ISSN 1682-8356
ansinet.com/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2019.523.529

Research Article
Pathogenicity of Kenyan Infectious Bursal Disease Virus Isolates in Indigenous Chickens

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Abstract

Background and Objective: The pathogenicity of infectious bursal disease virus varies from mild to very severe. The severity of the disease depends on the virus pathotype and breed of chickens affected, among other factors. Indigenous chicken ecotypes in Africa are generally known to be resistant to diseases. This study was performed to determine the pathogenicity of local IBDV isolates (E42, E19, E7 and E9) in indigenous chickens in Kenya. **Materials and Methods:** Local chickens were inoculated intraocularly with 10^4 EID_{50} of virus. Pathogenicity was determined by using the following: (1) Symptomatic index scores on an ascending scale of 0 (no signs) to 3 (most severe signs), (2) Histopathological lesion scores of the bursa of Fabricius, thymus, spleen, caecal tonsils and Harderian gland and (3) Bursal index, thymic index and splenic index scores based on organ to body weight ratios. **Results:** All isolates caused severe clinical disease, high mortality rates and severe pathological lesions, as observed with very virulent IBDV pathotypes. The mortality rates were as follows: E42 = 16.7%, E19 = 27.8%, E7 = 61.1% and E9 = 66.7%. The mean symptomatic index scores were highest on days 3 and 4 post-inoculation. The highest scores were 2.4 (isolate E9, day 4), 2.2 (isolates E19 and E7, day 4 for both) and 1.6 (isolate E42, day 3). The most damaged organ was the bursa of Fabricius, followed by the spleen, thymus and caecal tonsils, with a minimal effect on the Harderian gland in all isolates. The organ index scores did not vary significantly between isolates ($p \geq 0.05$). **Conclusion:** Indigenous chickens developed severe disease when infected with Kenyan IBDV isolates. All isolates were phenotypically the very virulent pathotype.

Key words: Bursal index, histopathological scores, symptomatic index, thymic index, very virulent IBDV

Received: June 14, 2019 Accepted: July 22, 2019 Published: October 15, 2019

Citation: W.U. Mutinda, P.G. Mbuthia, L.W. Njagi, L.C. Bebora and P.N. Nyaga, 2019. Pathogenicity of Kenyan infectious bursal disease virus isolates in indigenous chickens. Int. J. Poult. Sci., 18: 523-529.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indigenous chickens are an important resource and a tool for wealth creation in rural areas worldwide[1]. Because they are presumed to portray natural immunity to most infectious diseases, they are rarely subjected to any disease control management practices[2]. Loss of chickens due to poultry diseases in rural areas impoverishes women and youths who are the main keepers of indigenous chickens[3]. Outbreaks of infectious bursal disease (IBD), one of the most important viral diseases of chickens, cause major economic losses in chicken farms worldwide[4,5]. The disease is caused by infectious bursal disease virus (IBDV), which is classified into pathotypes. The most virulent pathotypes, commonly referred to as very virulent (vvIBDV) or hypervirulent strains, cause high mortality rates of up to 100% in White Leghorn SPF chickens, while classical or standard pathotypes cause less mortality and finally, subclinical strains cause almost no mortality at all[6]. Further, there is severe damage to the lymphoid organs, even in non-bursal lymphoid organs, in vvIBDV infections, while less damage to lymphoid organs is observed in infections caused by classical and subclinical strains[7,8]. Information on the pathogenicity of Kenyan IBDV isolates in indigenous chickens from previous research is scarce.

Variation in the pathogenicity of IBDV has been observed in different breeds of affected chickens[9]. Although, some outbreaks of IBD in indigenous chickens in Kenya have been reported to cause high mortality rates, indigenous chickens are still seen as resistant and are rarely vaccinated against this disease[10]. The differences among mortality rates in outbreaks of IBD in indigenous chickens, broiler and layer flocks were not statistically significant[10]. Molecular studies have shown circulation of vvIBDV strains in exotic and indigenous chickens in the neighbouring countries of Tanzania and Zambia[11]. In the current study, the pathogenicity of Kenyan IBDV isolates in specific-antibody-negative (SAN) indigenous chickens was determined.

MATERIALS AND METHODS

Viruses: Four IBDV isolates from outbreaks in Kenya where mortality rates were low, moderate and high, as shown in Table 1, were selected. The outbreaks presented with clinical signs of IBD: ruffled feathers, white watery diarrhoea, anorexia, reluctance to move, depression and death. Dead or very sick birds had haemorrhages in the breast, thigh and leg muscles and enlarged bursae of Fabricius (Bfs) that were oedematous, haemorrhagic or necrotic and atrophied. Outbreaks were confirmed by antigen detection in BFs using the agar gel precipitation test (AGPT) with standardized antigen (Cat No. RAA0123 (IBDV Antigen) Lot No. BR28/08) and standardized antisera (Cat No. RAB0124 (IBDV Type 1+ve serum) Lot No. BR25/08) from the Animal Health and Veterinary Laboratories Agency, United Kingdom.

Outbreak flocks selected for sampling were those that had no other disease confirmed in the flock. The isolates were designated as viruses E7, E9, E19 and E42. They were titrated in 11-day-old embryos and 50% embryo infective doses (EID_{50}) were calculated using the Reed-Muench[12]method.

Experimental chickens: Five-week-old SAN indigenous chickens (common normal feathered, naked neck indigenous chicken ecotypes found in Kenya) were used in this experiment. The chicks were from eggs obtained from an isolated local farm in Nairobi that was disease free and did not vaccinate against Gumboro disease. They were hatched and raised in a bio-secure environment at the University of Nairobi, transferred to an inoculation room at the age of 5 weeks and allotted to experimental groups. The birds inoculated with the same isolate were housed in one room separately from the others. Inside the rooms, birds were kept in isolation cages. Each group was assigned an attendant. Feed and water were provided *adlibitum.* Prior to inoculation, the birds were bled and harvested sera were confirmed to be free from antibodies against IBDV by the AGPT and ELISA as described in the 2008 OIE manual[13].

Animal welfare: The Biosecurity, Animal Use and Ethics Committee of the Faculty of Veterinary Medicine, University of Nairobi, gave permission to use chickens in this study. The chickens were managed as per international regulations and ethical considerations of animal welfare in animal experiments[14].

Experimental design: Two hundred sixteen (216) 5-week-old SAN chickens were allotted to nine groups designated

Table 1: Viral isolates recovered from chicks of different ages in infectious bursal disease outbreaks with varying mortality rates

Virus isolate code	Source (counties in Kenya)	Type of chicken affected	Age affected (weeks)	Number in flock	Mortality rate (%)
E7	Nairobi	Vaccinated layers	9	1154	80
E19	Kiambu	Layers	4	200	25
E9	Kwale	Vaccinated layers	4	300	75
E42	Kilifi	Indigenous chickens	14	100	100

numbers 1-8, with 18 birds in each group. Groups 1-4 were used for the pathogenicity study, while groups 5-8 were used for the mortality study. Each chicken from the first to the eighth groups was inoculated via the eye drop route with 10^4 EID_{50} of the respective viral isolate; one isolate was handled at a time to avoid cross contamination. For each isolate, a total of 18 birds were left as controls and termed the ninth group. In the pathogenicity study, three birds picked at random from each group were weighed and necropsy examination was undertaken at days 1, 3, 4, 8, 11 and 14 post-inoculation. The bursa of Fabricius, spleen, thymus, caecal tonsil and Harderian gland were collected and weighed and the average organ/body weight ratios were calculated. For the mortality study, the birds were kept for 2 weeks post-inoculation and monitored twice daily. Clinical signs were scored daily as described by Le Nouën *et al*.[15] with minor modifications. Dead birds were necropsied and samples were collected and processed for histopathological examination.

Quantification of clinical signs: Clinical signs were quantified by the symptomatic index as previously described by Le Nouën *et al*.[15] with minor modifications. Scores on this index ranged from 0-3 with increasing severity, as shown in Table 2. The mean symptomatic index (MSI) score of the surviving chickens was calculated daily.

Organ to body weight ratio: The lymphoid organs sampled (bursa of Fabricius, spleen, thymus, caecal tonsils and Harderian gland) were weighed separately. The average of each organ/body weight ratio or index was determined by the following formula: (organ weight in grams/body weight of individual bird in grams)×1000 as described by Tanimura *et al*.[16].

Histological processing: Sampled organs were fixed in 10% neutral buffered formalin. Sections were made and stained with haematoxylin-eosin following a conventional procedure as described by Luna[17]. Histological lesions were scored from 0-5 according to the index defined by Muskett *et al*.[18]; 0 = no damage, 1 = mild necrosis in isolated follicles, 2 = moderate generalized lymphocyte depletion or isolated follicles with severe depletion, 3 = over 50% of follicles with severe lymphocyte depletion, 4 = outlines of follicles remaining with few lymphocytes and an increase in connective tissue, cysts and thickened corrugated epithelium and 5 = loss of all follicular architecture with fibroplasia.

Data analysis: Descriptive statistics were generated and a two-way ANOVA (IBM SPSS software) was used to compare the effect of different isolates on the body weight, bursal index, thymic index, splenic index, Harderian gland index and caecal tonsils index scores on different days post-inoculation. The interaction effect was tested for statistical significance to determine whether the effect of the number of days post-inoculation on body weight or any of the organ index scores depended on the type of isolate inoculated. A p-value of less than 0.05 ($p<0.05$) was considered statistically significant[19].

RESULTS

The results showed that the four isolates tested produced severe disease with high symptomatic index scores (Table 3) in indigenous chicks with extensive damage to the bursa of Fabricius and other organs of the immune system.

Table 2: Symptomatic index scores based on clinical signs

Scores	Description of clinical signs
0	No clinical signs observed
1	Clinical signs of IBD (ruffled feathers) evident in a quiet bird; the bird is stimulated by a sudden change in environment (light, noise, or vicinity of experiment observer); appears normal and motility is not reduced
2	Clinical signs of IBD evident even when the bird is stimulated; dehydration, diarrhoea, dullness and reduced motility
3	Severe clinical signs of IBD with prostration or death

According to Le Nouën *et al*.[15] IBD: Infectious bursal disease

Table 3: Scores of some of the evaluated pathogenicity parameters during the peak of disease and mortality rates associated with the isolates

	Infectious bursal disease virus isolates			
Parameters evaluated	E19	E42	E7	E9
Symptomatic index scores on day 4*	2.2	1.4	2.2	2.4
BF histology lesions on day 4*	3.5	3.5	4.5	4.3
BF index score on day 4*	2.3	3.1	3.1	2.9
Mortality rates (%)	25	100	80	75

BF: Bursa of Fabricius, %: Percentage, day 4*: This was the day when the birds were very sick and the symptomatic index scores were the highest

Clinical signs and mortality rates: Clinical signs of ruffled feathers, diarrhoea, dullness, reduced motility, prostration and death were observed in all inoculated chickens with slight variation in the duration and severity. Chickens inoculated with isolate E9 showed these signs from day 3-9, while those inoculated with either isolate E7 or E42 showed the same signs from day 2-6 post-inoculation. Chickens inoculated with isolate E19 also showed the same clinical signs of disease but from days 2-7 (Fig. 1). The highest mortality rate was in chickens inoculated with isolate E9 at 66.7%, followed by E7 at 61.1% and E19 at 27.8% and the lowest mortality rate was in chickens inoculated with isolate E42 at 16.7%. The highest symptomatic index score was recorded on day 4 for all the isolates and it was highest in isolate E9 at 2.4, followed by E7 and E19 at 2.2 each and finally isolate E42 at 1.4 (Fig. 1).

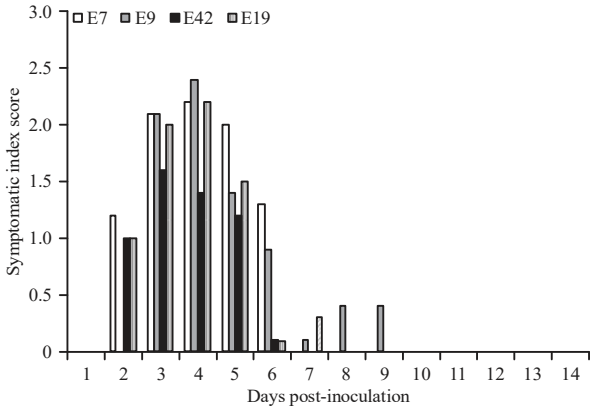

Fig. 1: The symptomatic index score (a measure of the severity of clinical signs according to Le Nouën *et al.*[12] per day per isolate)

Effect of the isolates on the weights of the bursa, spleen, thymus, caecal tonsils and body weight: The body weight of inoculated birds decreased starting from day 3 post-inoculation until day 8 and then rose to be comparable to the weight of control birds by day 14 (Fig. 2). The body weights had statistically significant differences on days 3, 4, 8, 11 and 14 post-inoculation ($p<0.0005$) but not among different isolates ($p = 0.54$).

The bursa index decreased starting from day 3 post-inoculation and continued to decrease steadily to day 14. The thymic index decreased to day 8 and then increased and the splenic index increased slightly throughout the experiment (Fig. 3). There was no statistically significant difference in the mean organ index between isolates: [bursal index ($p = 0.414$), thymic index ($p = 0.859$), splenic index ($p = 0.338$), Harderian index ($p = 0.105$) and caecal tonsils index ($p = 0.562$). Mean organ indices showed statistically significant differences between days 3, 4, 8, 11 and 14 post-inoculation for the bursal index ($p<0.0005$), thymic index ($p<0.0005$) and splenic index ($p = 0.001$) but not for the caecal index ($p = 0.089$) and Harderian gland ($p = 0.081$).

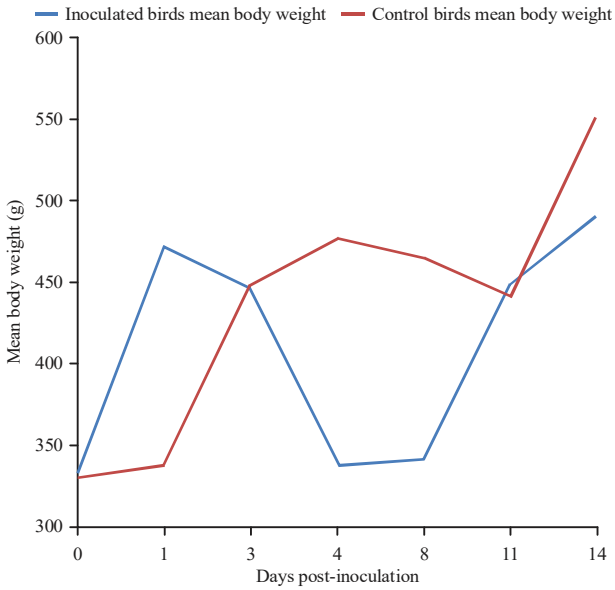

Fig. 2: Mean body weight of all inoculated birds compared with the control birds days post-inoculation
g: Grams

Pathologic changes induced by different isolates in the bursa of fabricius: Patches of focal necrosis were noted on day 1 post-inoculation in the bursa of birds inoculated with isolate E9, while birds inoculated with other isolates had no appreciable change in the bursa until day 3. The bursa of Fabricius was severely damaged by days 3 and 4 post-inoculation and there was some slight variation between isolates in severity of the damage. Isolate E7 had the most severe damage to the architecture of the BFs of inoculated birds, with an average score of 4.5 on days 3 and 4. Isolate E9 followed with a BF average score of 3.5 on day 3 that rose to 4.3 on day 4. This was followed by E19 at 3.5 and 4 on days 3 and 4, respectively and finally E42 at 3.5 on both days. Damaged bursae had haemorrhages, lymphocytic depletion, empty spaces, pyknotic and karyorrhexic lymphocytes and infiltration by granulocytes (Fig. 4). Fibroblasts and repopulation with lymphocytes were observed from day 8 and progressed to days 11 and 14 post-inoculation depending on the isolates. On day 14 post-inoculation, there was restoration of the normal architecture of the bursa of Fabricius (Fig. 5).

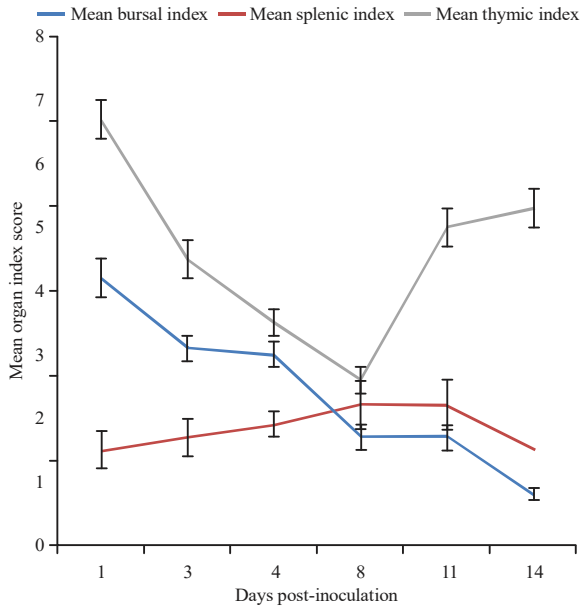

Fig. 3: Mean index scores for various organs in inoculated birds for different days post-inoculation

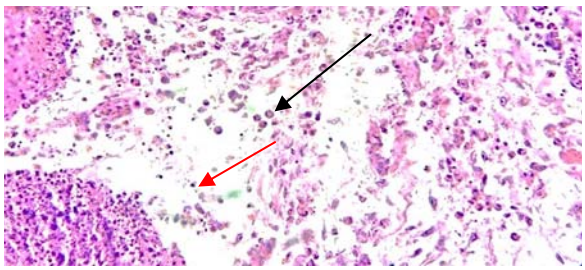

Fig. 4: Granulocytes (black arrow) and pyknotic lymphocytes (red arrow) in a bursa of Fabricius on day 3 post-inoculation with infectious bursal disease virus isolate E42 (×100 H and E)

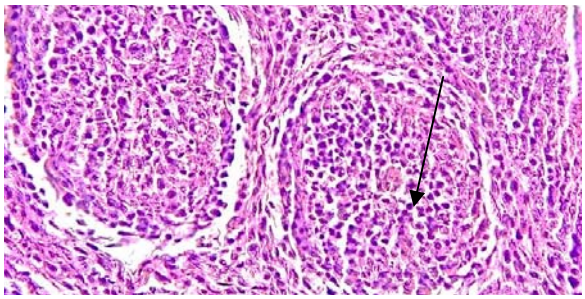

Fig. 5: Bursa of Fabricius architecture restored and repopulated with lymphocytes (black arrow) 8 days post-inoculation with infectious bursal disease virus isolate E42 (×100 H and E)

Pathologic changes in the spleen induced by different isolates: All the isolates caused diffuse degeneration and necrosis of lymphoid cells that were most evident in the peri-arterial sheath but with varying severity. There was minimal but appreciable pathology in the spleens of birds inoculated with isolates E7, E19 and E9 by the end of day 1 post-inoculation. The greatest pathology was observed on days 3 and 4 when scores were between 2 and 3 in all isolates. There was not much restoration to normal architecture in this organ on days 8, 11 and 14, unlike in the bursa where there was restoration. There was minimal variation among isolates regarding their effect on the spleen.

Pathologic changes in the thymus gland induced by different isolates: Lymphocytic depletion was observed on days 3, 4 and 8 with isolates E42, E7 and E19, after which the glandular architecture reverted to normal. Lymphocytic depletion with isolate E9 was observed on days 3, 4, 8 and 11. The highest average score was 2 on day 3 with isolate E42, followed by an average score of 1.7 on days 4 and 8 with isolate E9.

Pathologic changes in the caecal tonsils induced by different isolates: Karyopyknosis and depletion of lymphocytes along with infiltration with granulocytes were observed starting from day 3 with all isolates. The highest average score was 2.5 on day 3 with isolate E19, followed by an average score of 2 on the same day with isolate E9.

Pathologic changes in the Harderian gland induced by different isolates: All of the isolates had minimal effects on the Harderian gland. The organ was mostly highly populated with plasma cells. The highest score was 1.5 recorded on day 3 post-inoculation with isolate E7, followed by a score of 1 on day 4 with isolate E9. Isolate E19 yielded an average histological score of 0.5 on day 4. On day 8 post-inoculation, the score was 0.3 with isolate E9.Scores on all other days were 0 with all other isolates.

DISCUSSION

All four isolates in this study were pathogenic to indigenous chickens. There is variation in the pathogenicity of IBDV, from mild to very virulent[6]. Very virulent IBDV infections are characterized by severe clinical signs mainly of dullness, diarrhoea, prostration and death with an amplified and prolonged acute phase and high mortality rate[8]. These clinical signs were observed in this study and an exacerbated acute

phase was also observed. In acute experimental IBD following a 10^5 EID_{50} challenge, vvIBDV strains induce an approximately 50-100% mortality rate[13]. In this experiment, similar results were obtained with a lower dose of 10^4 EID_{50}; isolates E7 and E9 induced mortality rates of 61.1 and 66.7%, respectively.

Lesions in lymphoid organs were intense, as observed with vvIBDV pathotypes[7]. In IBDV infections, the bursa of Fabricius turns atrophic within 7 to 10 days but this atrophy is more rapid in vvIBDV infections, even at 3-4 days post-inoculation[8], as was observed with all of the isolates in this study. Furthermore, there is excessive inflammation due to the combination of activation of macrophages, production of pro-inflammatory cytokines and recruitment of heterophils, resulting in extensive lymphoid tissue damage in vvIBDV infections[20]. In addition, in vvIBDV infections, severe depletion of lymphoid cells was also observed in the non-bursal lymphoid tissues[21], as was observed with all the isolates in this study. All the isolates in this experiment produced severe clinical disease, severe lesions in the spleen and thymus gland, significant lesions on the caecal tonsils and even some observable pathology in the Harderian gland. In this study, the effect of the viruses on the thymus gland resulted in a decrease in the thymic index. Observations by other researchers found that a greater decrease in the thymic index and more severe lesions in the caecal tonsils, thymus and spleen occurred in chickens inoculated with vvIBDV[22]. In view of the above, the four isolates in this study demonstrated vvIBDV pathotype phenotypic behaviour when inoculated in indigenous chickens.

Genotypic and phylogenetic studies of IBDV isolates circulating in Tanzania and Zambia in the eastern and central southern African regions, respectively, showed that they belonged to the very virulent type[11]. This compares well with the findings of this study that all four IBDV isolates from outbreaks that occurred in different geographic areas of Kenya in the East African region were very virulent to inoculated susceptible indigenous chickens maintained in a bio-secure environment.

Isolate E42, which was found to be the least virulent of all isolates, had a 100% mortality rate in field outbreaks in indigenous chicks from which it was isolated. It is possible that the virus became attenuated in the laboratory or there was a relatively virulent and less virulent strain in the outbreak but the relatively less virulent strain was recovered.

This study has demonstrated that indigenous chickens in Kenya are highly susceptible to IBDV strains circulating in the region, confirming earlier observations made during outbreaks[10]. Indigenous chickens constitute 76% of the total poultry population in Kenya[23], they must be protected against IBD. Control of infectious bursal disease has been a challenge even in exotic chickens[24] that are produced under high-level biosecurity systems. Effective vaccination programs such asa priming with live vaccine followed by booster with killed vaccine regimen that yielded good results with experimental indigenous chickens in Kenya[25] should be applied.

CONCLUSION

This study has demonstrated that indigenous chickens in Kenya are highly susceptible to IBDV strains circulating in the region. The study has also demonstrated the very virulent phenotypic nature of the IBDV pathotypic strains circulating in Kenya.

ACKNOWLEDGMENT

The study was funded by The Government of Kenya through a grant approved by the National Council for Science Technology (RIG/03A).

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