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A serological survey for infectious bursal disease virus antibodies in free-range village chickens in northern Tanzania

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ABSTRACT

A study of infectious bursal disease (IBD) or 'Gumboro disease' seroprevalence rates in healthy, non-vaccinated indigenous scavenging chickens in northern Tanzania was conducted in November and December 2009 on 362 chickens raised in a traditional management system. Individual bird and flock-level information was collected using a semi-structured questionnaire, and serum samples were screened for IBD virus (IBDV) antibodies using the enzyme-linked immunosorbent assay (ELISA). The study revealed high rates of IBDV antibodies, yielding an overall seropositive rate of 58.8 % and with at least one positive bird detected in 82.8 % (74/90) of flocks. Univariate logistic regression analysis revealed that seropositivity to IBDV varied significantly ($\chi^2 = 16.1$, $P < 0.001$) between the study sites. The flock seroprevalence was found to vary from 37.5 % to 91 % between districts and from 75 % to 90 % between regions. The results of this study showed that IBD is an endemic and widely distributed disease in northern Tanzania.

Keywords: free-range chickens, infectious bursal disease, prevalence, risk factors, Tanzania.

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INTRODUCTION

Free-range poultry are an important constituent of Tanzanian's poultry industry, with 80 % of the country's 56 million chickens reared in traditional free-range systems in villages[20,21] Most flocks are small and of mixed age, distributed between many households and feed mainly by scavenging. This feeding habit exposes them to contact with wild birds, which might be a source of infectious bursal disease virus (IBDV) infection[16,32]. The seroprevalence of IBDV has been demonstrated in free-living wild birds in Japan including migratory species[24]. Very virulent IBDV has been isolated from wild birds in Korea[11].

Infectious bursal disease (IBD) or Gumboro disease is a highly contagious immunosuppressive viral infection of young chickens (3–6 weeks old) causing severe economic and production losses worldwide[22]. IBDV is the causative agent of IBD. IBDV is a double-stranded dsRNA virus that has a bi-segmented genome and belongs to the genus *Avibirnavirus* of the family *Birnaviridae*[26]. The virus is extremely lymphocidal and infects IgM-bearing B-lymphocytes in the bursa of Fabricius, leading to immunosuppression[1]. There are two distinct serotypes of IBDV, and within each serotype antigenic variation is considerable[9]. Only serotype 1 viruses cause economically significant immunosuppressive disease in young chickens[1,30]. Serotype 2 infects chickens and turkeys but does not cause clinical disease. 'Variant' strains of IBDV, which have major antigenic differences from the 'standard' strains, cause immunosuppression but not clinical disease in older chickens[10]. Continuous presence of IBDV in village poultry populations has been reported elsewhere[13,31]. The first IBDV isolates from various locations in Tanzania were characterized as very virulent (vv) type in 2007[13]. These 'variants' were found to be widely distributed throughout Tanzania and demonstrated great similarities with isolates from western Africa and European/Asian vvIBDV variants[14,19].

Some of the risk factors that have been associated with the maintenance of IBDV include carrier chickens, village poultry population dynamics, other poultry species, including wild birds, and heterogeneity of IBDV[13,29,30]. Transfer of the virus from house to house is through fomites. The virus is also released in the faeces, ingestion of which is the main method of bird-to-bird spread[33].

The epidemiology of IBDV in village chickens in Tanzania is insufficiently studied but it appears that IBDV is the most important recurring disease every year[2,31]. Although IBDV represents one of the most severe poultry diseases and is responsible for marked economic losses, few studies of IBDV have been done on chickens in Tanzania, which hinders the implementation of effective disease-control measures. For this reason, the aim of the present study was to determine the seroprevalence rate of IBDV and the factors that may be associated with risk of infection in free-range poultry in northern Tanzania.

MATERIALS AND METHODS

Study sites

This study was carried out in 4 regions (Tanga, Kilimanjaro, Arusha and Manyara) comprising 7 administrative districts (02'11–6'14S, 35'11–38'26E) in northern Tanzania.

The climate is sub-humid with temperatures ranging from 14°C to 23°C in high elevation areas and 30°C to 37°C along the northern coast of the Indian Ocean. The study areas experience 2 main seasons, the dry season from May to October, and the wet season from November to April. Rainfall ranges from 635 mm to 3.050 mm, with low rainfall in the low-lying areas and high rainfall on the high-altitude and plateau areas.

Household selection and data collection

Households for the study were selected based on the past experience of chicken keeping, possession of chickens and willingness to participate in the study. This sampling procedure included 40 villages and 90 households. Primary data related to chicken production were collected through consultative procedures from various sources including the respective district agriculture and livestock department offices. The secondary data, coupled with a checklist and farm inspection, were collected using semi-structured

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questionnaires which were completed at all selected households during a single visit. The questionnaire was administered in the national Swahili dialect by veterinary department staff members, who were trained in participatory research methodologies. Important household and flock-level data recorded included location, owner, source (brought in or homebred), history of IBD vaccination, health status at time of visit (subjectively classified as healthy or unhealthy), sex, housing (classified as permanent chicken Banda or dwelling), external parasite type and infestation were categorized as Yes or No. Birds were categorized as follows: chicks (1 to ≤3 months of age), growers (>3 to ≤9 months) and adults (≥9 months). The data were collected during November and December 2009.

Blood sampling and storage

Approximately 1.5 to 2 mℓ of blood was collected from the humeral region of the wing vein with a 3-mℓ syringe. The syringe was laid nearly horizontally until the blood clotted. After clotting, the syringe was returned to a vertical inverted position to permit the serum to ooze out. The sample was then kept at 37 °C for several hours or left overnight before the serum was removed. The separated serum was transferred to cryovials, labelled, and stored at –20 °C until the enzyme-linked immunosorbent assay (ELISA) was performed to detect antibodies against the IBD virus[26,27].

Serum analysis

The ELISA test was done following procedures outlined by the infectious bursal disease virus antibody test kit (ProFLOK Plus, Synbiotics Corporation, San Diego, CA, item no: 96-6500) and Snyder *et al.*[28] All serum samples tested in the present study were processed in a single well each, according to the standard protocol. Briefly, the ELISA plates (Co-star) were supplied pre-coated with IBDV antigen. The samples were added and the plates subsequently washed and soaked, followed by the addition of 100 $\mu\ell$ of goat anti-chicken IgG conjugate [HRP/IgG (H+L) PO] diluted at 1:100 into all wells and incubation at room temperature for 30 min. Following incubation, the plates were soaked, washed in PBS-T and developed by adding 100 $\mu\ell$ of ABTS-Hydrogen Peroxide/Substrate solution per well and incubated at room temperature for 15 min. Absorbance was measured using an ELISA reader (Multiscan Ex., Lab systems, Finland) at 405 nm. The reader was connected to a computer loaded with ELISA Data Interchange (EDI) software. Calculation of the sample to positive (sP) values was done manually using the following equation: [sP = (sample absorbance) – average normal control absorbance)/corrected positive control absorbance]. Sp values were manually converted to percentage positivity (PP) by using the following equation: [PP = Sp value of the sample/Sp value of the corrected positive control] × 100. Samples with Sp values of 0.299 and higher or equivalent to PP ≥30 % (cut-off) were considered positive for IBDV infection.

Data analysis

Collected data sets (birds, flock and laboratory) were entered, managed and analysed using Epi-Info, Version 6.04b[6] (Centres for Disease Control, Atlanta, USA). Descriptive statistics generated included counts, frequencies and proportions. Chi-square analysis was used to compare the association between dependent (seroconversion status: positive or negative) and independent variables (location, sex, age category, source, health status, level of ectoparasites infestation). In all chi-square tests a probability level of $P < 0.05$ was considered statistically significant.

RESULTS

Study flock characteristics

All the selected farms/flocks were visited, owner(s) interviewed and chickens sampled. A voluntary participating rate of 100 % was thus achieved. Overall, 362 birds of mixed ages and sex from 90 flocks/farms were examined. The number of birds examined and sampled per village varied from 2 to 29. No history of IBD vaccination was recorded in any of the surveyed sites. Thirty-two (8.8 %) birds had signs of illness (ruffled feathers, lacrimation) and drooping wings during sampling. The proportions of birds in each category of each variable investigated during the study are shown in Table 1.

Antibody response to IBDV exposure

Of 40 villages sampled, 35 had at least one individual bird IBDV seropositive, which implies a seroprevalence of 87.5 % (range, 66.6–100) among villages. The overall seroprevalence rate for IBDV antibodies was 58.8 % (213/362). Seroprevalence rates of 69.4 %, 66.7 %, 47.3 % and 54.8 % were found in Tanga, Kilimanjaro, Arusha and Manyara, respectively (Table 1). There was no statistically significant difference ($P > 0.05$) between sexes, source, health status, housing and age categories in IBD virus seroprevalence rates. However, a significantly lower seroprevalence was observed in birds infested with lice, 45.2 % (47/104), followed by tick infestation, 49.3 % (35/75), than flea-infested ones, 60.8 % (101/196). Of 90 flocks investigated, 74 (82.8 %) had at least one bird seropositive. The flock seroprevalence was found to vary from 37.5 % to 91 % between districts and from 75 % to 90 % between regions (Table 2).

DISCUSSION

There was evidence that exposure of indigenous free-range chickens and infection with IBDV was widespread in the 7 studied districts. Seroprevalence was significantly higher in Tanga and Kilimanjaro ($P < 0.05$) compared with Arusha and Manyara. This may suggest that free-range chickens in Kilimanjaro and Tanga are more at risk of IBD than in the Arusha and Manyara regions. In the Tanga region, seroprevalence to IBDV was highest in the Mkinga and Pangani districts, which are amongst the hottest, most humid and wettest districts in the region. Several studies have shown that IBDV is stable in the environment and capable of surviving for extended periods in contaminated farms[33]. The warm, humid coastal environment of the Tanga region may also favour the survival and spread of viruses. This observation warrants further investigation to elucidate the role of these climatic factors in virus survival and persistence.

The relatively higher overall seroprevalence rate of IBD virus antibodies in indigenous chickens may be attributed to a number of factors. The management system in traditional poultry production may favour widespread infection. Poor sanitary conditions, continuous exposure of chickens to range conditions and wild birds, nutritional deficiencies, the absence of vaccination in traditionally managed chickens, and contact of chickens of 1 village with those in other villages may facilitate the spread of IBDV. This is in agreement with a previous report[27]. The ease of contact at local open-air markets between chickens from different areas, which are then taken back to various localities, can undoubtedly facilitate the rapid spread and persistence of IBD among indigenous chickens.

The prevalence of IBDV antibody in birds reared under a free-range village management system in this study agrees with reports from other countries with similar chicken husbandry systems[5,25]. The overall individual bird-level seroprevalence of IBD in free-range village chickens in this study was higher than the reports of 30.7 %[17] and 30 %[23] in Sudan and Botswana respectively. Lower prevalence rates were also reported in indigenous village chickens in Cameroon

Table 1: **Seroprevalence and proportions of free-range village chickens in each category of each variable investigated in the study regions (November/December 2009).**

Variable	Number examined	(%)	Number (%) positive	Univariate analysis		
				OR	*P*-value	95 % CI, OR
Administrative district						
Pangani	100	27.6	69(69.0)	RF		
Mkinga	44	12.2	31(70.5)	4.2	0.001	1.70–8.38
Moshi	39	10.8	26(66.7)	2.1	0.002	1.01–6.91
Monduli	43	11.9	14(32.6)	0.8	0.620	0.43–9.12
Meru	50	13.8	32(64.0)	1.2	0.330	0.12–13.25
Ngorongoro	55	15.2	24(43.6)	0.9	0.560	0.32–11.67
Babati	31	8.6	17(54.8)	1.4	0.480	0.85–14.53
Administrative region						
Manyara	31	8.6	17(54.8)	RF		
Arusha	148	40.9	70(47.3)	0.8	0.340	0.81–8.22
Kilimanjaro	39	10.8	26(66.7)	2.5	0.002	1.13–4.61
Tanga	144	39.8	100(69.4)	3.2	0.001	1.35–6.74
Sex						
Female	293	80.9	172(58.7)	RF		
Male	69	19.1	41(59.4)	0.97	0.512	0.55–1.72
Age						
Adult	209	57.7	126(60.3)	RF		
Growers	126	34.8	75(59.5)	1.6	0.284	0.56–4.51
Chicks	27	7.5	12(44.4)	1.2	0.466	0.45–3.23
Health status						
Health	330	91.2	191(57.9)	RF		
Unhealthy	32	8.8	22(68.8)	0.62	0.157	0.26–1.43
Source						
Homebred	316	87.3	181(57.3)	RF		
Brought in	46	12.7	32(69.6)	1.70	0.114	0.83–3.53
Housing						
Chicken house	177	48.9	99(55.9)	RF		
Human dwelling	185	51.1	114(61.6)	1.27	0.272	0.83–1.98
Ectoparasite infestation						
No	130	35.9	83(63.8)	RF		
Yes	232	64.1	130(56.0)	0.71	0.147	0.45–1.15
Tick infestation						
No	291	80.4	178(61.2)	RF		
Yes	71	19.6	35(49.3)	0.62	0.068	0.35–1.08
Flea infestation						
No	166	45.9	112(57.1)	RF		
Yes	196	54.1	101(60.8)	0.86	0.272	0.55–1.34
Lice infestation						
No	258	71.3	166(64.3)	RF		
Yes	104	28.7	47(45.2)	0.46	0.001	0.28–0.75

OR = odd ratio; CI = confidence interval of OR; RF = reference factor.

(33.9 %)[5] and in backyard chickens in Zimbabwe (55 %)[15]. Similarly, the mean seroprevalence obtained was comparable to the reported prevalence of 60 % in village chickens in Sahel zone of Nigeria[8]. Similar studies revealed antibodies to IBDV to be distributed in poultry worldwide[7,22]. The positive samples found in unvaccinated flocks indicated that IBD virus was circulating in those farms.

Source, housing system, age and sex had no significant effect on IBD seroprevalence. This finding agrees with reports from other areas of the country and countries with similar back yard chicken production systems[15,18,32]. In contrast to our finding, significant effects of age (chicks) were reported by authors who detected higher seroprevalence in semi-scavenging reared chickens in Bangladesh.[3]

This study indicated that chickens infested with ectoparasites (lice and ticks) were all associated with a lower probability of infection with IBDV. It is not clear, however, why the detected probability of infection was low, considering the number and level of ectoparasite infestation recorded during our survey. This observation may warrant further investigation.

Poultry diseases such as IBD were shown to be the most important constraints for commercial and local chicken production in rural settings of Tanzania[12,18]. Vaccination of village chickens against diseases such as IBDV is rarely undertaken, therefore the antibodies detected are most likely due to natural infection[8]. It is therefore recommended that IBD antibodies in other domestic birds such as ducks and turkeys be investigated. Preventive measures such as regular vaccination of young chicks and parent stocks should be instituted. Consistently, good husbandry practices such as good housing should be encouraged as stress is a major predisposing factor of the disease. It is vitally important that further detailed studies focus on the seasonality of IBD virus infection and strain identification so that preventive and control programmes can be designed.

Table 2: **Flock-level seroprevalence of IBD by region and district (November/December 2009).**

Region	District	Number of flocks examined	Number positive (%)
Arusha	Ngorongoro	11	10(90.9)
	Meru	16	14(87.5)
	Monduli	8	3(37.5)
Tanga	Mkinga	7	6(85.7)
	Pangani	26	22(84.6)
Manyara	Babati	11	9(75.0)
Kilimanjaro	Moshi	11	10(90.9)
Overall		90	74(82.8)

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