribed to the pattern of natural coccidiosis infection in poultry. This agrees with an earlier study by Muazu *et al.* (2008) indicating a higher mortality of 52% in young chicks of age 2 - 3 weeks.

The clinical signs (ruffled feathers, bloody diarrhoea, frank blood and listlessness) that commenced by the second week after vaccination and eventually waned by the 4th week in the vaccinated group showed close relationship with the mortality trends. These observations are consistent with earlier reports on clinical coccidiosis after coccidial vaccination (Kitandu *et al*. 2005; Sharman *et al*. 2010; Usman *et al*. 2011). Contrastly, the unvaccinated group witnessed mild to severe signs of coccidiosis with concomitant rapid onset within the first two weeks peaking at weeks 2 and 5 compared to the vaccinated counterpart, which corroborate the reports of Dogo *et al.* (2007).

The increased mean gross lesion scores noted in the unvaccinated group implies that the birds were not primarily protected from coccidiosis and its secondary opportunistic infections. Remarkably, this study shows that coccidial vaccine used protected the vaccinated group as

evidenced by their reduced mean gross lesion scores. These findings on improvement of mean gross lesion scores by coccidial vaccine agrees with earlier reports on coccidial vaccine trials (Allen and Fetterer, 2002; Kitandu *et al*. 2005; Conway and Mckenzie, 2007; Sharman *et al*. 2010; Price and Barta, 2010; Usman *et al*. 2011; Blake and Tomley, 2014; Singh *et al*. 2014). The above studies demonstrated the effectiveness of various live attenuated vaccines in protecting birds against coccidiosis. Also the result from this study is in congruence with the findings documented from related study using a similar brand of coccidial vaccine (Imocox) by Akande *et al*. (2012). Furthermore, the success rate in the use of Livacox vaccine to protect birds against coccidiosis could be the reason for the high adoption rates in the use of the vaccine by farmers as reported by Oladoja and Olusanya (2007) in Ijebu Area of Ogun State, Nigeria. This preliminary observations demonstrated the feasibility of protection of pullets against coccidiosis using Livacox vaccine in Plateau State.

CONCLUSION

This study demonstrates that the use of Livacox vaccine could result in the protection of birds against coccidiosis and may be a leverage for improved egg production in layers. However, it is envisaged that there is need for further studies to determine the impact of vaccination in layers in the poultry industry.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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