This is the peer reviewed version of the following article: [Osmani MG, Thornton RN, Dhand NK, Hoque MA, Milon S, Kalam MA, Hossain M, Yamage M. Risk factors for highly pathogenic avian influenza in commercial layer chicken farms in Bangladesh During 2011. Transboundary and emerging diseases. 2014 Dec 1;61(6):e44-51. doi: 10.1111/tbed.12071], which has been published in final form at http://onlinelibrary.wiley.com/doi/10.1111/tbed.12071/abstract . This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

Risk factors for Highly Pathogenic Avian Influenza (HPAI) in commercial layer chicken farms in Bangladesh during 2011

M.G. Osmani[1], R.N. Thornton[2], N K Dhand[3], M. A. Hoque[4], Sk. M. A Milon[2], M.A. Kalam[1], M. Hossain[1], M. Yamage[2]

[1] Department of Livestock Services, Epidemiology Unit,
Department of Livestock Services, Krishi Khamar Sharak, Farmgate, Dhaka 1215, Bangladesh
[2] FAO, Avian Influenza Unit, Department of Livestock Services, Krishi Khamar Sharak, Farmgate, Dhaka 1215, Bangladesh
[3] University of Sydney, 425 Werombi Road, Camden, NSW 2570, Australia
[4]Chittagong Veterinary and Animal Science University,Zakir Hossain Road, Khulshi, Chittagong,4202, Bangladesh

Correspondence:
Corresponding author, email: ron.thornton@windowslive.com, current affiliation AsureQuality, New Zealand.

Summary
A case control study was conducted during 2011 involved 90 randomly selected commercial layer farms infected with highly pathogenic avian influenza type A subtype H5N1 (HPAI) and 175 control farms randomly selected from within 5km of infected farms. A questionnaire was designed to obtain information about potential risk factors for contracting HPAI and was administered to farm owners or managers. Logistic regression analyses were conducted to identify significant risk factors. A total of 20/43 risk factors for contracting HPAI were identified after univariable logistic regression analysis. A multivariable logistic regression model was derived by forward stepwise selection. Both unmatched and matched analyses were done. The key risk factors identified were numbers of staff, frequency of veterinary visits, presence of village chickens roaming on the farm and staff trading birds. Aggregating these findings with those from other studies resulted in a list of 16 key risk factors identified in Bangladesh. Most of these related to biosecurity. It is considered feasible for Bangladesh to achieve a very low incidence of HPAI. Using the cumulative list of risk factors to enhance biosecurity pertaining to commercial farms would facilitate this objective.

Key words:
Avian influenza; case-control study; logistic regression; risk factors, observational studies.

Introduction

Highly pathogenic avian influenza type A subtype H5N1 (HPAI) was first reported in Bangladesh in March 2007. Since then, it has become established throughout the country with a low incidence, clustering, and a pronounced seasonal pattern characterised by a near-absence of reported cases outside a 4-month peak in the cold dry season (Ahmed et al., 2011).

The annual tally of reported HPAI cases in Bangladesh at the start of this study in July 2011 was 158 cases in layer farms, three in broiler farms and three in backyard village flocks. Most of the commercial poultry farms in Bangladesh are type 3 small-scale farms according to Food and Agriculture Organization of the United Nations classification (FAO, 2005). These farms in Bangladesh typically have poor biosecurity. However, they do differ amongst themselves markedly in this respect, and it is conceivable this might at least partially explain why one farm might become infected whilst its near neighbour might not. Any study about risk factors needs therefore to take into account relative differences amongst a generally poorly protected farming sector.

Broiler farms greatly outnumber layer farms (Dolberg, 2008). The low HPAI incidence rate in broiler farms was thought to be due to their short production cycles, since these farms are in most other respects similar to layer farms. The small number of infected backyard flocks chickens was thought likely to have been due to under-reporting.

HPAI has had serious economic consequences in Bangladesh. About $2.5 million has been disbursed in compensation alone to affected farmers since the first cases were notified in 2007, and it has made poultry protein less available to the very poor. Only six people are known to have contracted the disease in Bangladesh but this country is nevertheless regarded as presenting a high pandemic risk. It is therefore important to control outbreaks effectively and to eradicate HPAI if possible.

It has long been known that avian influenza is spread mainly by contact with infected birds, their products and fomites, and that improvements to biosecurity on farms and in live bird markets are therefore important for the control of this disease (Halvorson, 2009). In Bangladesh, a training program dramatically increased the awareness amongst farmers about the principles of effective biosecurity, and circumstantial evidence suggested that this training had a direct effect in lowering the incidence of disease (Mondal et al., 2012). However, farmers and market authorities are typically slow to improve biosecurity even when they can see a general sense in doing so. In part, this is because they balance the cost of biosecurity enhancements against what they perceive as a tenuous link with potential savings in their particular case. In this respect it is helpful to have solid rather than circumstantial evidence about risk factors for infection with avian influenza. It is also helpful if risk factors can be ranked by importance because the most important ones can then be identified and implemented properly.

Case-control studies are well suited for helping to identify and rank risk factors during disease outbreaks provided they are interpreted carefully with respect to local conditions and practices and with respect to potential response bias (Elwood, 2007). Results have been published from three case control studies conducted in Bangladesh (Biswas et al., 2009a; Biswas et al., 2009b; Osmani et al., 2008). This study supplements the previous ones by analysing a more extensive set of data from outbreaks that occurred during the year 2011. It was closely modelled on the previous studies but it had increased statistical power and it built on experiences gained from the previous studies. Its results are intended to be considered alongside findings from the other case-control studies and also from outbreak investigations to increase understanding about the transmission of the HPAI in Bangladesh.

Materials and methods

Study design

A sample of 100 commercial layer case farms and 200 controls (1:2) was calculated to provide 95% confidence of detecting an odds ratio of ≥3 with 80% statistical power assuming a minimum of 5% of control farms had been exposed to the risk factor of interest (Elwood, 2007). Broiler farms were not included because only three had been affected during the year of the study (Dr Abu Hannan, DLS, personal communication). Village outbreaks were not included partly because only three had been reported at the time of the study and partly because different risk factors operate in village flocks so they require their own specialised study (Biswas et al., 2009a).

The case farms were selected randomly using computer-generated random numbers from the total of 158 cases that had been officially reported in layer farms by the time the study started. These farms had been identified by typical clinical signs, diagnosed as HPAI positive using the Anigen test (Anigen® Rapid AIV Ag Test, Animal Genetics Incorporated, Kyonggi-do, Korea) and subsequently confirmed using a one-step real time PCR test (RT-PCR) for H5N1 (Lee et al., 2001). Two control farms were selected randomly from a list of unaffected farms when possible within 5km of each case farm. A control farm was defined as one that had not experienced HPAI outbreak during the year of the study. The owners of the case and control farms were contacted by the principal author, and all were cooperative in agreeing to participate in this study.

A questionnaire identified farms by name and map coordinates and collected data about 43 putative risk factors for HPAI. The questions were grouped in relation to farm characteristics, risk movements and biosecurity. The questionnaire (available on request) was tested on five farms prior to administration and five veterinarians were trained to apply it during face to face interviews with farm owners or managers. The questions were written in English but the interviews were conducted in Bengali. Enumerator performance was monitored during field visits and data were validated from about 10 farms each. The interviews were conducted from 4 – 9 months after outbreaks had occurred (between January and May 2011), so respondents were encouraged during the interviews to remember details around the month of the incident of interest to help them recall details accurately. Control farmers were encouraged to relate to equivalent periods of interest. Activities tended to be routine and stable, so it was not difficult to obtain comparable information.

Data analysis

Completed questionnaires were evaluated before being accepted and data were then entered into a purpose-built Microsoft Access 2007 database. Data were validated visually and then using descriptive statistics. All statistical analyses were done using R (R: A Language and Environment for Statistical Computing; http://www.R-project.org). Selected variables were rejected from further analysis if more than 10% of their records were missing. Questionable data were discussed with the enumerators or farmers. Farm locations were plotted in ArcMap 9.3 (ESRI®ArcMap™9.2) using XY points recorded on site.

Accepted risk factor variables were screened using univariable logistic regression analysis with HPAI status recorded as a binary outcome. A likelihood ratio (LR) chi-square test significance level of $p \leq 0.30$ was used to select variables for possible inclusion in a multivariable logistic regression model.

Collinearity was assessed for all pairs of initially selected variables. Strengths of association were measured using Spearman's rho and its significance for pairs of numerical or ordinal variables, and the contingency coefficient and its significance for the remaining pairs of nominal variables. The null hypothesis of statistical independence between scores from pairs of variables was rejected if their association was statistically significant at the $p<0.05$ level. Associations were considered to be significant if Spearman's rho or the contingency coefficient was ≥ 0.7. If there were several such associations, variable thought likely to be most useful for control were to be selected. Otherwise, both highly correlated variables were to be concluded and their effects on the model tested.

Lowess curves were created by plotting the values of each selected numerical variable against the log odds of being a case farm to evaluate the assumption of linearity. If the association was not linear, the data were categorized for inclusion in the full model.

The final model was built using stepwise forward selection of variables. Variables were retained in the full model if their LR chi square test was significant at $p \leq 0.05$. All possible two-way interaction terms were added one at a time to the finally selected main variables and retained if their LR chi-square test was significant at $p \leq 0.05$. Confounding was then assessed by adding as yet unselected putative confounding variables into the final model. A variable so added was considered to be a potential confounder if it caused ≥10% change in the pre-existing regression coefficients. The overall fit of the final model was evaluated using the Hosmer-Lemeshow goodness-of-fit test.

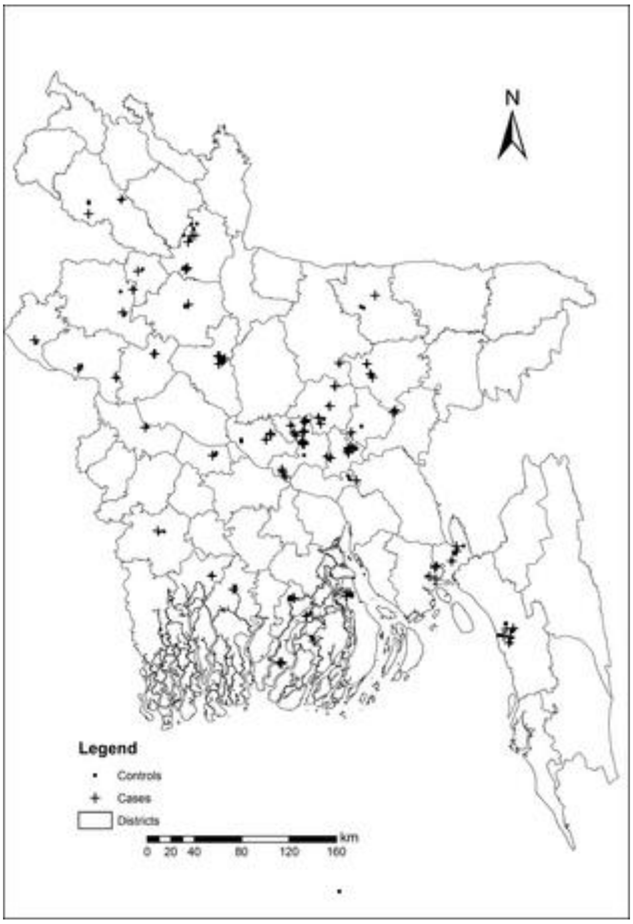
Where possible, controls had been selected within 5km of case farms. For comparative purposes, a conditional logistic regression analysis was therefore performed with cases and controls matched by location.

Results

Mapping

Data were available from 90 infected and 175 control layer farms (Figure 1).

Figure 1: Map of farms enrolled in the case-control study.



Logistic regression model

The risk factors included in the study questionnaire are listed in Table 1.

Table 1: Case-control risk factors explored when interviewing commercial layer farmers in Bangladesh

Farm characteristics: HPAI status, Distance from live bird market, Distance from main road, Distance from nearest town, Farm area, Farm Dwellings, Number of broiler farms within 0.5km, Number of farms nearby, Number of layers, Number of staff, Pond nearby, Source of birds, Staff contract, Type of neighbouring farms, and Type of replacement stock.

Risk movements: Feed transport, Site of product sale, Site where vehicles loaded, and Transport mode of products off.

Biosecurity: Cleaning of egg trays, Clothes changed upon entry, Company representative visits, Disposal of dead birds, Disposal of litter, Ducks roaming on farm, Entrances, Feed composition, Feed source, Fence, Foot baths used upon entry, Frequency of disinfecting sheds, Secure gate, Sharing of egg trays, Sheds cleaned and disinfected before restocking , Shoes changed upon entry, Staff own their own poultry, Staff trade birds, Technical staff visits, Vaccinator source, Vegetation, Vet visits, Village chicken roaming on farm, and Wild birds on farm.

A total of 20/43 variables were selected for inclusion in the full model following univariable analysis and a very conservative LR chi square $p \leq 0.3$ (Elwood, 2007). These were: number of layers, number of staff, type of replacement stock, frequency of veterinary visits, frequency of industry representative visits, frequency of technical staff visits, type of feed transport, method for transporting eggs and birds to sale, site for loading vehicles, place of sale for eggs and birds, cleaning and disinfection of egg trays, density of vegetation on property, number of entrances, presence of a secure gate, presence of roaming village chickens, policy for changing shoes upon entry, frequency of shed disinfection, method of disposal of dead birds, method of disposal of litter and whether or not staff traded birds (Table 2). Two of the selected variables were numerical and the remainder were either nominal or ordinal. High levels of association had been noted between 'number of layers' paired with: 'number of staff', 'type of feed transport', 'method of disposing of dead birds' and 'method of disposal of litter'. These variables were not excluded but their effects were closely monitored during multivariable analysis.

Table 2: Univariable HPAI risk factors statistically significant at p≤0.3

		Farm status		
Variable	**Levels**	**Control**	**Case**	**p (LR chi square)**
Number of layers	records	173	87	<0.05
	missing values	2	3	
Type of	day old chicks	162	85	0.1
replacement stock	pullets or both	11	5	
	missing values	2	0	
Number of staff	records	171	86	<0.05
	missing values	14	4	
Vet visits	only on call	137	63	0.1
	infrequent but	9	3	
	scheduled	12	15	
	weekly	17	9	
	monthly			
Company	never	109	47	0.1

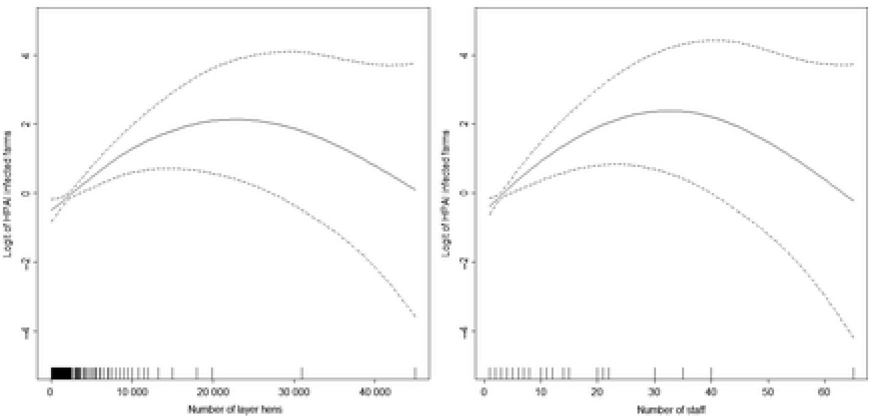
representative	weekly	10	12	
visits	monthly	54	28	
	missing values	2	3	
Technical staff	no visits prior 2wk	53	44	<0.05
visits	some visits prior 2wk	120	44	
	missing values	2	2	
Feed transport	own vehicle	15	15	0.2
	shared vehicle	65	26	
	feed company	16	8	
	vehicle	79	40	
	rickshaw van	0	1	
	missing values			
Transport mode of	own vehicle	28	18	0.3
products off	shared trader vehicle	143	63	
	both	4	4	
	missing values	0	5	
Site where vehicles	outside farm	93	39	0.1
loaded	inside farm gate	82	49	
	missing values	0	2	
Site of product sale	local market	63	25	0.2
	distant market	112	53	
	missing values	0	3	
Cleaning of egg	daily	57	31	0.3
trays	occasionally	106	46	
	never	12	10	
	missing values	0	3	
Vegetation	dense	43	32	0.1
	light	113	45	
	no vegetation	18	12	
	missing values	1	1	
Entrances	one	134	68	0.1
	two	28	9	
	three or more	12	11	
	missing values	1	2	
Secure gate	yes	104	47	0.2
	no	68	42	
	missing values	3	1	
Village chicken	yes	74	43	0.3
roaming on farm	no	99	45	
	missing values	2	2	
Shoes changed	changed by all	75	46	0.3
upon entry	only staff change	60	28	
	not changed	40	15	
	missing values	0	1	
Frequency of	occasionally	75	31	0.3
disinfecting sheds	once each day	59	32	
	twice or more each	40	27	
	day	1	0	
	missing values			
Disposal of dead	on ground	40	22	0.1
birds	buried	117	65	

	in pond	18	3	
Disposal of litter	biogas or composted	25	9	0.1
	disposed of fresh	98	53	
	drained to pond	38	13	
	other	14	15	
Staff trade birds	yes	13	14	<0.05
	no	162	75	
	missing values	0	1	

The relationship between the scores for the numerical variables 'number of layers' and 'number of staff' and their log odds of being a case premises were not linear (Figure 2). These data were therefore categorized prior to entry into the final model (Dohoo et al., 2003). 'Number of layers' was categorized using cut points 0, 1000, 2000, 3000, 45,000. 'Number of staff' was categorized using cut points 0, 1, 2, 5, 65.

The final model derived after stepwise forward selection of the 20 variables selected after univariable analysis is presented in Table 3. The selected main variables were categorized number of staff, frequency of veterinary visits, staff trading birds and the presence of roaming village chickens on the farm. There were no statistically significant two-way interaction terms. No confounding variables were identified. The Hosmer-Lemeshow goodness of fit statistic for the final model was (H-L = 11.5, df =8, p=0.2), suggesting that the model had an acceptable overall level of fit.

Figure 2: Values of the two selected numerical variables plotted against the logit of their respective outcomes.



Conditional logistic regression analysis did not substantially alter the odds ratios of the variables in the final unmatched model. Their confidence intervals tended to be broader but not so as to alter the interpretation of the model.

Table 3: Final logistic regression model for identifying key risk factors for HPAI infection on commercial layer farms in Bangladesh

Variable	Category	OR	95% confidence intervals	LRT p value
Number of staff	0 - 2	1		0.001
(categorized)	3 - 5	12.2	1,1, 4.3	
	6 – 65	5.1	2.2, 12.2	
Veterinary visits	on call only	1	1	0.01
	infrequent but scheduled	0.6	0.1, 2.0	

	weekly	3.0	1.2, 7.3	
	monthly	1.2	0.4, 3.1	
Staff trade birds	yes	1	1	0.02
	no	0.3	0.1, 0.8	
Village chickens roaming	roaming on farm	1	1	0.05
	none present	0.6	0.3, 1.1	

Discussion

When this study was initiated during July 2011, there had been 158 reported cases in Bangladesh that year and 524 cases reported since March 2007. The disease had therefore become established, widely spread and economically significant but with a low incidence and with a pronounced seasonal trough consistently present for several months during the dry season. Active surveillance was efficient and effective as monitored within FAO and World Bank funded projects, control systems appeared to be adequate as monitored through FAO field visits, and compensation was realistic enough to encourage early reporting by farmers as was consistently noted on field visits. Keepers of backyard poultry, and workers within live bird markets, tended to be quite poorly informed about avian influenza, and they were inclined to under-report cases or to trade in sick birds (Sarker et al., 2011; Sultana et al., 2012). However, the small commercial farmers in this study had become well informed over the years since HPAI had first appeared in Bangladesh and as a result they had become noticeably more able and more inclined to provide reliable information during field studies (Dr MA Kalam, FAO, personal communication). The poultry value chain in Bangladesh was well understood (Dolberg, 2008), many of the risk factors for HPAI had been described, and surveillance and control procedures had also been written in conformance with international standards. At the time of the study, control of HPAI in Bangladesh was therefore considered by national and international experts to be both technically and logistically feasible. Nevertheless, both passive surveillance and the implementation of basic biosecurity in commercial farms were known to be defective, and these were two important weaknesses. Two key requirements for effective control were therefore: (1) ranking of risk factors for infection with HPAI to allow more incisive advice to be given about implementing them, and (2) commitment by all informed stakeholders to early reporting and diligent control under official guidance. This study contributed towards knowledge about the key risk factors for HPAI on commercial layer farms.

Case-control studies are subject to misclassification and recall bias because they are retrospective and they rely on information from respondents who may have imperfect recall who may want to distort the truth (Dohoo et al., 2003). They are nevertheless efficient and well suited for describing multifactorial causes of rare diseases (Elwood, 2007), and they have proven useful for identifying and ranking the importance of risk factors for the spread of highly infectious diseases in both developed and developing countries (Biswas et al., 2009a; East et al., 2006; Firestone et al., 2011; Kung et al., 2007; McClaws et al., 2009; Thomas et al., 2005).

This case-control study supplemented two previous ones done in commercial layer farms in Bangladesh (Biswas et al., 2009a; Osmani et al., 2008). Those two had been conducted soon after HPAI had entered the country. They had therefore necessarily enrolled fewer cases (33 and 50, respectively) and their data had been collected when the disease situation in Bangladesh was less well understood, but these smaller studies had nevertheless provided useful information. Useful information about HPAI risk factors has, in fact, been elicited from a case-control study in Hong Kong involving only 16 cases (Kung et al., 2007).

The list of putative risk factors in this study was mostly adapted from those used in the two previous case-control studies in Bangladesh. The statistical power of the present study was increased by enrolling an eventual total of 90 case and 175 control farms. Power is the ability to demonstrate an association given that one exists, so it is an important study attribute which can be readily enhanced by increasing the number of cases and/or controls (Elwood, 2007).

Bias was minimized by selecting study farms randomly from all known cases and by use of carefully designed and piloted questionnaires that were administered in face-to-face interviews by veterinarians who had been trained for the task. Enumerator performance was monitored and their data were evaluated prior to acceptance. Recall was aided by focussing the time of interest to the month of the incident being investigated. Most farmers in Bangladesh identify closely with their farms and most therefore had good recall about events when questioned. The investigators had nevertheless been taught to sift replies intelligently particularly with respect to what they could observe on site. Each investigator had been assigned farms in their home territory. Their assigned farmers did not therefore perceive them as being alien and they tended to talk freely.

Data were collected for 43 putative risk factors. Consideration could have been given to focussing on smaller list of key variables given inherent biases in the data (Thomas et al., 2005). However, weaknesses in the data were thought more likely to blur distinctions between cases and controls (decreased power) rather than to create spurious associations.

The final model identified the following main explanatory variables for infection with HPAI: number of staff (categorized), frequency of veterinary visits, staff trading birds and the presence of chickens roaming on the farm. A matched analysis conditional upon location resulted in slightly changed odds ratio estimates with wider confidence intervals but it did not alter the interpretation of the model.

There are many logical reasons why risk of infection with HPAI might increase with increased numbers of staff. For example, more staff means more movements on and off the farm, a greater likelihood of off-site contact with infected birds and a decreased ability to train staff well and to monitor their biosecurity practices. However, no interaction terms were statistically significant.

Weekly veterinary visits was a risk factor (OR = 3.2) compared with less frequent visits. This risk factor was not identified in previous case-control studies involving HPAI outbreaks in commercial farms (Biswas et al., 2009a) and backyard flocks (Biswas et al., 2009b) in Bangladesh or in a case-control study in Hong Kong (Kung et al., 2007). Veterinarians are commonly asked to vaccinate against presumptive acute Newcastle disease outbreaks, but avian influenza can resemble Newcastle disease and outbreak investigations have documented several cases where veterinarians have vaccinated on farms subsequently known to have been infected with avian influenza. Most veterinarians did not decontaminate thoroughly between farms so they were an obvious risk through fomite spread.

Having staff that did not trade birds was protective (OR = 0.3). This risk factor was not identified in previous case-control studies involving HPAI outbreaks in commercial farms (Biswas et al., 2009a) and backyard flocks (Biswas et al., 2009b) in Bangladesh or in a case-control study in Hong Kong (Kung et al., 2007). This risk factor was logical since trading increases contact rates with outside birds, and live bird markets in particular in are believed to be high risk areas in Bangladesh.

Not having roaming village chickens on the farm was protective (OR = 0.3) against infection with avian influenza. Equivalent risk factors were identified in two previous case-control studies conducted in Bangladesh. One study identified accessibility to feral and wild animals as a risk factor for infection with HPAI in commercial farms (Biswas et al., 2009a) whilst another identified contact with pigeons and sharing of night shelters between chickens and ducks as risk factors for HPAI outbreaks in backyard poultry (Biswas et al., 2009b). Wandering village chickens had been identified as risk factors concurrently by outbreak investigators.

These results, when combined with those from other case-control studies conducted in Bangladesh (Biswas et al., 2009a; Osmani et al., 2008), have suggested the following 16 important risk factors for HPAI on commercial farms: frequent veterinary visits, village chickens roaming on the farm, staff trading birds, proximity to towns, proximity to live bird markets, type of replacement stock, proximity of backyard flocks, use of an external vaccinator, mixing own feed, loading egg trays inside farm, irregular cleaning when re-stocking, failure to bury dead birds, draining litter directly into adjacent ponds, access to feral animals (poor fencing), type of vehicle used to transport eggs to the market and use of footbaths. Outbreak investigations, which are a different type of study with their own strengths and weaknesses, conducted in Bangladesh have suggested the following major risk factors: village chickens and ducks wandering on farms (poor fences), recent visits by industry-related professionals (particularly outside vaccinators), egg trays and vehicles visiting live bird markets (Biswas et al., 2008; Thornton, 2011). Wild birds had not been explored as a potential risk factor for the occurrence of HPAI in this study because it was difficult to do so because farmer information about wild birds tended to be unreliable.

The incidence of HPAI in Bangladesh was considered to be low during this study, and surveillance was considered to be adequate though far from perfect. Most of the important risk factors identified in this study related to routine biosecurity practices that would have been easy and cheap to implement in Bangladesh. A national focus on improving biosecurity should therefore result in greatly enhance success in HPAI control. The importance of biosecurity is acknowledged by commercial farmers and in live bird markets in Bangladesh(Sarker et al., 2011) but its implementation has nevertheless been poor. This failing is not unique to Bangladesh but exists throughout Asia (Azhar et al., 2010; Cristalli and Capua, 2007) for reasons that are complex and hard to address. There has, however, been an increasing focus on biosecurity in Bangladesh. For example, a World Bank funded initiative exists there to demonstrate and encourage the adoption of improved biosecurity in small commercial farms (Dr Tesfai Tseggai, FAO, personal communication).

Enhancing biosecurity is a recognized global strategy for control of HPAI (FAO, 2008), and in this respect Bangladesh is fully aligned. The same FAO strategy also recommends that vaccination be considered as a control tool. In line with this, the Veterinary Authorities have implemented a pilot vaccination program in commercial layer poultry in two of Bangladesh's 64 districts.

Acknowledgments
We particularly thank the Bangladesh Department of Livestock staff for help in liaising with farmers and for providing insights into local risk factors. We also thank Dr Md Giasuddin from the Bangladesh National Reference Laboratory for Avian Influenza for expert advice. We are grateful to Mark Stevenson from the Massey University Epicentre and Simon Firestone from Sydney University for their advice in using R statistical program. Michael Ward from the University of Sydney is also acknowledged for his contributions to the design of this study.

References

Ahmed, S., Ersboll, A., Biswas, P., Christensen, J., Toft, N., 2011, Spatio-temporal magnitude and direction of highly pathogenic avian influenza (H5N1) outbreaks in Bangladesh. PLoS ONE 6, e24324.

Azhar, M., Lubis, A.S., Siregar, E.S., Alders, R.G., Brum, E., McGrane, J., Morgan, I., Roeder, P., 2010, Participatory disease surveillance and response in Indonesia: strengthening veterinary services and empowering communities to prevent and control highly pathogenic avian influenza. Avian Diseases 54, 749-753.

Biswas, P., Christensen, J., Ahmed, S., Barua, H., Das, A., Rahman, M., Giasuddin, M., Hannan, A., Habib, A., Debnath, N., 2009a, Risk factors for infection with highly pathogenic influenza A virus (H5N1) in commercial chickens in Bangladesh. veterinary Record 164, 743-746.

Biswas, P., Christensen, J., Ahmed, S., Das, A., Rahman, M., Barua, H., Giasuddin, M., Hannan, A., Habib, M., Debnath, N., 2008, Avian influenza outbreaks in chickens, Bangaldesh. Emerging Infectious Diseases 14, 1909-1912.

Biswas, P., Christensen, J., Ahmed, S., Das, A., Rahman, M., Barua, H., Giasuddin, M., Hannan, A., Habib, M., Debnath, N., 2009b, Risk for infection with highly pahtogenic avian influenza virus (H5N1) in backyard chickens, Bangladesh. Emerging Infectious Diseases 15, 1931-1936

Cristalli, A., Capua, I., 2007, Practical problems in controlling H5N1 high pathogenicity avian influenza at village level in Vietnam and introduction of bisecurity measures. Avian Diseases 51, 461-462.

Dohoo, I., Martin, W., Stryhn, H., 2003, Veterinary epidemiologic research.

Dolberg, F. 2008. Poultry sector country review: Bangladesh (Rome, FAO), pp. 1-61.

East, I., Kite, V., Daniels, P., Garner, G., 2006, A cross sectional survey of Australian chicken farms to identify risk factors associated with seroposivity to Newcastle-disease virus. Preventive Veterinary Medicine 77, 199-214.

Elwood, M., 2007, Critical appraisal of epidemiological studies and clinical trials, Third Edition. Oxford University Press, 1-570 pp.

FAO 2005, A global strategy for the progressive control of highly pathogenic avian influenza (HPAI).

FAO, 2008, The global strategy for prevention and control of H5N1 highly pathogenic avian influenza - 2008.

Firestone, S., Schemann, K., Toribio, J.-A., Ward, M., Dhand, N., 2011, A case-control study of risk factors for equine infuenza spread onto horse premises during the 2007 epidemic in Australia. Preventive Veterinary Medicine 100, 53-63.

Halvorson, D., 2009, Prevention and management of avian influenza outbreaks: experiences from the United States of America. Rev. sci. tech. Off. int. Epiz. 28, 359-369.

Kung, N., Morris, R., Perkins, N., Sims, L., Ellis, T., Bissett, L., Chow, M., Shortridge, K., Guan, Y., Peiris, M., 2007, Risk for infection with highly pathogenic influenza A virus (H5N1) in chickens, Hong Kong, 2002. Emerging Infectious Diseases 13, 412-418.

Lee, M., Chang, P., Shien, J., Cheng, M., Shieh, H., 2001, Identification and subtyping of avian influenza viruses by reverse transcription-PCR. Journal of Virological Methods 97, 13-22.

McClaws, M., Ribble, C., Martin, W., Wilesmith, J., 2009, Factors accociated with the early detection of foot-and-mouth disease during the 2001 epidemic in the United Kingdom. Canadian Veterinary Journal 50, 53-60.

Mondal, S., Tardif-Douglin, D., Ryan-Silva, R., Magnani, R., 2012, Controlling highly pathogenic avian influenza, Bangladesh. Emerging Infectious Diseases 18, 2083-2085.

Osmani, M., Rahman, M., Loth, L., Kalam, M., Hossain, M., Khan, F., Khan, M., 2008, A case control study to determine risk factors of HPAI outbreaks in commercial poultry farms in Bangladesh, In: A study on highly pathogenic avian influenza in Bangladesh. pp. 24-64.

Sarker, S., Talukder, S., Chowdhury, E., Das, P., 2011, Knowledge, attitudes and practices on biosecurity of workers in live bird markets at Mymensingh, Bangladesh. ARPN Journal of Agricultural and Biological Science 6, 12-17.

Sultana, R., Rimi, N., Azad, S., Islam, M., Khan, M., Gurley, E., Nahar, N., Luby, S., 2012, Bangldeshi backyard poultry raiser's perceptions and practices related to zoonotic transmission of avian influenza. Journal of Infection in Developing Countries 6, 156-165.

Thomas, M., Bouma, A., Ekker, H., Fonken, A., Stegeman, J., Nielson, M., 2005, Risk factors for the introduction of high pathogenicity Avian Influenza virus into poultry farms during the epidemic in tghe Netherlands in 2003. Preventive Veterinary Medicine 69, 1-11.

Thornton, R. 2011. Investigation of HPAI outbreaks in Bangladesh during 2011: End of Assignment Report (FAO), pp. 1-22.