Indian J. Virol. (July–September 2013) 24(2):272–277
DOI 10.1007/s13337-013-0135-2

ORIGINAL ARTICLE

Survivability of Highly Pathogenic Avian Influenza H5N1 Virus in Poultry Faeces at Different Temperatures

Baleshwari Kurmi · H. V. Murugkar · S. Nagarajan · C. Tosh · S. C. Dubey · Manoj Kumar

Received: 19 June 2012 / Accepted: 2 May 2013 / Published online: 15 May 2013

Abstract Highly pathogenic avian Influenza (HPAI) is an important zoonotic disease and is becoming a great threat to poultry industry. India has experienced continual outbreaks of H5N1 HPAI virus since February, 2006 especially in Eastern India. Survivability in poultry faeces is an important determinant in evaluating the persistence of the virus in the poultry sheds and their vicinity. In this paper, survivability of Indian H5N1 HPAI virus in dry and wet poultry faeces at 42, 37, 24 and 4 °C, respectively is reported. The effect of different temperatures was determined by linear regression model and defined in terms of linear equation. The virus survived up to 18 h at 42 °C, 24 h at 37 °C, 5 days at 24 °C and 8 weeks at 4 °C in dry and wet faeces, respectively. The coefficients of determination (R^2) values for dry and wet faeces revealed that the difference in viral persistence in dry and wet faeces at all temperatures was not very marked. Results of the present study indicated that H5N1 HPAI virus may remain viable for extended periods of time in faeces at low temperatures and may act as a long term source of influenza virus in the environment.

Keywords Avian Influenza · Faeces · H5N1 · Poultry · Temperature

Introduction

Highly pathogenic avian influenza (HPAI) virus has become a great threat to poultry and public health because of its virulence, speed of inter-flock transmission and lack of prophylactic measures due its variable genetic composition. In India, the first outbreak of H5N1 avian influenza was reported in 2006 [7] in Maharashtra, Gujarat and Madhya Pradesh [20]. However after a successful culling operation these areas have not encountered any outbreak since last 4 years [7]. Subsequently in other parts of India, outbreaks of the HPAI were reported from Manipur [26] and from Assam, Sikkim and West Bengal [16]. Repeated outbreaks of the HPAI in West Bengal Assam and Tripura have since been reported [17, 18].

Aquatic wild birds are the natural reservoir of influenza viruses [1, 9, 22, 27]. Avian influenza viruses (AIVs) mainly replicate in the gastrointestinal and respiratory tracts of the birds and are excreted from the oral cavity, nostrils, conjunctiva, and cloaca of infected birds [5, 25]. These excretions lead to heavy environmental contamination and persistence of virus on animate and inmate objects. Due to the large amount of H5N1 excreted though poultry faeces, viral persistence in this medium is of great concern for the spread of the virus and designing control measures [5].

AIVs are able to survive for fairly long period in bird faeces [19, 25]. The environmental persistence of virus depends on many factors such as, local environmental conditions, viz. temperature, salinity and organic matter [4]. Several reports suggest that environment may be contaminated from respiratory secretions and faeces and may remain infective at ambient temperatures for weeks or months, even longer in the refrigeration conditions [24]. Isolation of many AIVs subtypes, including H5 has been reported from unconcentrated surface water [10], mud and soil swabs and from aquatic birds [14] and environments where previous outbreaks have been previously documented [12].

B. Kurmi · H. V. Murugkar · S. Nagarajan · C. Tosh · S. C. Dubey · M. Kumar (✉)
High Security Animal Disease Laboratory, Indian Veterinary Research Institute, Anand Nagar, Bhopal 462 021, India
e-mail: mjoshi_43@yahoo.co.in

In India, the disease occurrence showed a pattern that may be correlated with variation in some environmental factors.The available reports are inconclusive to determine the duration of time for which AIV may remain infectious in faeces. So the present study was designed to assess the persistence of H5N1 virus in the poultry faeces under different temperature conditions which are in accordance with Indian temperature conditions.

Materials and Methods

Virus

Highly Pathogenic Avian influenza virus A\Ck\Sikkim\151466\2008H5N1 accessed from repository of HSADL Bhopal was used in this study. The virus was propagated in embryonated chicken eggs (ECEs) and EID_{50} of the infective allantoic fluid was estimated as per Reed and Muench method [21]. The EID_{50} of the stock virus was estimated to be $10^{10.33}$/ml.

Fecal Samples

Fresh fecal sample was collected from SPF chickens maintained in the SPF unit of the laboratory and was divided into two parts; one part was used as such as the wet faeces and another part was dried aseptically in the oven at 50 °C to bring the moisture level below 20 % and used as the dry faeces. In order to rule out the presence of any inherent toxicity or infective agent in the fecal sample, both the dry and wet fecal samples were processed and inoculated in eggs. The eggs were incubated at 37 °C for 5 days. All the embryos were found to be alive up to 5 days and the haemagglutination test of harvested allantoic fluids was negative indicating that the eggs were free from any infection.

Spiking of the Virus in the Faeces and Titration of Virus in Virus-Faeces Mixture

The spiking of faeces (dry and wet) was done in order to ensure a final virus concentration of around 10^6 EID_{50}/ml, which is often shed by the infected chickens in the faeces [8]. Briefly, both dry and wet faeces were spiked with the original virus (EID_{50} $10^{10.33}$/ml, diluted 1:100) in the ratio of 1 part diluted virus: 1 part faeces and triturated with mortar pestle to ensure proper mixing. The spiked faeces were incubated at 37 °C for 30 min to ensure proper adsorption of the virus to the faeces. EID_{50} of the virus-faeces mixture (dry and wet faeces) was estimated as above [21] and was confirmed to be 10^6 and $10^{6.5}$/ml for dry and wet faeces, respectively.

Temperature Treatments of the Virus-Faeces Mixtures

Survivability of virus in faeces was examined at 24, 37, 42 and 4 °C. Spiked faecal samples (100 mg) were aliquoted into the 2 ml screw capped vials. Four sets of 30 vials were made for both dry and wet faeces; each set was stored at the four different temperatures mentioned above. The samples at 4 °C were the processed for isolation of virus every week; 24 °C every 24 h and 37 and 42 °C were processed every 6 h. Positive control and negative control for both dry and wet faeces was kept at −80 °C and processed along with the treated samples.

Processing of Treated Faeces

After incubation at different temperatures as described above the spiked fecal samples were processed for virus isolation by inoculating into ECEs [28] at the specified time intervals. For each specified time interval, the positive and negative control faecal samples were also processed simultaneously for egg inoculation.

Hemagglutination (HA) Test

Twenty-five microlitres of 1× phosphate buffered saline was dispensed into each well of a plastic V bottomed microtitre plate (M/s. Greiner, Germany). In the first well 25 µl of harvested allantoic fluid was dispensed and two fold serial dilutions of virus were made from 1:2 to $1:2^{12}$. Further, 25 µl PBS was dispensed in each well and finally, 25 µl of 1 % (v/v) chicken RBCs were dispensed in all the wells. The last row of the microtitreplate was kept as RBC control, which was prepared by dispensing 50 µl of PBS and 25 µl of 1 % chicken RBCs. The plate was gently tapped for mixing and incubated at the room temperature (20–25 °C) for 30 min in a biosafety cabinet. The HA titer was determined by tilting the plate and observing the presence or absence of tear shaped streaming of the RBCs against the RBC control. The reciprocal of highest dilution giving complete haemagglutination (no streaming) was taken as the end-point HA titer (http://www.oie.int/downld/AVIAN%20INFLUENZA/A_AI-Asia.him).

HA-negative allantoic fluids were repassaged up to third passage and tested for HA before considering the allantoic fluids to be negative for virus isolation. The experiment was replicated thrice.

Statistical Analysis

The survivability of virus was determined by calculating the percent infectivity [13] as determined by the percentage of infected (Haemagglutination positive) embryos out of the total number of embryos inoculated. The variables

considered for the statistical analysis consisted of percent infectivity as dependent variable and time as the independent variable. The effect of different temperatures on the survivability of H5N1 was determined by linear regression model and defined in terms of linear equation. Coefficient of determination (R^2) indicated the goodness of fit of a model.

Results

Survivability at 42 °C

The duration of survivability was determined in terms of the percent infectivity to the ECEs in first passage and recovery of the virus in subsequent passages as described earlier [13]. The virus lost 60–65 % infectivity within 12 h of incubation (Table 1) and could not be detected in first passage after 12 h, on second passage the virus was recovered up to 18 h but after 18 h virus was not detected by HA test as well as in RT-PCR [11] and real time RT-PCR even after the third passage. The linear regression analysis indicated that the expected survival of virus was 21 h against the observed value of 18 h. The coefficients of determination (R^2) values for dry and wet faeces were estimated to be 0.9486 and 0.9446, respectively, indicating that the difference in the expected values and the observed values of the virus in dry and wet faeces at 42 °C was not significant (Table 3).

Survivability at 37 °C

At 37 °C, virus lost 80–85 % infectivity within 18 h in first passage. On second passage the virus was recovered only up to 24 h (Table 1). The regression analysis showed that virus was inactivated faster at 37 °C as the observed survival of virus was 24 h for both dry and wet faeces and it was consistent with expected value of 30.6 and 26.27 h, in dry and wet faeces, respectively. The coefficients of determination (R^2) values for dry and wet faeces were estimated to be 0.7439 and 0.9311, respectively (Table 3).

Table 1 Percent infectivity of H5N1 avian influenza virus in dry and wet faeces at 42 and 37 °C

Time (h)	42 °C		37 °C	
0	Dry	Wet	Dry	Wet
6	100	100	100	100
12	73.4	66.7	73.4	57.2
18	40	35.8	73.4	46.7
24	0	0	14.4	21.4
30	0	0	0	0
36	0	0	0	0

Survivability at 24 °C

At 24 °C, the virus was detected only up to 4th day in both dry and wet faeces after the first passage and the virus lost about 80 % infectivity within 4 days (Table 2). However the virus was recovered up to 5 days in dry and wet faeces in second passage. The virus was not detected after 5 days in both dry and wet faeces even after the third passage. Linear regression analysis indicated that the expected survival of virus was 5.7 days in dry and 5.9 days in wet faeces against the observed value of 5 days. The coefficients of determination (R^2) values for dry and wet faeces were estimated to be 0.9393 and 0.9402, respectively indicating that the difference in the expected values and the observed values of the virus in dry and wet faeces at 24 °C was not significant (Table 3).

Survivability at 4 °C

At 4 °C the virus was found to survive up to 8 weeks in dry and wet faeces. In first passage the virus was recovered up to 7th week in dry and up to 6th week in wet faeces. The virus lost 80 % infectivity on 6th week in wet faeces and 90 % infectivity on 7th week in dry faeces (Table 2). After 8 weeks virus could not be recovered in subsequent passages. Regression analysis indicated very less difference in expected survival value and observed value and was corroborated by the R^2 values of 0.9526 and 0.9299, for dry and wet fecal samples, respectively. The estimated duration of persistence was 8.5 weeks in dry and 8.85 weeks in wet faeces against the observed value of 8 weeks (Table 3).

Discussion

Environmental temperature plays a very important role in the survivability of the Avian Influenza virus. India is a tropical country and the environmental temperature varies in the range of 24–42 °C in many parts of the country. Survivability of the H5N1 virus in wet and dry faeces at different temperatures i.e., 4, 24, 37 and 42 °C was studied, which corresponded to the range of environmental temperatures encountered at various geographical locations in the country. In this study, virus was observed to have survived for only up to 24 and 18 h at 37 and 42 °C, respectively and up to 5 days and at 24 °C in both wet and dry faeces. Survivability of virus in poultry manure at the ambient temperature as reported by various workers has shown a lot of divergence. The findings of the present study were in agreement with previous observations [13] wherein loss of infectivity of AIV H7N2 was reported within 24 h at the temperatures ranging from 30–37 °C in commercial chicken manure and at 15–20 °C within 2–23 days. Survivability of the virus in the present

Table 2 Percent infectivity of H5N1 avian influenza virus in dry and wet faeces at 24 and 4 °C

Type of faeces	Temperature	Time (days)	Percent infectivity	Temperature	Time (weeks)	Percent infectivity
Dry	24 °C	0	100.0	4 °C	0	100.0
Wet			100.0			100.0
Dry		1	78.6		1	100.0
Wet			80.0			100.0
Dry		2	57.2		2	90.0
Wet			66.8			93.4
Dry		3	46.7		3	84.8
Wet			42.8			74
Dry		4	21.5		4	60.0
Wet			21.5			60.0
Dry		5	0		5	40.0
Wet			0			33.34
Dry		6	0		6	33.34
Wet			0			20.0
Dry		7	0		7	10.0
Wet			0			0
Dry		8	0		8	0
Wet			0			0

study at 37 and 42 °C was however, considerably higher than that observed by Songserm and coworkers [23], who reported that the H5N1 virus was inactivated within half an hour at these temperatures in the presence of sunlight and up to 4 days in shade at 25–32 °C. This implies that exposure of the virus infected faeces to sunlight could result in hastened inactivation process due to faster drying and radiation effects of the sunlight. In contrast, Chumpolbanchorn et al. [6] found that the H5N1 virus could not be recovered after 24 h at 25 °C in chicken manure. In contrast, Beard and his coworkers [2] reported that HPAI (H5N2) at 25 °C could be detected even after 7 days. The contrasting and varying observations regarding the effect of temperature on virus survivability during the present study and those reported by various workers implies the complexity of the factors influencing the virus survivability in the chicken faeces. The organic content of the faecal material, amount of moisture, exposure to the sunlight, residual disinfecting chemicals may all play a role in avian influenza virus survivability.

Linear regression analysis of the virus infectivity in the embryonated eggs indicated that the observed persistence of the virus did not deviate from the expected values for all the temperatures The linear regression model indicated that the difference in the persistence of the virus in dry and wet faeces at all temperatures was not very marked and there was a negative correlation between the virus survival and temperature and the virus was highly susceptible to the higher temperature.

Analysis of outbreaks of H5N1 avian influenza in Eastern India indicated that favorable climatic conditions could have ensured the persistence of the virus and could be one of the major reasons for repeated outbreaks. The temperatures in the western India reach as high as 45 °C during the summer season and is also very dry. In contrast, the temperatures in eastern region of the country hover around 35 °C even in the peak summer season. In addition, almost all the outbreaks had been reported during the peak winter months where the temperatures are considerably low in the region. Favorable temperature coupled with high humidity in the region makes it more conducive for the longer persistence of the virus in the environment [6].

The mechanism of the effect of temperature is not clearly understood. It may kill or disable the viruses by two possible ways. High temperature may alter the envelope or damage the proteins and nucleic acid. Enveloped viruses can be rendered harmless when their envelope is destroyed, because the virus no longer has the recognition sites necessary to identify and attach to host cells [29]. High temperatures also denature the proteins, and can result in fatal mutations or can halt the process of protein synthesis, by damaging RNA [3]. The positive correlation between the colder temperatures and the persistence of the virus has been comprehensively established [2]. Extremely low temperatures in the environment results in the prolonged survivability of the virus and enhances the risk of recurrent bouts of outbreaks in the same geographical regions. Persistence studies of the virus at 4° C was carried out for two reasons; one was to see the effect of low temperature environment on the survivability of the virus, and the other was to see the feasibility of the isolation of the virus from

Table 3 Survivability of H5N1 avian influenza virus in dry and wet faeces

		LRM equation	E	O	R^2
42 °C	Dry	y = −4.5567*x + 97.36	21.6 h	18 h	0.9486
	Wet	y = −4.445*x + 93.832	21.5 h	18 h	0.9446
37 °C	Dry	y = −2.8427*x + 86.809	30.6 h	24 h	0.7439
	Wet	y = −3.3181*x + 87.32	26.2 h	24 h	0.9311
25 °C	Dry	y = −15.354*x + 91.723	5.7 days	5 days	0.9393
	Wet	y = −15.734*x + 93.943	5.9 days	5 days	0.9402
4 °C	Dry	y = −12.239*x + 108.3	8.85 weeks	8 weeks	0.9526
	Wet	y = −12.438*x + 105.89	8.5 weeks	8 weeks	0.9299

Y % infectivity, *x* persistence in hours, *E* expected time required for virus to become undetected, *O* observed time required for virus to become undetected, * decrease in infectivity/h, R^2 coefficient of determination

the fecal samples that are generally stored at 4 °C in the laboratories. In this study the virus survived up to 8 weeks at 4 °C in both wet and dry faeces. The results are in agreement with observation of previous studies [2] that showed that the infectivity of H5N2 virus at 4 °C could be detected after 35 days. The regression analysis indicated very less difference in expected survival value and observed value and was corroborated by the R^2 values of 0.9299 and 0.9526, for dry and wet fecal samples, respectively. The regression analysis also indicated very less difference in the persistence of virus in wet and dry faeces. Lu et al. [13] reported that AIV H7N2 retained its infectivity at refrigeration temperature for more than 20 days in experimental field chicken manure.

The variation in the fecal contents, storage conditions of the samples, exposure to environmental factors, such as humidity, sunlight, etc., can all play a role in the variation observed as regards to the survivability of the virus [23]. The survivability depends on the medium in which the virus is present. The present study showed that persistence of virus in the water greatly differs from that in faeces, as in many studies it was reported that the virus survives longer in the water. The AIVs persisted in distilled water from 126 to 207 days at 17 °C to 30–102 days at 28 °C [24]. Another study indicated that a H7N3 subtype preserved its infectivity when incubated at 4 °C for 35 days [15]. One important consideration in this aspect from the epidemiology point of view could be the close association of the persistence of virus in the faeces and the water bodies present in the many parts of the country.

Our study did not show significant difference in the survivability of the virus in wet and dry faeces. This may be due to small amount of the sample taken which might lead to rapid dehydration of the faeces during incubation. However in large amounts, moisture may protect the virus in wet faeces and survival may be longer in the wet faeces than the dry faeces [6].

The observations of the present study that virus in poultry faeces is susceptible to inactivation within 1 day of the exposure at the temperatures of 37 and 42 °C indicates the usefulness of the hot summer months in many parts of the country where these levels of temperatures are maintained for more than 3 months. In addition to the successful culling and post culling operations, non recurrence of outbreaks in Western India Maharashtra, Gujarat and Madhya Pradesh after 2006 could partly be due to exceedingly high environmental temperature (up to 45 °C) in the summer that followed the outbreak in February. Such high temperatures could have inactivated any residual virus that may be present in the area. In contrast, repeated outbreaks of HPAI in West Bengal, Assam and Tripura [16–18] could be correlated with presence of large number water bodies and favourable environmental conditions i.e. lower temperature and humidity that might have played a role in persistence of the virus in the environment. To control influenza outbreaks or a pandemic, it is of utmost importance to identify and characterize the different factors and biological properties of avian strains that could promote influenza virus persistence and transmission.

Acknowledgments The authors are thankful to Indian Council of Agricultural Research and Director, Indian Veterinary Research Institute, Izatnagar, Bareilly for supporting this study and providing financial help to carry out this study.

References

1. Alexander DJ. A review of avian influenza in different bird species. Vet Microbiol. 2000;74:3–13.
2. Beard CW, Brugh M, Johnson DC. Laboratory studies with Pennsylvania avian influenza viruses (H5N2). Proceedings of the US Animal Health Association, 4th Worth, TX, USA, 1984;88:462–473.
3. Benedictis DP, Beato MS.Capua I. Inactivation of avian influenza viruses by chemical agents and physical conditions: a review. Z Pub H. 2007;54:51–68.
4. Brown JD, Swayne DE, Cooper RJ, Burns RE, Stallknecht DE. Persistence of H5 and H7 avian influenza viruses in water. Avian Dis. 2007;51:285–9.
5. Centers for Disease Control and Prevention Key facts about avianinfluenza (bird flu) and avian influenza A (H5N1) virus. 2005. http://www.cdc.gov/flu/avian/gen-info/pdf/avian_facts.pdf. Accessed 5 May 2012.
6. Chumpolbanchorn K, Suemanotham N, Siripara N, Puyati B, Chaichoune K. The effect of temperature and UV light on infectivity of avian influenza virus (H5N1, Thai field strain) in

chicken fecal manure. Southeast Asian J Trop Med Pub Health. 2006;37:102–5.
7. Dubey SC, Nagarajan S, Tosh C, Bhatia S, Krishna L. Avian Influenza: a long-known disease and its current theat. Ind J Anim Sci. 2009;72:113–40.
8. Forrest HL, Jeong-Ki K, Webster RG. Virus shedding and potential for interspecies waterborne transmission of highly pathogenic H5N1 influenza virus in sparrows and chickens. J Virol. 2010;84:3718–20.
9. Hinshaw V, Webster RG, Turner B. The perpetuation of orthomyxoviruses and paramyxoviruses in Canadian waterfowl. Can J Microbiol. 1980;26:622–9.
10. Hinshaw V, Webster R, Turner B. Waterborne transmission of influenza a viruses. Intervirol. 1979;11:66–9.
11. Lee MS, Chang PC, Shien JH, Cheng MC, Shieh HK. Identification and subtyping of avian influenza viruses by reverse transcription PCR. J Virol Methods. 2001;97:13–22.
12. Li KS, Guan Y, Wang J, Smith GJD, Xu KM, Duan L, Rahardjo AP, Puthavathana P, Buranathai C, Nguyen TD, Estoepangestie ATS, Chaisingh A, Auewarakul P, Long HT, Hanh NTH, Webby RJ, Poon LLM, Chen H, Shortridge KF, Yuen KY, Webster RG, Peiris JSM. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature. 2004;430:209–13.
13. Lu H, Castro AE, Pennick K, Liu J, Yang Q, Dunn P, Weinstock D, Henzler D. Survival of avian influenza virus H7N2 in SPF chickens and their environments. Avian Dis. 2003;47:1015–21.
14. Markwell DD, Shortridge KF. Possible waterborne transmission and maintenance of influenza viruses in domestic ducks. Appl Env Microbiol. 1982;43:110–6.
15. Muhammad K, Das P, Yaqoob T, Riaz A, Manzoor R. Effect of physicochemical factors on survival of avian influenza virus (H7N3 type). Int J Agric Biol. 2001;4:416–8.
16. Murugkar HV, Nagarajan S, Tosh C, Bhatia S, Venkatesh G, Jain R, Kumar S, Khandia R, Pandey M, Tripathi S, Kulkarni DD, Dubey SC. H5N1 virus outbreaks in poultry in India. Vet Rec. 2008;162:255.
17. Nagarajan S, Murugkar HV, Tosh C, Behera P, Jain R, Tripathi S, Khandia R, Gupta V, Kulkarni DD, Dubey SC. Avian influenza virus (H5N1) in chickens in India. Vet Rec. 2009;164:128.
18. Nagarajan S, Tosh C, Smith DK, Peiris JSM, Murugkar HV, Sridevi R, Kumar M, Katare M, Jain R, Syed Z, Behera P, Cheung CL, Khandia R, Tripathi S, Guan Yi, Dubey SC. Avian influenza (H5N1) virus of clade 2.3.2 in Domestic Poultry in India. PLoS One. 2012;7(2):e31844. doi:10.1371/journal.pone.0031844.
19. Nazir J, Haumacher R, Ike AC, Marschang RE. Persistence of avian influenza viruses in lake sediment, duck faeces, and duck meat. Appl Env Microbiol. 2011;77(14):4981–5.
20. Pattnaik B, Pateria AK, Khandia R, Tosh C, Nagarajan S, Gounalan S, Murugkar HV, Shankar BP, Shrivastava N, Behera P, Bhagat S, Peiris JSM, Pradhan HK. Phylogenetic analysis revealed genetic similarity of the H5N1 avian influenza viruses isolated from HPAI outbreaks in chickens in Maharashtra India with those isolated from swan in Italy and Iran in 2006. Curr Sci. 2006;91:77–81.
21. Reed LJ, Muench H. A simple method for estimating the 50 % end points. Am J Hyg. 1938;27:493.
22. Slemons RD, Johnson DC, Osborn JS, Hayes F. Type A influenza viruses isolated from wild free flying ducks in California. Avian Dis. 1974;18:119–24.
23. Songserm T, Jam-on R, Sae-Heng N, Meemak N. Survival and stability of HPAI H5N1 in different environments and susceptibility to disinfectants. In: Schudel A, Lombarrd M, editors. Development of biological, vol. 24. Switzerland: Karger; 2006. p. 254.
24. Stallknecht DE, Shane SM, Kearney MT, Zwank PJ. Persistence of avian influenza viruses in water. Avian Dis. 1990;34:406–11.
25. Swayne DE, Jackwood MP. Pathobiology of avian influenza virus infections in birds and mammals avian influenza. In: Swayne DE, editor. Avian Influenza. Ames: Blackwell; 2008. p. 87–103.
26. Tosh C, Murugkar HV, Nagarajan S, Bhatia S, Pateriya AK, Behera P, Jain R, Kumar S, Khandia R, Vanamayya PR, Dubey SC, Ahlawat SPS. Outbreak of avian influenza virus H5N1 in India. Vet Rec. 2007;161:279.
27. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. Microbiol Rev. 1992;56:152–79.
28. WHO, The Writing Committee of the World Health Organization. Consultation on Human Influenza A/H5. Avian influenza A (H5N1) infection in humans. New Eng J Med. 2005;353:1374–85.
29. York JL. Enzymes: classification, kinetics, and control. In: Devlin TM, editor. Textbook of biochemistry with clinical correlations. 4th ed. New York: Wiley-Liss; 1997. p. 127–78.