Original Article

A seroepidemiological investigation on major viral and bacterial pathogens in small-scale chicken flocks in the Mekong delta region of Vietnam

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Abstract

Small-scale commercial chicken farming represents an important source of income to rural households in the Mekong Delta region of Vietnam. A wide range of vaccines are currently administered to flocks, but is not based on empirical knowledge on pathogen circulation. We collected serum samples and vaccination data from a random sample of 267 small-scale native chicken flocks in Dong Thap to determine serological profiles by ELISA against 8 pathogens: Avian Influenza (AI), Chicken Anaemia virus (CAV), Infectious Bursal Disease (IBD) virus, Infectious Bronchitis virus (IBV), Newcastle Disease virus (NDV), *O. rhinotracheale* (ORT), *P. multocida* (PM) and *M. gallisepticum* (MG). The aims were: (1) to describe the vaccines used; (2) to investigate the relationship between titres and vaccination status; and (3) to identify diseases most likely to be circulating in the area by investigating the seroprevalence in unvaccinated flocks. A total of 33 commercial vaccines against 6 different pathogens had been used, and flocks had been vaccinated against a median of 4 [IQR 3-5] pathogens each. In decreasing order, the highest titres among unvaccinated flocks corresponded to CAV (97.0%), followed by IBD (88.6%), IBV (66.5%), ORT (56.9%), NDV (45.5%), MG (50.9%), AI (21.4%) and PM (2.7%). Given the frequency of clinical cases confirmed in diagnostic laboratory, results support maintaining vaccination programmes against IBD, IBV, NDV and AI. Since MG is commonly detected, inclusion of vaccination against MG is recommended. Ongoing serological monitoring of chicken flock should be carried in conjunction with vaccination data in order to adapt disease control measures to circulating pathogens.

Keywords: bacterial pathogen, chicken, seroepidemiology, viral pathogen, Vietnam

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Introduction

In the Mekong Delta region of Vietnam, small-scale commercial chicken farming represents an important source of income to rural households. In the area, it is common to raise chicken flocks using native breeds managed as all-in-all-out and raised over a 3-5 month period. Economic losses due to disease and mortality in flocks in the area are extremely high. A recent study reported a median cumulative mortality of 20.9 per 100 purchased birds over the production cycle, the highest mortality corresponding to the 5-10 week period (Carrique-Mas *et al.,* 2019). Most published diagnostic research in the region has focused on the detection and characterization of Highly Pathogenic Avian Influenza (HPAI), which has been circulating in Vietnam and other countries of the Greater Mekong region since 2003 (Suttie *et al.,* 2019), and is still active in Vietnam. A recent study of chicken flocks in Mekong Delta small-scale chicken flocks detected *A. paragallinarum,* MG, IBD and IBV in >20% flocks experiencing morbidity and mortality (Van *et al.,* 2020).

It is believed that an optimized vaccination program is key to successful disease control in poultry production (Marangon and Busani 2007). In the Mekong Delta region vaccination practices differ considerably from flock to flock, and farmers's decisions with regards to vaccination are often not knowledge-based and may be biased. From an immunological and economic perspective, there is a limit as to how many vaccines can be administered to flocks. Serological tests are performed with two aims, disease diagnostics and to evaluate exposure to pathogens and/or vaccination (Butcher 2002). Evaluating serum antibody titres from representative birds is an important flock disease management tool. In small-scale farming systems typical of LMICs serological testing is rarely, if ever, performed due to economic and logistics constraints. The correct interpretation of such test results requires the integration with age and vaccination data.

We investigated a random selection of small-scale chicken flocks in the Mekong Delta at the end of the production cycle in order: (1) to describe the vaccines used; (2) to investigate the relationship between titres and vaccination status; and (3) to identify diseases most likely to be circulating in the area by investigating the seroprevalence in unvaccinated flocks. We further related seroprevalence results to the frequency of detection of these pathogens in flocks in a previous study. This information is a first step to prioritize disease control strategies in small-scale chicken flocks in the area.

Materials and Methods

Study area and farm selection: The study was carried out in two districts (Cao Lanh and Thap Muoi) within Dong Thap province in the Mekong Delta region of Vietnam. The human population in the province was 1.7 M and the chicken population 5.1 M (2019). The area has a tropical climate with average daily temperatures ranging from 25°C to 28°C and a rainy season spanning May to October. Farms raising single age chicken flocks (i.e. all-in-all-out) for meat (with ≥100 birds) were randomly selected and their owners were invited to participate in a longitudinal study (Carrique-Mas and Rushton 2017).

Sample and data collection: Selected farms were visited from February 2017 to June 2019. We defined 'study flocks' as flocks restocked and followed up over their lifespan. Data on flock numbers and vaccination were collected over three regular visits during the production cycle (restocking with day-old chicks, mid-production and end of production). On the last visit, one representative bird was selected from the flock and ~0.5ml blood was collected from it by puncture from its brachial vein. Blood samples were placed in a cold box after collection, and were immediately transported to the laboratory. In the laboratory, samples were allowed to clot, and serum was decanted into 2 ml cryovials and were stored at -20 °C until further testing. Information on vaccination and number of chickens was collected using purposefully-designed record books. All visits and sample collections were conducted by trained veterinary staff affiliated to the Sub-Department of Animal Health and Production of Dong Thap (SDAH-DT).

Serological tests: Chicken serum samples were tested for the presence of IgY antibodies against the following eight pathogens: AI, CAV, IBD, IBV, NDV, ORT, PM and MG. All tests were performed using ELISA commercial kits from IDEXX (PM) (IDEXX, Maine, USA) and BioChek (Reeuwijk, The Netherlands) (all other pathogens). The manufacturers' instructions were carefully followed in all cases, and positive and negative controls provided by the manufacturer were used for each plate.

Data analyses: The ELISA optical density (OD) readings were interpreted as positive/negative result based on cut-off values provided by the manufacturer. Seroprevalence was modelled by a polynomial logistic regression as a function of age and vaccination status. The optimal degree of the polynomial regression was searched incrementally from degree 1 by likelihood ratio tests. The association between titres and vaccination (Y/N) was investigated by building linear models with serological titre (log_{10} transformed) as outcome, 'farm' as a random effect and 'vaccination' (Y/N), with 'flock size' (log) and 'age' as co-variables (Hens *et al.,* 2012). Since flocks were vaccinated with variable number of doses, we investigated the variable No. of doses (0. 1. >1) in replacement of 'vaccination'.

Results

Flock and vaccination data: The median number of flocks investigated per farm was 2 [Interquartile range, IQR, 1-4] per farm. In total, we investigated 267 flocks raised in 100 farms. Flocks were restocked with a median of 300 chickens [IQR 200-500]. Chickens were a median of 17 [IQR 15-18] weeks of age at the time of sampling (end of production) Flocks were vaccinated against a median of 4 [IQR 3-5] infectious diseases. Ten (3.7%) flocks had been vaccinated against one, 25 (9.4%) against two, 81 (30.3%) against three, 91 (34.1%) against four, and 60 (22.5%) against five diseases. A total of 256 (95.9%) flocks had been vaccinated against

NDV, followed by Avian Influenza (AI) (84.3%), IBD (83.5%), fowlpox (43.1%), fowl cholera (PM) (30.3%) and IBV (25.1%). 74.9% of all vaccine doses were given between weeks 1 and 4 of life. For NDV, HPAI, fowlpox, PM and IBV most (>60%) vaccinated flocks had been vaccinated with one dose only. In contrast, for IBD, 52% vaccinated flocks received two or more doses. A total of 33 commercial vaccines were identified (Supplementary Table 1). The number of commercial vaccines against each pathogen were: NDV (14), IBD (7), IBV (6), HPAI (3), PM (2) and fowlpox virus (1). All vaccines were live attenuated, except PM and AI vaccines, which consisted of inactivated (injectable) formulations.

Seroprevalence, vaccination and hazard rates: In decreasing order, the highest seroprevalence in unvaccinated flocks was CAV (97.0%), followed by IBD (88.6%), IBV (66.5%), ORT (56.9%), NDV (45.5%), MG (50.9%), AI (21.4%) and PM (2.7%) (Table 1). A significant increase of seroprevalence by age was detected for IBV and MG, but not for all other pathogens (Fig 1). Weekly hazard rates (HR) (i.e. probability of a flock becoming infected by week) for these pathogens were 0.071 (95% CI 0.061-0.083) (IBV) and 0.043 (95% CI 0.036-0.050) (MG). For ND, IBV, IBD, and AI titres did not differ between vaccinated and unvaccinated ones. However, titres against PM-vaccinated chickens were higher than for non-vaccinated ones (coef.=0.301; p=0.003). Flocks vaccinated with two doses of AI had a higher titre than those vaccinated with one shot or not vaccinated (coef. 0.324; p=0.007). In all other cases, vaccination with two doses did not result in a higher titre. Irrespective of age, the size of the flock (log) was associated with a higher titre for IBD (coef. =0.139; p=0.0028), NDV (coeff. =0.204; p=0.0015), CAV (coeff. 0.129; p=0.0026) and MG (coeff. =0.408; $p<0.001$). The flock size was highly correlated with the age, indicating that larger flocks generally were sold later (Pearsons's corr 0.370; $p<0.001$).

Table 1 Seroprevalence by vaccination status and estimated monthly hazard rates calcaulated from seroprevalence data of 267 small-scale chicken flocks in the Mekong Delta of Vietnam.

Pathogen	All flocks (n=267)	Vaccinated	Unvaccinated	Weekly HR (95% CI)
NDV	169 (63.3%)	164/256 (64.0%)	5/11 (45.5%)	NC
AI	81 (30.3%)	72/225 (32.0%)	9/42 (21.4%)	NC
IBD	253 (94.8%)	214/223 (96.0%)	39/44 (88.6%)	NC
PM	12 (4.5%)	7/81 (8.6%)	5/186 (2.7%)	NC
IBV	185 (69.3%)	52/67 (77.6%)	133/200 (66.5%)	0.121 (0.101-0.145)
CAV	259 (97.0%)	NC	259/267 (97.0%)	NC
ORT	152 (56.9%)	NC	152/267 (56.9%)	NC
MG	136 (50.9%)	NC	136/267 (50.9%)	0.078 (0.066-0.093)

NC = Not calculated.

Supplementary Table 1 Summary of vaccines and dosing procedure of 267 small-scale chicken flocks in the Mekong Delta of Vietnam.

Pathogen	Strain	No. vaccines	No. of flocks vaccinated with 1 dose	No. of flocks vaccinated with >1 dose	Total vaccinated
NDV	F	1	87	90	177
	B1	3	46	16	62
	LaSota	6	36	11	47
	Mukteswar	1	12	3	15
	AVF/HR	1	10	1	11
	Clone 45	1	1	2	3
	Not specified	1	2		2
Total		14	97	159	256
HPAI	NIBRG-14	1	138	83	221
	RE 6	2	4		4
Total		3	142	83	225
IBD	Winterfield 2512	3	74	89	163
	LZD 228-JAC3	1	27	11	38
	228E	1	10	21	31
	Intermediate standard	1	2	2	4
	Cheville 1/68	1	1	2	3
Total		7	89	134	223
Fowlpox	Weybridge	1	115		115
Total		1	115		115
PM	PA.1 and PA.2	1	67	4	71
	Serotype A:1	1	14		14
Total		2	72	9	81
IBV	H120	5	50	21	71
	H52	1	2		2
Total		6	40	27	67

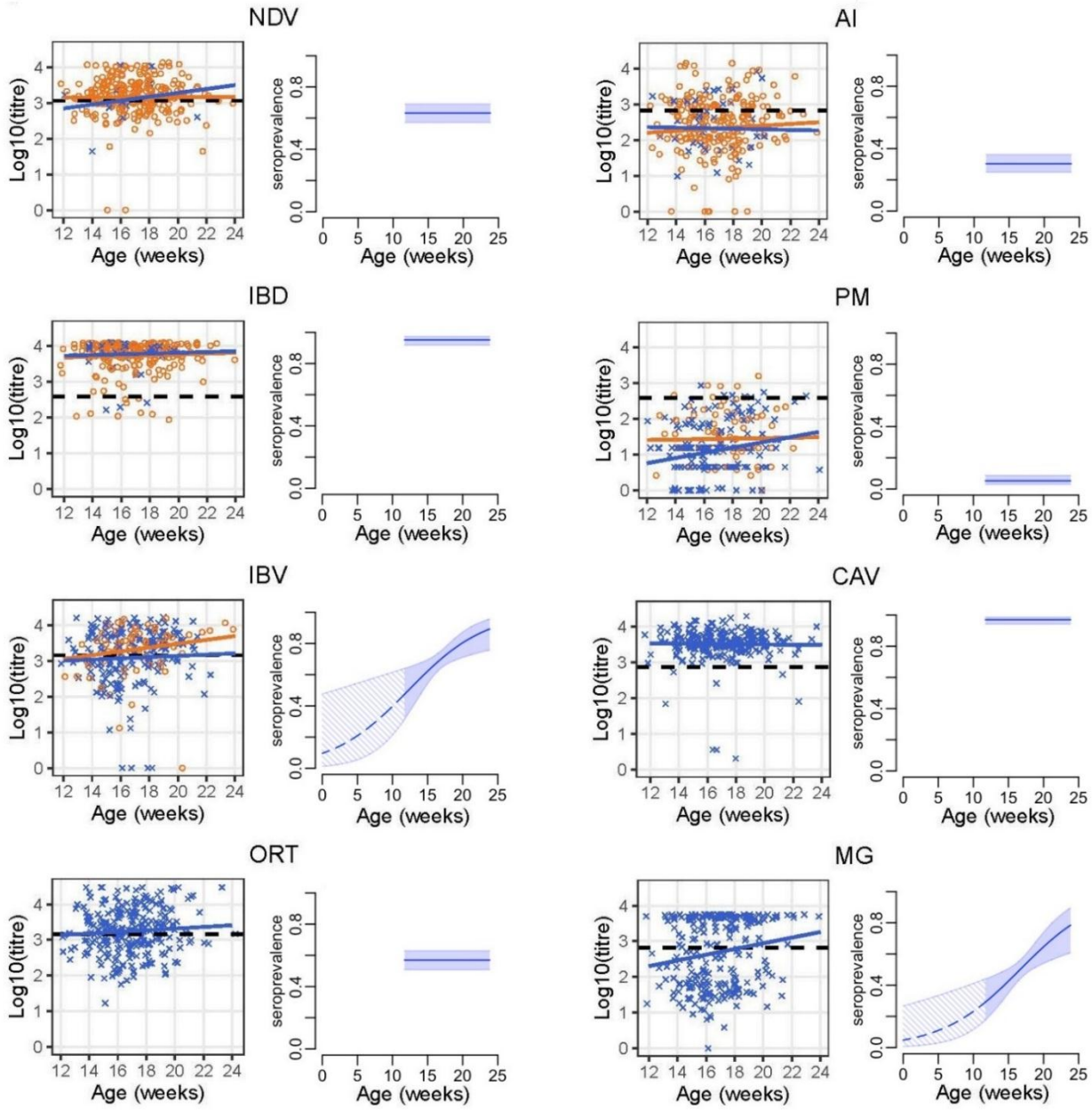

Figure 1 Titres and seroprevalence by age for each of the 8 pathogens investigated. Vaccinated chickens are displayed in orange; the unvaccinated ones in blue. The black horizontal lines indicates the cut-off value for positivity as indicated by the manufacturer. Continuous lines indicate linear regression.

Discussion

The serological results from unvaccinated flocks confirmed widespread circulation of all eight pathogens investigated in Mekong Delta chicken flocks. For most pathogens seroprevalence values among unvaccinated flocks ranged from high to very high, being most extreme for CAV and IBD (>88%). In contrast, serological data for PM, indicated low-level circulation (2.7% prevalence). The high titres identified in our study were consistent with a high rate of detection of some of these pathogens in diseased flocks in the area as reported in a previous study: MG (26.2%), IBD (24.6%), IBV (21.3%), ORT (13.1%) and HPAI (4.9%) (Van *et al.*, 2020). However, in that study, ND was not detected and CAV was not included in the diagnostic panel. Furthermore, no information was available about vaccination, since farmers did not keep any records. A possible explanation for this discrepancy is the predominance of low pathogenicity strains in the study area, in contrast with strains from northern Vietnam, that were most ND strains were classified as 'velogenic' (highly virulent) in a previous study (Choi *et al.*, 2014).

A surprising finding was the almost non-existing association between vaccination and serological titres, except for PM titres. This probably reflects that, in many flocks, vaccines were administered as single dose. It is also likely that some titres may have decayed over time, given the relatively long period elapsing from vaccination to serological testing. Other possible reasons for this may include vaccine degradation due to incorrect storage, or an administration procedure with regards to dosing or timing of application. For IBD, NDV, CAV and MG the observed higher titres in

large flocks suggested higher risk of disease introduction in such flocks. These findings are consistent with a higher mortality in larger chicken flocks in the area reported previously (Carrique-Mas *et al.,* 2019). Specifically a higher risk of HPAI has been shown to been shown in larger flocks (Otte J. *et al.,* 2008).

Combined with results from diagnostic investigations in flocks the area, our serological data supports maintaining vaccination against IBD and IBV, ND and HPAI in small-commercial flocks. It would be particularly important to improve IBV vaccine coverage, since only 27% flocks had been vaccinated against this pathogen. Unfortunately, the diversity of vaccines used and administration methods did not allow estimating the protective effect of the vaccines used. However, in general we saw a lower incidence of disease in flocks vaccinated against >4 pathogens (data not shown). However, vaccination behaviour also correlates with good husbandry practices and biosecurity (authors' observation). Vaccination against ND, IBV and IBD have traditionally formed the basis of vaccination programmes in Vietnam (Bui *et al.,* 2001). Vaccination against Highly Pathogenic Avian Influenza (HPAI) using inactivated injectable vaccines was further introduced in 2005. In many Vietnamese provinces HPAI vaccines are generally supplied by the veterinary authorities free of charge to small-scale farmers, and therefore this pathogen often reaches a high coverage. Given the diversity of circulating pathogens it is imperative to step up biosecurity standards, since it is not possible to vaccinate against all pathogens, and biosecurity standards in such units are typically poor. The implications of the observed high CAV titres merit more research. Given the high seroprevalence for MG, its relatively frequent detection in diseased chickens, and the challenges of providing MG-free day-olds, we recommend incorporating this pathogen to the vaccination programme of long-cycle meat birds raised in the area.

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