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Mortality rate and clinical features of highly pathogenic avian influenza in naturally infected chickens in Bangladesh

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Summary

A retrospective study was performed to assess the mortality rates and clinical signs in commercial and backyard chickens naturally infected with the highly pathogenic avian influenza (HPAI) H5N1 virus in Bangladesh.

All of the 33 commercial and 25 backyard farms, recorded during the first wave of HPAI outbreaks (January to November 2007) in Bangladesh, were enrolled in this study. The farm profile data, stock information and major clinical signs recognised by the farmers and/ or farm attendants, were collected through a prototype questionnaire. The investigating veterinarians' retrospective appraisals of the clinical signs were recorded by interview. After the clinical onset, the incidence rates of mortality in breeder, commercial broiler, commercial layer and backyard chickens were, respectively: 0.0215, 0.0341, 0.0179 and 0.0703 per chicken-day at risk. The four major clinical signs observed were cyanotic combs and wattles, oedema of the head and face, drowsiness and huddling and ecchymotic discoloration of the leg shanks. The first sign (cyanotic combs and wattles) was equally observed in chickens from commercial and backyard farms (p = 0.44) but the second (oedema of the head and face) was more common in backyard farms (p = 0.03).

Keywords

Avian influenza – Bangladesh – Chickens – H5N1 – Highly pathogenic avian influenza – Mortality – Natural infection – Poultry.

Introduction

Highly pathogenic avian influenza (HPAI) H5N1 virus poses a serious threat to poultry and to human livelihoods, as well as to public health in many countries of the world. This danger is more noticeable in a few developing countries (Bangladesh, Egypt, Indonesia and Vietnam) (5), where the virus seems to be firmly entrenched and where poultry are mostly reared in a production system categorised by the Food and Agriculture Organization of the United Nations (FAO) as poultry production system 3 (6). Bangladesh has a massive backyard chicken

population, being reared by 89% of rural households (4). The country predominantly relies on passive surveillance, in which an affected farmer/smallholder informs the Department of Livestock Services (DLS) about a suspected outbreak. Enhancing farmers' recognition of the clinical signs in chickens infected with the virus could substantially improve the proportion of cases reported and allow a more rapid response to outbreaks.

Bangladesh was officially declared affected by HPAI H5N1 virus on 22 March 2007, but an earlier epidemiological study has revealed that the first HPAI outbreak could have begun in mid-January 2007 (2). By November 2007, 58 HPAI outbreaks were reported during a first wave of the disease (2) and, by May 2009, 47 of the 64 districts of the country had recorded a total of 324 HPAI outbreaks (www.mofl.gov.bd). This countrywide dispersal of the virus and persistence of outbreaks could indicate hidden infections, due to a deficiency in disease reporting. There are no pathognomonic signs of HPAI (7, 9, 10, 12) but the predominant clinical signs, along with the mortality pattern, might be important aids in enhancing people's awareness of the disease while, at the same time, improving passive surveillance. This study determines the incidence rates of mortality and compiles the clinical signs in chickens that are naturally infected with HPAI H5N1 virus in small commercial and backyard farms in Bangladesh.

Materials and methods

An overview of case reporting and confirmation of highly pathogenic avian influenza

A farm was considered infected with HPAI H5N1 virus when the National Reference Laboratory for Avian Influenza (NRL-AI) confirmed the presence of H5N1 in samples collected from a chicken. In Bangladesh, government-subsidised Veterinary Services are provided to farms through the DLS. This department maintains an *upazila* livestock office in each *upazila* (a lower administrative unit of Bangladesh), comprising a state veterinary surgeon (SVS) and supporting staff to deliver animal health services. During the HPAI crisis, poultry farms reporting high mortality were visited by the SVS to examine the chickens. If HPAI was suspected, clinically affected and dead chickens were sent to the Central Disease Investigation Laboratory (CDIL), or any of the seven Field Disease Investigation Laboratories (FDILs) of the DLS, which provide government-subsidised diagnostic services.

At the CDIL or FDIL, oropharyngeal swabs were taken from the dead chickens to test for avian influenza A virus antigen, using the avian influenza type A quick antigen detection kit (Synbiotics Corporation, San Diego, California). If this test was positive, tracheas from the dead chickens were referred to the NRL-AI, where H5 was detected using reverse transcription polymerase chain reaction (RT-PCR) from pieces of tracheal tissue. Viral RNA extraction and purification were carried out with an RNA extraction kit (Rneasy Mini Kit, Qiagen, Germany), using the standard procedure. Reverse transcription polymerase chain reaction was conducted with the extracted products by using the Quiagen one-step RT-PCR kit on a thermal block (ASTEC, Japan), with a primer set HA oligo 5´(5´-ACACATGCYCARGACATACT-3´) and HA oligo 3´(5´-CTYTGRTTYAGTGTTGATGT-3´), described by Lee *et al.* (8). The product was electrophoresised in 1.5% agarose and visualised with an ultraviolet transilluminator (Biometra TI 1, Germany). If the results were confirmed positive, they were reported to the DLS so that measures could be taken to contain the outbreak, including declaring an infection zone (of 1-km radius) around the affected farm, banning traffic movement and culling of all birds on the infected farm and within the zone of infection.

Data collection

All the 58 HPAI-affected farms (HPAI-AFs) registered in Bangladesh by 17 November 2007 were retrospectively investigated. A list of the farms was collected from the DLS epidemiology unit. Interviews were conducted with the farmers by questionnaire to elicit important farm profiles. These included information on:

- stock
- management practices
- biosecurity
- flock health history
- putative sources of infection
- cleansing and disinfection
- compensation
- veterinary follow-up.

The stock information from each farm before the clinical onset of HPAI and during culling was verified by the DLS because compensation was provided from the authority to every farmer whose chickens were culled. The farmers provided the same stock information for this study. All the veterinarians who had investigated the 58 farms were interviewed to record the clinical signs that they had observed. The median time that elapsed between an outbreak and the interview was 71 days (minimum 1; maximum 178). Two trained veterinarians were deployed to interview farmers, attendants and veterinarians in the local Bengali language. Global positioning system coordinates from each farm were collected during farm visits and entered into a digitised map of Bangladesh, using a geographic information system program, Arc View 9.1 (ESRI, 2004, USA).

Data analysis

All data from the interviews were entered into a spreadsheet programme (Excel 2000, Microsoft Corporation) and STATA-7 (STATA Corporation, Texas, 2001) for summary and data management. The incidence rate of mortality for chickens from each production category was calculated as follows. The number of chickens that died between the clinical onset (when the first clinical signs appeared) of HPAI and the culling of chickens was employed as the numerator. The number recorded before the clinical onset multiplied by the mean number of days that elapsed between the clinical onset and culling was used as the denominator. The difference in magnitude of a clinical sign of HPAI shown in naturally infected chickens on commercial farms and those on backyard farms was tested in a single level (2 × 2) contingency table analysis (significance determined using the chi-square statistic).

Results

Farm profiles and incidence rates of mortality

Figure 1 portrays the spatial distributions of the 58 HPAI-AFs. Six HPAI-AFs belonged to FAO production system 2 and the rest to systems 3 and 4 (5). At the start of the outbreaks, the mean age (and range) of chickens on the breeder, broiler and layer farms was 37.2 (31–70), 4.5 (3–6) and 34.3 (6–75) weeks, respectively. Three breeder and ten layer farms were located in isolated places and the others were all found in residential areas. Chickens from 30 of the 33 commercial farms were reported to have been vaccinated against Newcastle disease but this was the case in only seven backyard farms. On backyard farms, chickens were reared in scavenging systems on homesteads and, at night, were kept in small shelters made of bamboo, wood, iron sheets etc. to protect them from predation and theft. Ducks were also kept on ten of the backyard chicken farms, pigeons on two, and geese on two.

Table I shows the farm profiles, including production categories plus the types of chickens reared and the incidence rates of mortality in both commercial and backyard chickens naturally infected with HPAI H5N1 virus. The incidence rates of mortality in breeder, broiler, layer and backyard chickens during the overt phase of the disease, before culling, were 0.0215, 0.0341, 0.0179 and 0.0703 per chicken-day at risk, respectively. This means that approximately 2%, 3%, 2% and 7% of breeder, broiler, layer and backyard chickens died each day, once infected, under the prevailing farm conditions in Bangladesh.

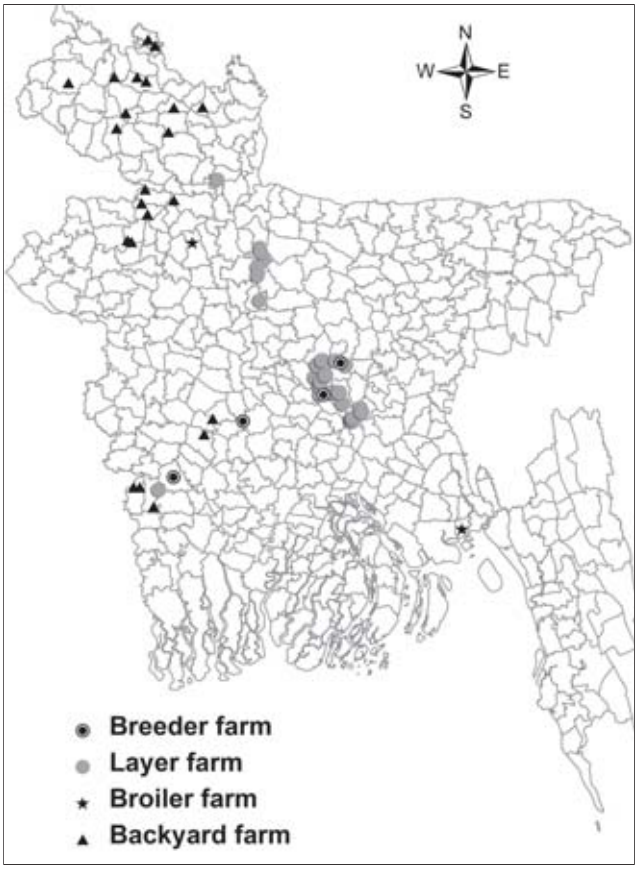

Fig. 1
Map of Bangladesh showing locations of farms affected by highly pathogenic avian influenza (15 January to 17 November 2007)
Four breeder, two broiler, 27 commercial layer and 25 backyard farms were affected

Clinical signs

The nine major signs observed in the chickens on the HPAI-AFs are summarised in Table II. Owing to a lack of records, data were not available to calculate the fall in egg production during the overt phase. The two most pronounced signs were cyanotic combs and wattles and oedematous heads and faces. The first sign was evenly detected on commercial and backyard farms ($p = 0.44$), but the second was detected significantly more often in the backyard farms ($p = 0.03$). One clinical sign; namely, drowsiness and huddling, was more apparent on backyard farms ($p = 0.02$). In contrast, diarrhoea and neurological dysfunctions were more common on the commercial farms ($p < 0.05$). Chickens on approximately 20% of farms from both production sectors displayed ecchymotic discoloration of the leg shanks. Indurate crops assessed by external palpation were observed in chickens on only 16% of backyard farms.

Table I
Incidence rates of mortality in commercial and backyard chickens naturally infected with highly pathogenic avian influenza subtype A H5N1 virus in Bangladesh (January to November 2007)

Farm category	No.	Bird type (no. of farms)	Total no. of chickens (min.; max.)		IR per chicken-day at risk (CI)[a]
			Before clinical onset	Before culling	
Breeder	4	Fayoumi (1), Hubbard Classic (2), Starcross (1)	121,959 (3,543; 61,667)	99,639 (3,353, 50,597)	0.0215 (0.0212, 0.0218)
Commercial broiler	2	Hubbard Classic (2)	7,800 (1,800; 6,000)	5,483 (1,000, 4,483)	0.0341 (0.0328, 0.0356)
Commercial layer	27	BV300 (1), Fayoumi (2), Hisex Brown (2), Hisex White (5), Hyline White (8), Isa Brown (2), Lohman White (3), Starcross (4)	83,942 (1,000; 11,975)	67,832 (282, 11,572)	0.0179 (0.0177, 0.0182)
Backyard	25	Fayoumi (2), indigenous (20), Sonali (3)[b]	5,069 (3; 1,970)	3,501 (0, 1,908)	0.0703 (0.0669, 0.0739)
Total	**58**	**–**	**218,770 (3; 61,667)**	**176,455 (0, 50,597)**	**0.0240 (0.0237, 0.0242)**

a) Mean days between clinical onset of highly pathogenic avian influenza and culling of breeder, commercial broiler, commercial layer and backyard chickens = 8.5, 8.7, 10.7 and 4.4 days, respectively
b) F1 generation of Fayoumi (hen) × Rhode Island Red (cock) breeds
CI: confidence intervals
IR: Incidence rates

Discussion

No pathogenicity index of any of the H5N1 isolates from Bangladesh was carried out, but the H cleavage site of some selected isolates contained poly basic amino acids (PQGERRRKKRGLF), a characteristic feature of HPAI A viruses (1). The authors note that vaccination against avian influenza is not practised in Bangladesh.

While information is available on clinical and pathological features in chickens experimentally infected with HPAI H5N1 virus (7, 9, 10, 12), none of the signs is considered pathognomonic. To the authors' knowledge, published information is lacking on the clinical signs and incidence rates of mortality in commercial and backyard chickens naturally infected with HPAI H5N1 virus. The incidence rates of mortality in chickens naturally infected with HPAI H5N1 virus in this study indicate that approximately 2% of breeders, 3% of broilers, 2% of layers and 7% of backyard chickens die each day during the overt phase of HPAI. Stocking densities, rearing practices and other environmental conditions in Bangladesh could influence this daily mortality and explain why the mortality rate in naturally infected chickens is lower than that generally observed in experimental infections.

The clinical signs in naturally infected chickens described in this study reveal that more than 75% of farms in any

Table II
Clinical features in chickens on commercial and backyard farms naturally infected with highly pathogenic avian influenza H5N1 virus in Bangladesh (January to November 2007)

Clinical sign	No. (%) of farms with this clinical sign			P	Comment
	Commercial (*n* = 33)	Backyard (*n* = 25)	Total (*n* = 58)		
Cyanotic combs and wattles	25 (76%)	21 (84%)	46 (79%)	0.44	–
Diarrhoea	8 (24%)	1 (4%)	9 (16%)	0.04	Whitish and greenish faeces
Drowsiness and huddling	5 (15%)	11 (44%)	16 (28%)	0.02	Broiler chickens also show signs of feather picking
Ecchymotic discoloration of the leg shanks	7 (21%)	5 (20%)	12 (21%)	0.91	–
Oedema of the head and face	17 (56%)	20 (80%)	37 (64%)	0.03	–
Excessive lacrimation	4 (12%)	6 (24%)	10 (17%)	0.24	–
High mortality and sudden death	9 (27%)	5 (20%)	14 (24%)	0.52	Death < 4 h
Indurate crop	0	4 (16%)	4 (7%)	–	Palpable from outside
Nervous signs	9 (27%)	1 (4%)	10 (17%)	0.02	7 had torticollis, 2 had paralysis and 1 had a drooping wing
Others	5 (15%)	4 (16%)	9 (16%)	0.93	Each sign in ≤ 2 farms

production category may have chickens with cyanotic combs and wattles, while approximately 55% of commercial farms and 80% of backyard farms may have chickens with oedema of the head and face. Thus, in Bangladesh, any commercial farm with chickens with cyanotic combs and wattles, accompanied by a daily mortality exceeding 2%, and any backyard chicken flock showing cyanotic combs and wattles and/or oedema of the head and face, with a daily mortality of 7% or more, would be highly suspect for HPAI. Raising farmers' awareness and encouraging their active participation could have a significant impact on finding hidden HPAI outbreaks, a vital step in the enhancement of passive surveillance in Bangladesh. However, as there is no pathognomonic sign for HPAI, samples from each suspected case should be tested at the NRL-AI for a definitive diagnosis. However, a tentative diagnosis based on the incidence rate of mortality and clinical signs would also allow early precautionary measures to be put in place before laboratory confirmation.

Owing to restraints on resources, passive surveillance for HPAI was initiated in Bangladesh in 2007–2008, with FAO support. Although active surveillance later began in some selected *upazila* (www.aitubd.org/content.php?page=OngoingActivities), the country still largely depends on passive surveillance. In the past, any suspected HPAI cases were reported to DLS by the affected farmers. Because of the low mortality rate in chickens during the early course of the disease, as evidenced in this study, many HPAI cases may not have been reported, contributing to widespread dispersal of the virus. Vaccination against the disease is banned in Bangladesh. Thus, since they are immunologically naive to infection and kept in poor biosecurity conditions, chickens in Bangladesh are much more prone to HPAI H5N1 infection. However, again, due to the low mortality rate and slow spread of the virus within a flock, such clinical events could go unreported. Under-reporting of HPAI cases may well be a probable cause of HPAI H5N1 persistence in the country since 2007.

Once a positive diagnosis for HPAI is made on a farm in Bangladesh, a ban is imposed on human, animal and vehicle movements within a 1-km radius (the infected zone) of an HPAI-AF. For that reason, this study relied upon information about clinical signs provided by the DLS investigating veterinarians. Those signs reported by farmers were cross-verified with the veterinarians' own observations. It was interesting to note that both the veterinarians' and the farmers' observations matched very closely on at least two signs: cyanotic combs and wattles, and diarrhoea.

In production system 2 flocks, chicken mortality followed a distinct pattern. Deaths usually began in a single pen of a shed and, when most of the chickens in that pen had died, mortality started in adjacent pens, with chickens in the most remote pens being the last to die. It may be relevant to mention that, on the HPAI index farm in Bangladesh, there were three sheds and the distance between any two sheds was less than 40 m. Seventy-one days passed between the appearance of HPAI in chickens in one shed and the culling of chickens from all the sheds, but none of the chickens in the other two sheds was clinically affected during that time. This slow spread of the virus in Bangladesh is difficult to explain and demands a thorough study on virus survival under similar environmental conditions.

This study observed a higher incidence of mortality in chickens in backyard flocks. This may be explained by sub-optimal feeding, concurrent infections or other management factors but the results do not lend support to the common belief that indigenous birds are less susceptible to H5N1 than modern hybrids.

Indurate crops, a clinical sign observed in the smallholding chickens (Table II) in this study, have never been reported. However, as pathological investigations were not performed on these crops, the possible mechanism involved cannot be explained. Inaccessibility to the infection zones, which prevented detailed gross and subsequent microscopic examination of the organs, constituted a major limitation of this study.

Highly pathogenic avian influenza H5N1 has been reported in poultry or wild birds in 63 countries worldwide (11). Officially, Bangladesh reported its first HPAI outbreak in early 2007; by April 2010, a total of 356 outbreaks had been reported in 49 of the 64 districts (www.mofl.gov.bd/daily_birdflu_report.pdf). Effective control measures resulted in the cessation of HPAI occurrences in most countries that reported the disease. But, as in a few other resource-limited countries, the virus persisted in Bangladesh (3). Under-reporting of cases may be one cause of such prolonged persistence. A pro-active role by farmers in reporting suspected HPAI cases to DLS is the key to a successful passive HPAI surveillance programme. However, if farmers are to become more actively involved in the reporting process, they should first be made aware of how to diagnose probable cases of HPAI. The mortality rates and clinical signs reported from this study may be very useful in such a task.

Conclusion

The incidence rates of mortality in breeder, broiler, layer and backyard chickens that were naturally infected with the HPAI H5N1 virus in Bangladesh were, respectively: 0.0215, 0.0341, 0.0179 and 0.0703 per chicken-day at risk. The four major clinical signs observed in backyard chickens were cyanotic combs and wattles, oedema of the

head and face, drowsiness and huddling and ecchymotic discoloration of the leg shanks. The first sign, cyanotic combs and wattles, was equally observed in commercial chickens.

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Influenza aviaire hautement pathogène au Bangladesh : taux de mortalité et caractéristiques cliniques de la maladie chez des poulets atteints d'infection naturelle

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Résumé

Une étude rétrospective a été conduite au Bangladesh afin de déterminer le taux de mortalité et les signes cliniques chez des poulets de basse-cour infectés naturellement par le virus H5N1 de l'influenza aviaire hautement pathogène (IAHP).

La totalité des 33 élevages commerciaux et des 25 élevages familiaux concernés par la première vague de foyers d'IAHP (de janvier à novembre 2007) a participé à l'étude. Un questionnaire prototype a permis de recueillir des informations sur les caractéristiques des élevages, les stocks de volailles ainsi que les principaux signes cliniques observés par les éleveurs et/ou les auxiliaires d'élevage. L'évaluation rétrospective des signes cliniques par les vétérinaires participant à l'enquête a été consignée au cours d'entretiens. Après l'apparition des signes cliniques, les taux de mortalité à un jour enregistrés dans les élevages de reproducteurs, les élevages commerciaux de poulets de chair, les élevages commerciaux de poules pondeuses et les élevages familiaux de poulets étaient respectivement de 0,0215, de 0,0341, de 0,0179 et de 0,0703 par poussin à risque. Les quatre signes cliniques prépondérants étaient une cyanose de la crête et des caroncules, un œdème de la tête et de la face, une somnolence, une tendance à se blottir et des ecchymoses et décoloration des pattes. Le premier de ces signes (cyanose de la crête et des caroncules) était aussi fréquent dans les élevages commerciaux que familiaux ($p = 0,44$) ; en revanche, l'œdème de la tête et de la face était plus fréquent dans les élevages familiaux ($p = 0,03$).

Mots-clés

Bangladesh – Infection naturelle – Influenza aviaire – Influenza aviaire hautement pathogène – Mortalité – Poulet – Virus H5N1 – Volaille.

Tasa de mortalidad y características clínicas de la influenza aviar altamente patógena en pollos infectados por vía natural en Bangladesh

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Resumen

Los autores describen un estudio retrospectivo realizado en Bangladesh para analizar las tasas de mortalidad y los signos clínicos en pollos industriales o de granja infectados por vía natural por la cepa H5N1 del virus de la influenza aviar altamente patógena (IAAP).

El estudio englobaba las 33 explotaciones industriales y 25 pequeñas granjas registradas durante la primera oleada de brotes de IAAP en Bangladesh (enero a noviembre de 2007). Mediante un cuestionario-tipo se reunieron datos sobre las características de la explotación, los animales presentes en ella y los principales signos clínicos detectados por los productores y/o gerentes. También se entrevistó a los veterinarios investigadores para que hicieran una valoración retrospectiva de la sintomatología. Tras la aparición de los signos clínicos, las tasas de incidencia de mortalidad en aves reproductoras, asaderas industriales, ponedoras industriales y de granja fueron, respectivamente, de 0,0215, 0,0341, 0,0179 y 0,0703 por pollo-día expuesto al riesgo de infección. Los cuatro principales signos clínicos observados fueron: cresta y barba cianótica; edema craneal y facial; somnolencia y postración; y decoloración equimótica de las patas. El primero de ellos (cresta y barba cianótica) se observó por un igual en los pollos de explotaciones industriales y de granja ($p = 0,44$), pero el segundo (edema craneal y facial) resultó más frecuente en la segunda clase de explotaciones ($p = 0,03$).

Palabras clave

Aves de corral – Bangladesh – H5N1 – Infección natural – Influenza aviar – Influenza aviar altamente patógena – Mortalidad – Pollos.

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