Serological evidence of egg drop syndrome 1976 in backyard poultry flocks in Southwestern Nigeria

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Poultry, viruses,
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Summary

In Nigeria, egg drop syndrome 1976 (EDS'76), unlike other poultry diseases, has been given little attention as a cause of economic losses due to decreased egg production, particularly in backyard poultry flocks. This study aimed to investigate the presence of EDS'76 virus in backyard poultry flocks in Oyo and Osun states, Southwestern Nigeria. Blood samples were collected in 24 farms from 218 apparently healthy, unvaccinated birds which comprised 30 Japanese quails, 75 turkeys, 30 ducks, 57 indigenous chickens and 26 guinea fowls. Indirect ELISA was used to detect anti-EDS'76 virus antibodies in sera from the birds, and poultry owners were interviewed on their purposes with regard to bird raising. Overall, 139 (63.8%) sera were positive for EDS'76 with 26.7% (8/30), 90.7% (68/75), 33.3% (10/30), 89.5% (51/57) and 7.7% (2/26) from Japanese quails, turkeys, ducks, indigenous chickens and guinea fowls, respectively. Some of the farmers practiced placing eggs from guinea fowls under brooding indigenous hens for natural incubation, or sold eggs from turkeys and ducks to commercial hatcheries. Our findings suggest that these bird species serve as reservoirs of EDS'76 virus with the probable involvement of backyard poultry in its transmission, particularly to commercial poultry and other birds in Southwestern Nigeria. Thus, backyard poultry should be included in anti-EDS'76 vaccination schedules in the country.

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■ INTRODUCTION

The 'backyard' poultry is estimated to account for over 90% of the total poultry population in rural and suburban areas in Nigeria. In backyard poultry production, the extensive management system is usually practiced, i.e. birds are allowed to roam free, or different poultry species are raised in the same cages due to limited breeding space. This small-hold poultry provides an important source of income, family assets and high quality animal proteins in these areas, with little or no capital investment (Jagne et al., 1991). However, this production system tends to expose the birds to infectious diseases (Adebiyi and Fagbohun, 2017). These diseases can reduce productivity and cause high mortality rates. This often originates from poor disease control because of farmers' non-affordability of veterinary services and low financial capacity. Among the infectious diseases of poultry, egg drop syndrome 1976 (EDS'76) causes economic losses due to its direct effect on egg production (Cha et al., 2013).

This syndrome caused by EDS'76 virus, a double-stranded non-enveloped DNA virus within the genus *Atadenovirus* in the *Adenoviridae* family (Benko and Harrach, 1998), was first described in laying hens and is characterized by the production of soft-shelled and shell-less eggs by apparently healthy birds (McFerran et al., 1978). This economically important poultry disease that causes decreased egg production and occasionally respiratory distress has been recognized worldwide in different wild and domestic birds (Brash et al., 2009; Cha et al., 2013). Although EDS'76 virus is mainly transmitted

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through contaminated eggs, sporadic outbreaks have also been traced to contact of chickens with other birds, contaminated fomites and droppings (Clark, 2019). Commercial hens are routinely vaccinated against EDS'76 whereas most small backyard flocks are not (Clark, 2019).

Several studies show that all ages and breeds of chickens are susceptible to EDS'76 although differences in the response may occur (McFerran and Smyth, 2000; Cha et al., 2013). In addition, antibodies have been detected in other species of birds such as cattle egrets, owls, pheasants, geese and Muscovy ducks (McFerran and Smyth, 2000).

In Nigeria, EDS'76 unlike other poultry diseases is given little attention as a cause of economic losses in poultry industry due to decreased egg production. Emphasis has been on the control of viral diseases such as Newcastle disease, infectious bursal disease, infectious bronchitis and avian influenza (Monne et al., 2015; Adebiyi and Fagbohun, 2017) without any attention given to the prevention and control of EDS'76 virus infection particularly in backyard poultry flocks. This is probably because the affected birds are apparently healthy (Ezema et al., 2008). In addition, there are sparse studies reporting serological evidence of EDS'76 in backyard poultry in Nigeria with the majority of such reports carried out in commercial chicken flocks (Nawathe et al., 1980; Elayo et al., 2010; Igbokwe et al., 2020). Hence, this study aimed to investigate the presence of this virus in backyard poultry flocks in Southwestern Nigeria.

■ MATERIALS AND METHODS

This study was carried out in line with the National Code for Health Research Ethics and was approved by the Oyo State Ministry of Health Research Ethics Committee (AD13/479/346).

Farmers, and animal sampling

A total of 218 adult unvaccinated apparently healthy birds from backyard flocks were used in this cross-sectional study. The birds comprised 30 Japanese quails, 75 turkeys, 30 ducks, 57 indigenous chickens and 26 guinea fowls from 24 backyard poultry flocks in Oyo and Osun states, Southwestern Nigeria. The poultry owners were interviewed on the major purposes of bird raising.

About 2 ml of blood was aseptically collected from the jugular or brachial vein of each bird. The blood was centrifuged at 1500 *g* for 5 min and separated sera stored at -20°C until tested.

Detection of EDS'76 antibodies

The sera were screened using an indirect enzyme-linked immunosorbent assay (iELISA) kit (Green Spring, Shenzhen Lvshiyuan Biotechnology, China) that detects avian EDS'76 virus immunoglobulin antibodies. The iELISA was carried out according to the manufacturer's instructions and the optical density (OD) was read with an ELISA reader at 450 nm (630 nm as reference).

According to the manufacturer's protocol, the positive control average OD 450 nm value is ≥ 0.6, whereas the negative control is < 0.15. To determine whether a sample is positive or negative, the OD value of a particular sample must be higher or lower, respectively, than the addition of 0.2 to the OD value of negative control; in addition, where OD of negative control is < 0.05, it is calculated as 0.05

Statistical analysis

Results were analyzed with GraphPad Prism version 7.0 (San Diego, USA). Data were subjected to descriptive statistics and one-way ANOVA at the level of significance $p < 0.05$.

■ RESULTS AND DISCUSSION

Results revealed that out of the 218 sampled birds, 26.7% (8/30), 90.7% (68/75), 33.3% (10/30), 89.5% (51/57), and 7.7% (2/26) were positive for EDSV'76 antibodies in Japanese quails, turkeys, ducks, indigenous chickens, and guinea fowls, respectively (Table I). EDSV'76 seroprevalence in all species was 63.8% (139/218). Although the seroprevalence varied between birds, differences were not significant. In addition, most (45.8%) of the poultry owners kept birds as a source of income, whereas 37.5% and 16.7% raised them for sustenance and mixed purposes, respectively. Other findings were that some farmers placed eggs from guinea fowls under brooding indigenous hens for natural incubation; some sold turkey and duck eggs to commercial hatcheries for poult and duckling production, others were either reluctant to allow sample collection from their birds, others yet outrightly refused.

Since its initial description, EDS'76 has been a major cause of loss in egg production as a result of production of eggs with abnormal shell shapes and sometimes shell-less eggs (Van Eck et al., 1976; Salihu et al., 2010). The detection of EDSV'76 antibodies in apparently healthy birds in this study revealed the circulation of the virus in Japanese

Table I: Seroprevalence of egg drop syndrome in backyard poultry species in Southwestern Nigeria /// *Séroprévalence du syndrome chute de ponte chez des volailles villageoises du sud-ouest du Nigeria*

Species	Sampling site	Num. sampled	Num. positive (%)
Quail	Ikire	10	3
	Ikire	20	5
Sous-total		30	8 (26.7)
Turkey	Adeoyo	14	13
	Alexander	23	23
	Soka	6	3
	Olomi	15	14
	Oluyole	7	7
	Ikire	10	8
Sous-total		75	68 (90.7)
Duck	Odo ona	13	3
	Ologuneru	2	0
	Orita aperin	3	2
	Apata	9	2
	Apata	3	3
Sous-total		30	10 (33.3)
Indigenous chicken	Odo ona	9	7
	Akako	3	3
	Ona ara	12	10
	Ologuneru	5	4
	Ita aperin	4	4
	Fatimoh	3	3
	Adabeji	3	2
	Apata	6	6
	Gada	12	12
Sous-total		57	51 (89.5)
Guinea fowl	Sasha	12	1
	Bode	14	1
Sous-total		26	2 (7.7)
Total		218	139 (63.8)

quails, turkeys, ducks, indigenous chickens and guinea fowls raised in backyard poultry systems in Oyo and Osun states. Since these birds were unvaccinated against EDS'76, the detected antibodies could only have resulted from seroconversion following natural infection with the virus. Thus, the birds could serve as reservoirs shedding the virus in the environment, playing a crucial role in the epidemiology of the disease. In addition, since the birds showed no clinical signs of disease and were apparently healthy, they may have played a carrier role in the transmission of the virus particularly to commercial poultry (Adebiyi and Fagbohun, 2017) and perhaps to other birds. This finding is consistent with previous reports of infection with EDS'76 in poultry elsewhere (Brash et al., 2009; Cha et al., 2013), including the fact that most backyard poultry are not vaccinated against EDS'76 (Clark, 2019), and that affected birds are apparently healthy (Ezema et al., 2008). The backyard poultry system is usually confronted with poor management, lack of veterinary care and poor disease control which contribute to low productivity and high mortality rates (Salihu et al., 2010). Most of the birds in this study were allowed to scavenge which could allow for unrestricted spread of the disease and contribute to the high prevalence of EDSV'76 antibodies in these birds. Similarly, evidence of EDS'76 infection in free-range flocks that had contact with wild fowls, geese or ducks has been reported (Salihu et al., 2010).

Furthermore, backyard poultry production where the extensive management system is usually practiced, i.e. birds are allowed to roam free, or where different poultry species are raised in the same cages because of the limited breeding space, tend to expose birds to infectious diseases (Adebiyi and Fagbohun, 2017). This possibility of virus transmission from these birds to commercial poultry and other birds may be of veterinary importance considering that passage of EDSV'76 through chickens may result in increased pathogenicity (Brugh et al., 1984).

The practice by some small-hold farmers to place eggs from guinea fowls under brooding indigenous hens may ensure endemicity of EDS'76 within backyard poultry in the study area, as the "virus is excreted through the cloaca and originates in the oviduct and will be present in and on eggs for up to three weeks" (Smyth and Adair, 1988). Furthermore, studies show that "following infection *in vivo*, there was no excretion until onset of lay, when the unmasking of the virus resulted in virus excretion and rapid spread" (Kaleta et al., 2003; Bidin et al., 2007).

Additionally, some farmers incubated eggs of turkeys and ducks in commercial hatcheries. These practices may cause transmission of the virus to egg-producing flocks and wide spreading of EDS'76 in poultry. "This is due to the presence of virus on the exterior of eggs, leading to contamination of trays and trolleys. In many cases, this equipment is not adequately cleaned or disinfected before being returned from the egg-packing plants to other farms at random" (McFerran and Smyth, 2000).

■ CONCLUSION

The findings of this study revealed that egg drop syndrome '76 virus circulates in Japanese quails, turkeys, ducks, indigenous chickens and guinea fowls in Oyo and Osun states, Southwestern Nigeria, indicating that these bird species serve as reservoirs of EDS'76 virus. They underscore the importance of the routine surveillance for EDS'76 in different avian species. Backyard poultry production, being an important economic activity for small-hold farmers in Southwestern Nigeria, should be given adequate attention in disease control and prevention and considered in EDS'76 vaccination schedules in the country. Also, there is a need to include backyard poultry for further studies to determine the genotype of EDSV'76 circulating in the country.

Conflicts of interest

The study was carried out without conflicts of interest.

Author contributions statement

AIA conceived and designed the study. AIA and AFF collected samples. AIA conducted the experiment. AIA and AFF analyzed the results. AIA drafted the first version of the manuscript. Both authors read and approved the manuscript.

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Résumé

Adebiyi A.I., Fagbohun A.F. Mise en évidence sérologique du syndrome chute de ponte 1976 dans les élevages de volailles villageoises du sud-ouest du Nigeria

Au Nigeria, le syndrome chute de ponte 1976 (EDS'76), contrairement à d'autres pathologies aviaires, n'a reçu que peu d'attention en tant que cause de pertes économiques dues à la diminution de la production d'œufs, en particulier chez les volailles villageoises. Cette étude a eu pour objectif de rechercher la présence du virus du EDS'76 chez les volailles villageoises des états de Oyo et Osun, au sud-ouest du Nigeria. Des échantillons sanguins ont été prélevés dans 24 élevages sur 218 oiseaux apparemment sains et non vaccinés, dont 30 cailles japonaises, 75 dindes, 30 canards, 57 poulets indigènes et 26 pintades. Le test ELISA indirect a été utilisé pour la présence d'anticorps contre le virus du EDS'76 dans les sérums des oiseaux, et les propriétaires des volailles ont été interrogés sur leurs objectifs en matière d'élevage. Au total, 139 (63,8 %) sérums ont été positifs pour EDS'76, dont 26,7 % (8/30), 90,7 % (68/75), 33,3 % (10/30), 89,5 % (51/57) et 7,7 % (2/26) provenaient respectivement de cailles japonaises, de dindes, de canards, de poulets indigènes et de pintades. Certains éleveurs plaçaient les œufs de pintades sous des poules indigènes pour une incubation naturelle ou vendaient les œufs de dindes et de canards à des couvoirs commerciaux. Nos résultats suggèrent que ces espèces d'oiseaux servent de réservoirs du virus du EDS'76 et que les volailles villageoises sont probablement impliquées dans sa transmission, notamment aux volailles commerciales et aux autres oiseaux du sud-ouest du Nigeria. Les volailles villageoises doivent donc être incluses dans les programmes de vaccination contre EDS'76 dans le pays.

Mots-clés : volailles, virus, syndrome de chute de ponte '76, Nigeria

Resumen

Adebiyi A.I., Fagbohun A.F. Detección serológica del síndrome de caída de puesta 1976 en granjas de aves de corral de las aldeas del suroeste de Nigeria

En Nigeria, el síndrome de caída de puesta 1976 (EDS'76), a diferencia de otras patologías aviares, ha recibido poca atención como causa de pérdidas económicas debidas a la disminución de la producción de huevos, especialmente en las aves de corral de las aldeas. El objetivo de este estudio era investigar la presencia del virus del EDS'76 en las aves de corral de las aldeas de los estados de Oyo y Osun, en el suroeste de Nigeria. Se tomaron muestras de sangre en 24 granjas a 218 aves aparentemente sanas y no vacunadas, entre ellas 30 codornices japonesas, 75 pavos, 30 patos, 57 pollos autóctonos y 26 pintadas. Se utilizó la prueba ELISA indirecta para detectar la presencia de anticuerpos contra el virus EDS'76 en el suero de las aves, y se encuestó a los propietarios de las aves sobre sus objetivos de cría. Un total de 139 (63,8 %) sueros fueron positivos para el EDS'76. Correspondían al 26,7 % (8/30) de las codornices japonesas, el 90,7 % (68/75) de los pavos, el 33,3 % (10/30) de los patos, el 89,5 % (51/57) de los pollos autóctonos y el 7,7 % (2/26) de las pintadas. Algunos granjeros colocaban los huevos de pintada bajo gallinas autóctonas para su incubación natural o vendían los huevos de pavo y pato a incubadoras comerciales. Nuestros resultados sugieren que estas especies de aves sirven de reservorio del virus EDS'76 y que es probable que las aves de corral de las aldeas estén implicadas en su transmisión, especialmente a las aves de corral comerciales y a otras aves del suroeste de Nigeria. Por lo tanto, las aves de corral de las aldeas deberían incluirse en los programas de vacunación contra el EDS'76 en el país.

Palabras clave: aves de corral, virus, síndrome de caída de la postura '76, Nigeria