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REGULAR ARTICLES

Risk factors associated with infectious bursal disease vaccination failures in broiler farms in Kenya

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Abstract Immunization together with application of biosecurity measures are the principal methods of preventing infectious bursal disease outbreaks in high-risk areas. However, outbreaks in vaccinated chicken flocks have been reported in many parts of the world as a result of factors of vaccine virus, animal, or vaccine handler. In Kenya, such outbreaks have been reported, but the causes have not been studied. This study aimed at determining the risk factors associated with vaccine handling leading to vaccine failure in broiler flocks in Kwale County, Kenya. Structured questionnaires and visual observations were used to collect data from 83 broiler farms, 6 breeding farms, and 17 vaccine outlets. Relative risk (RR) analysis was used to determine the association between identified potential risk factors and vaccination failure. Results show that vaccines were properly handled in all vaccine outlet shops. Breeding farms maintained high levels of biosecurity and employed standard vaccine handling practices. Basic biosecurity practices were poor in broiler farms. Broiler farms failed to meet all the recommended standard procedures for vaccine storage, reconstitution, and administration. Risk factors included poor vaccine storage (RR=8.7) and use of few drinkers to administer vaccine (RR=5.8); traces of disinfectants in drinkers used to administer live vaccine (RR=2.8); use of wrong vaccine—infectious bronchitis instead of infectious bursal disease vaccine (RR=2.1); and use of improper diluents (RR=1.6). Broiler farmers need training on basic farm biosecurity measures and standard vaccine handling practices.

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Introduction

Infectious bursal disease virus (IBDV) is an environmentally stable immunosuppressive virus (Mahgoub et al. 2012, pp. 2047–2057). Jackwood (2011) found that IBDV can provide an early natural exposure to chickens as young as 2 weeks of age. Earlier researchers found that immunization together with application of biosecurity measures are the principal methods of infectious bursal disease (IBD) prevention (Müller et al. 2012, pp. 133–139). Vaccination failures have been reported in many different parts of the world (Müller et al. 2012; Adamu et al. 2013, pp. 420–433) and attributed to different reasons. Some of the reasons are improper handling and administration of vaccine, virus antigenic differences, live attenuated vaccine virus potency, and residual maternal antibody interference with vaccine virus (Islam et al. 2008, pp. 22–30). Zorman et al. (2011) reported on field outbreak in broiler flock that had shown no significant antibody response to vaccination with intermediate vaccines. The aim of this study was to determine the farm level risk factors associated with vaccine handling that are involved in IBD outbreaks in vaccinated broiler chicken flocks in Kwale, Kenya. The study was on outbreaks of the severe form of IBD, commonly known as *gumboro* that the farmers could recognize.

Materials and methods

Study design

The study was conducted in Msambweni and Matuga divisions in Kwale, Kenya. It covered broiler farmers, breeders, and

agrovet shops serving the farmers. Structured questionnaires were administered, and visual observation was made to collect data on the control of infectious bursal disease virus from 83 broiler farmers, 6 breeding farm managers, and 17 staff of vaccine outlets. Biosecurity measures observed in the farms were recorded. Farmers were assessed by identification of IBD, and only those who identified it correctly were interviewed. The interview dealt on IBD vaccination schedules in operation, cold chain maintenance, vaccine storage and transportation, and vaccine reconstitution and administration. Data on incidences of IBD outbreaks in vaccinated flocks and the types of IBDV vaccines in the market was captured. Veterinary vaccines in use in Kenya are registered with the Pharmacy and Poisons Board which ensures quality, safety, and efficacy. The outlets are inspected at regular intervals to ensure compliance with laws and regulations regarding storage and dispensing of pharmaceuticals. Information on vaccination schedules that breeding farms recommended for their chicks was also collected. All data was analyzed using Statistical Package for Social Science (SPSS).

Calculation of relative risk

The association between suspected risk factors and vaccination failure was determined using relative risk analysis as was used earlier by Nyaga et al. (1980) and described by Leon (2004). Relative risk (RR) value was calculated as the probability of infectious bursal disease outbreak occurring in vaccinated broilers where the risk factor was present compared to the probability of outbreak in broilers where the risk factor was absent.

The same procedure was used to determine relative risk estimate for two, three, or even more test factor levels using one factor as reference point.

Interpretation of relative risk

The calculated relative risk value was interpreted as described by Leon (2004). If the relative risk was equal to 1, then the risk of vaccination failure in those who had the factor was equal to the risk in those who did not have the factor, and therefore there was no association between the factor and vaccination failure. Where the relative risk was less than 1, risk in those who had the factor was less than in those who did not have the factor. In the case where the relative risk was greater than 1, the risk in those who had the factor was greater than in those who did not have the factor. The confidence intervals for the estimated relative risk (R') were used to determine the significant deviation from 1.0. Furthermore, Fisher's exact test and chi-square were applied to each risk factor, and the significance was assessed.

Results

Vaccinations at broiler farms

Vaccinations were done by farmers and their farmworkers. Education of personnel handling vaccines and vaccinating birds was none (25 %), primary (33 %), secondary (31 %), and graduates (11 %). Seventy-four out of 83 broiler farmers (89.2 %) vaccinated their flocks against IBD, and 59.5 % (44/74) experienced outbreaks. None of these 74 farmers followed all the standard recommended procedures for vaccine storage, reconstitution, and administration.

Five (6.02 %) farmers confused infectious bronchitis (IB) with infectious bursal disease (IBD). They used the combined Newcastle/infectious bronchitis vaccine to protect against Newcastle disease and infectious bursal disease. Four of these farms experienced IBD outbreaks. Those who used non-IBD vaccines were 2.1 times more likely to get an outbreak than those who used IBD vaccines ($R'=2.1$).

Vaccinations at the breeding farms

In all the breeding farms, vaccinations were done by farm managers. The managers were veterinary technical personnel trained at certificate, diploma, or degree levels and handled vaccines according to standard recommended procedures. The farms obtained their vaccines directly from the suppliers. None of the breeding farms experienced IBD outbreaks.

Packing of vaccines for the broiler farms in agrovet shops

All the 17 vaccine outlet (agrovet) shops interviewed said that they packed vaccines in ice for their clients. Two of them had backup generators. The rest kept their vaccines in cool boxes during periods of prolonged blackouts. There was no association between agrovet shops and vaccination failure ($p>0.05$).

All the gumboro vaccines encountered in the agrovet shops were intermediate strains but from different companies and countries. The M.B. strain (Assia Pharmaceuticals) and CH/80 (Murphy Chemicals) were stocked by six (6/17; 35.29 %) shops, an intermediate strain (Hester Pharmaceuticals, India), marketed by Ultimate Vet, was stocked by 8 (8/17; 47.06 %) shops, while D78 (HighChem) and BUR 706 (Rhone Merieux) were found in one agrovet shop.

Operators and activities of the agrovet shops

Agrovet shops were operated by animal health assistants (41 %), pharmacy technicians (41 %), nontechnical people (12 %), and veterinarians (6 %). Farmers visited the agrovet shops with sick birds and /or carcasses for assistance on diagnosis and intervention measures. The agrovet operators made diagnosis and prescribed medicines. Occasionally,

postmortems were done in the backyard of the agrovet shops. Farmers rarely went to the veterinary clinics for advice. Farmers purchasing vaccines were served on the same counters where sick birds/carcasses were received and examined.

Vaccine packaging and storage from outlets and in the farm

Storage temperatures for all vaccines were indicated as 4–8 °C. Farmers and agrovet shop operators reported that vaccines were packed in ice from shops to farms. Majority (98 %) of the farmers said that the ice did not melt before reaching the farm. At the farms, vaccines were stored in different cooling facilities overnight, 2 weeks, or longer, and 8 farmers stored these in the fridge freezers, 6 in deep freezers, and 14 in the fridge.

Farmers who stored vaccines in the fridge freezer were more likely to get the outbreak than those who stored them in the fridge (R'=5.4) as shown in Table 1. There was an association between vaccine storage in nonrecommended temperature and IBD outbreak (RR=8.7). This association was found to be statistically significant (p=0.0193 chi-square test and p=0.0261 Fisher's exact test).

Diluents used to reconstitute vaccines and infectious disease outbreaks

Different types of diluents were used to reconstitute the IBD vaccines. These were tap water (35), rainwater (19), distilled water (14), well water (2), and other types of water including river water (4). Rainwater and tap water yielded a relative risk estimate above unity (R'=2.1 and 1.9, respectively), therefore more likely to get outbreak (Table 2). However, this association was not statistically significant (Fisher's test, p=0.501; chi-square test, p=0.335). Eight farmers indicated that irrespective of the source of their water, they sanitized the water with sodium hypochlorite and glutaraldehyde solutions (Waterguard® and Omnicide®). All eight farms experienced infectious bursal disease outbreaks in their vaccinated flocks.

Table 1 The different places where farmers stored gumboro vaccines in the farms and the outbreak reports in flocks vaccinated with such vaccines

Storage	IBD outbreak	Absence of IBD outbreak	Total	Vaccine failure[a] (%)	Relative risk estimate
Not stored	27	17	44	61.4	1.2
Fridge freezer	7	1	8	87.5	5.4
Deep freezer	6	0	6	100	Not computable
Fridge	9	7	16	56.3	1
Total	49	25	74	66.2	

IBD infectious bursal disease

[a] Percentage occurrence of disease in vaccinated flocks

Table 2 Frequency of outbreaks and the estimated relative risk for the different types of water used to reconstitute the vaccines

Type of water	IBD outbreak	No IBD outbreak	Total	Relative risk (R')
Tap water	25	10	35	1.9
Rain water	14	5	19	2.1
Well water	0	2	2	0
Others	2	2	4	0.8
Distilled water	8	6	14	1
Total	49	25	74	

R' denotes risk of outbreak on using one type of water against the risk of outbreak on using distilled water to reconstitute the vaccine

Three farmers heated water and used it while hot to reconstitute the vaccine. They had outbreaks of infectious bursal disease.

Number of drinkers used to administer the vaccine

Thirty-four farms used less than three 3-L drinkers per 100 birds to administer the vaccines (Table 3). These were more likely to get outbreak in their vaccinated flocks than those who used more than three drinkers (R'=5.8). Farmers who used few drinkers to administer the vaccine would walk around to disturb the flock during administration to ensure that each bird got some of the vaccine water. Use of less than three drinkers to administer the vaccine was a statistically significant risk factor associated with disease outbreaks (Fisher's, p=0.0015; chi-square, p=0.001).

Twenty-five farms indicated that they washed vaccine administration drinkers with antiseptic/disinfectant sodium hypochlorite solution (Jik®) and glutaraldehyde (Omnicide®). Washing drinkers with disinfectant was associated with vaccination failure (RR=2.8). Those who washed drinkers with disinfectants were 2.8 times more likely to get outbreaks than those who did not, but the association was not statistically significant; both p values (0.0733 from chi-square and 0.118 from Fisher's test) were slightly above 0.05. None of the farmers used skimmed milk or any other stabilizer in the reconstitution of the vaccine. Majority of the farmers (90.6 %) removed drinking water from the birds before

Table 3 Frequency of outbreaks and the estimated relative risk (R') for use of few drinkers to administer vaccines

Drinkers per 100 birds	IBD outbreak	No IBD outbreak	Total	Relative risk estimate (R')
Less than 3	29	5	34	5.8
3 or more	20	20	40	1
Total	49	25	74	

Table 4 Frequency of outbreaks and the estimated relative risk for different durations taken to consume vaccine water

Duration taken to finish vaccine water	IBD outbreaks	No outbreak	Total	Relative risk (*R*′)
>6 h	3	2	5	1.02
2–6 h	10	3	13	2.3
<30 min	14	5	19	1.9
30 min–2 h	22	15	37	1

R′ indicates the risk of outbreak in one duration relative to the risk of outbreak in the 30-min–2-h duration which was used as a reference period for *R*′ calculations

administering the vaccine. Failure to thirst the birds for 1–2 h was associated with IBD outbreaks (RR=1.2).

In 78.4 % of the farms, birds consumed the vaccine water within 2 h or less after reconstitution. More than half of the respondents indicated that birds finished the vaccine water within the recommended 30 min–2 h in duration. Farms which took 2–6 h to administer the vaccine water were more likely to get outbreaks in their vaccinated flocks than those who took 30 min–2 h (*R*′= 2.3), as shown in Table 4. This association, however, was not statistically significant ($p \geq 0.05$ both chi-square and Fishers' tests).

Source of day-old chicks and the timing of vaccination

Six breeding farms (H 1–6) marketed their day-old chicks in Kwale. High levels of biosecurity practices were employed in the breeding farms. The six breeding farms used different vaccination schedules on their parent stock. They recommended different vaccination schedules for chicks originating from them (Table 5). Failure to follow the vaccination program recommended by the breeders was associated with IBD outbreaks (RR=1.5).

Supply of water and feed to the birds

Thirty-six farms indicated that they vaccinated against IBD and provided clean water and feed ad libitum to the birds, while 38 did not. Table 6 shows the relative risk values of the factors investigated in this study.

Discussion

Infectious bursal disease is one of the economically important diseases that affect chickens worldwide. Outbreaks were found to occur frequently in vaccinated flocks in Kwale District of Kenya. Similar observations have been made in other parts of the world (Müller et al. 2012, pp. 133–139). Improper vaccine transportation and storage, inappropriate diluents, disinfectants, and sanitizers (chlorine and glutaldehyde) in diluents, or use of hot water to reconstitute the vaccine could inactivate the vaccine virus, leaving the birds susceptible to IBD. Infectious bursal disease virus is resistant to many environmental stresses (Guan et al. 2010, pp. 919–922). Doing postmortems of IBD sick birds at agrovet shops could lead to possible carry-over by farmers of live virus through formite contamination to their farms. All these activities were associated with disease outbreaks and were of critical biological importance and potential risk factors. In addition, most RR values were above unity; therefore, vaccination failure was more likely to occur where these activities were practiced. Furthermore, the computed *p* values and confidence interval for these factors lie on the borderline, meaning that the activities are worth noting and could develop problems. Some of these findings are similar to those reported by other researchers (Islam et al. 2008, pp. 22–30) on IBD breakdown, namely outbreaks due to cold chain breakdown, simultaneous vaccination and disinfection, and vaccination using inappropriate drinking water. Use of few drinkers, failure to thirst birds before administering vaccines, or birds

Table 5 Hatchery and farm vaccination programs and disease outbreaks in broiler farms

	IBD vaccination schedules for breeder hens				Vaccination schedule for progeny chicks using intermediate vaccine		Status of infectious bursal disease outbreaks in progeny chicks	
	1st dose D.P.H.	2nd dose D.P.H.	3rd dose W.P.H.[a]	Others W.P.H.[a]	1st dose D.P.H.	2nd dose D.P.H.	Outbreaks present	Outbreaks absent
H1	10	22	8–9	16–18	14	28	6	4
H2	7	28	–	–	14	21	1	1
H3	14	26	19	38	17	–	14	7
H4	18	26	18	–	–	–	1	0
H5	1	7	11[a]	18[a] and 16	1	14	0	2
H6	18	26	18	–	14–16	21–24	27	11

D.P.H. days post hatching, *W.P.H.* weeks post hatching, *H* hatchery, *IBD* infectious bursal disease

[a] Days post hatching

Table 6 A summary of the risk factors of infectious bursal disease vaccine failure

Risk factor	Risk factor present		Risk factor absent		Total	Relative risk (RR)
	Outbreak	No outbreak	Outbreak	No outbreak		
Use of wrong (non-IBD) vaccine	4	1	45	24	74	2.1
Birds starved of water and feed	25	13	24	12	74	0.96
Vaccine not packed in ice from source	0	0	49	25	74	–[a]
Storage of the vaccine not within the recommended temperature at the farm	13	1	36	24	74	8.7
Use of nondistilled water as diluent to reconstitute vaccine	41	19	8	6	74	1.6
Use of less than 3 drinkers per 100 birds	29	5	20	20	74	5.8
Washes drinkers with disinfectant	20	5	29	20	74	2.8
Birds not thirsted for 1–2 h before administering vaccine	24	11	25	14	74	1.2
Did not use skimmed milk	49	24	0	1	74	–[a]
Multivitamins not given at time of vaccination	49	25	0	0	74	–[a]
Vaccine water not consumed in 30 min–2 h in duration	27	10	22	15	74	1.8
Does not follow breeder's vaccination schedule	20	8	29	17	74	1.5

IBD infectious bursal disease

[a] RR was not computable

taking too long to consume the vaccine water could lead to uneven uptake of the vaccine, insufficient immune response, and ultimately vaccination failure.

Farmers in this study did not provide broiler chicks with multivitamins. Multivitamins are known to be antistress and immune stimulators as found by other researchers (Khan et al. 2003, pp. 192–196). Sources of stress encountered on the farms included heat stress, failure to provide feed and water ad libitum, and poor ventilation.

All the vaccines encountered were imported intermediate strain live vaccines. Importation of veterinary vaccines for use in Kenya is strictly limited to registered products. Registration of vaccines is a function of the Pharmacy and Poisons Board. Vaccination failure is a worldwide problem which has made it urgent to develop new vaccination strategies to counter emergence of new strains of IBDV (Mahgoub et al. 2012, pp. 2047–2057). Zorman et al. (2011) showed that intermediate strain vaccines failed to protect birds from field outbreaks in IBD high-risk area. This was in agreement with earlier results by Rautenschlein et al. (2005) and Yahia et al. (2008). Live vaccines may cause bursal atrophy, and, depending on their intrinsic characteristics or on the vaccination procedures, some may not induce full protection (Müller et al. 2012, pp. 133–139). Further studies to determine the ability of vaccines sold to farmers to protect against field strains in Kenya are recommended.

High immunity in breeding flocks is beneficial in protecting the offspring from field virus challenge during the critical first 2 weeks of life when the bursa is most vulnerable to damage induced by IBDV (Lemiere et al. 2013, pp. 46–51). The six breeding farms in this study practiced different vaccination regimes. Chicks entering the market from these hatcheries would have different levels of maternal antibodies and would need to be put under different vaccination schedules. A common observation was that a farmer could get chicks from one hatchery yet use the vaccination schedule recommended by a different hatchery, either issued with previous batch of chicks or borrowed from a neighbor. Residual maternal antibodies have been found to neutralize the vaccine virus and cause vaccination failure (Block et al. 2007, pp. 401–409). Timing of vaccinations could therefore be a potential risk factor of IBD vaccine failure in broilers in Kwale.

Breeding farms had no outbreaks in their vaccinated flocks. This may be because they maintained high levels of biosecurity and employed qualified animal health technicians to administer the vaccines to their flocks. In contrast, there was poor biosecurity practice in the commercial broiler farms.

In conclusion farmers failed to store, reconstitute, and administer the vaccines properly. Advice on disease control was sourced from noncompetent personnel in the vaccine outlet shops. Vaccination schedules recommended by the breeders were not followed. Training of farmers on proper handling of vaccines during storage, reconstitution, and administration is recommended. In addition, vaccination schedules should be harmonized and farmers, trained to improve biosecurity measures at the farm level.

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