Journal of Advanced Veterinary and Animal Research
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2018.e269
A periodical of the Network for the Veterinarians of Bangladesh (BDvetNET)

June 2018
Vol 5 No 2, Pages 204-210.

Original Article

Seroprevalance of Influenza A in swine population of Rangamati and Khagracchari districts

Md. Karim Uddin[1,#], Md. Shamim Ahasan[1], Mohammad Rafiqul Islam[2], M. M. Mafizul Islam[3] and Md. Fazlul Hoque[1]

• **Received:** Jan 12, 2018 • **Revised:** April 2, 2018 • **Accepted:** April 5, 2018 • **Published Online:** May 3, 2018

AFFILIATIONS

[1]*Department of Medicine, Surgery and Obstetrics, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh.*

[2]*Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka 1212, Bangladesh.*

[3]*Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.*

CORRESPONDENCE:

[#] Md. Karim Uddin,
Department of Medicine, Surgery and Obstetrics, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh.
E-mail: karim.vetmed@gmail.com

ABSTRACT

Objective: This study is conducted to assess the seroprevalence and associated risk factors (e.g., age, sex, bio-security practices and management system) of Influenza A virus in swine population of Rangamati and Khagracchari Districts
Materials and methods: Prevalence study Influenza A in swine population was conducted over a period of six months Rangamati and Khagracchari Districts between July to December 2013. 180 blood samples were collected from pigs, and the samples were tested for the detection of Influenza antibody using Indirect ELISA method.
Results: Total numbers of samples were 180 and numbers of positive cases were 22.Then the overall seroprevalence between the aforesaid districts was found to be 12.22%. Results of the investigation revealed that the seroprevalence of influenza A was 15% in Rangamati district, 10% in Khagracchari district . The highest seroprevalence was found in Rangamati district (15%) and the lowest seroprevalence was (10%) found in Khagracchari district. On the basis of sex, seroprevalence rate of influenza A was found 14.29% in male pigs and 9·76% in female pigs.
Conclusion: The study confirms that influenza virus is circulating in the pig populations of hill tracts area of Bangladesh. Our study had a number of limitations. Veterinarians, researchers and health officials will get new information from this research which will be helpful for developing prevention strategy for combating against this disease.

KEYWORDS

Influenza A; Seroprevalence; Swine; Pigs

How to cite: Uddin MK, Ahasan MS, Islam MR, Islam MMM, Hoque MF. Pathogenic potentials and shedding probability of *Salmonella enterica* serotype Kentucky in experimentally infected backyard chicken. Journal of Advanced Veterinary and Animal Research. 2018; 5(2):204-210.

INTRODUCTION

Influenza A virus is a single-stranded negative-sense RNA virus with 7 to 8 segmented genome encoded with eleven distinctive viral proteins (Lamb, 2001; Olsen et al., 2006). The replication of influenza virus of avian and human origin in swine has led to the coming out of novel reassortant viruses by changing its nature antigenically (Ito et al., 1998). For viral replication transmission is the first step in the host. For successful transmission of virus, a contact between virus source and susceptible host is required (Thomas and Weber, 2001).

Swine tracheal epithelial cells contain both α-2,3 and α-2,6 sialic acids receptors, (Nelli et al., 2010; Trebbien et al., 2011), Consequently, swines are susceptible host to influenza viruses of avian, swine, and human type and act as vectors of variout influenza viruses for genetic reassortment, resulting in evolving of new influenza virus sub-types (Imai and Kawaoka, 2012) and thus, swine has been called as mixing vessel where reassortant influenza virus can take place. The recent emergence of a pandemic influenza A virus of human type bearing genome thought to be of swine origin frazzled again the concern in the swine influenza epidemiology (Smith et al., 2009) and they possibly an intermediary host for the adjustment of avian type viruses to mammals and a mixing vessel for the evolving of reassortment viruses(Van Reeth et al., 2012). On pig farms swine influenza is very common, similar to that observed in humans, but affects the animals all year round, whatever the season.

As in humans, the intensity of clinical signs in pigs is inconsistent and can be influenced by many factors such as type of virus, the age and protected level of the infected pig, weather, the type of housing, and also co-infections (Khatri et al., 2010; Deblanc et al., 2013; Fablet et al., 2013). The lungs are found to be the predilection site of flu virus in pigs is confined to the air way. This respiratory virus replicates in epithelial cells of the respiratory system (Brown, 2000); thus nasal secretions are the prime source of excretion of influenza virus. Characteristic clinical signs of the acute infection are pyrexia, anorexia, inactivity and reluctance to rise after 1 to 3 days of an incubation period (Olsen et al., 2006).

To preserved viral epitopes of virus, and protecting partially with an genetically distinct viral strains can arise even though both strains may exhibit low cross-reactive antibodies this virus stimulates mucosal and systemic cellular immune responses when natural infection occurs (De Vleeschauwer et al., 2011). Kyriakis et al. (2010) recommended that preceding protection to the recognized strains of virus due to infection, partly immunized pigs in European countries against pandemic influenza virus.

Many years ago, diagnosis of influenza was basically done by clinical signs, because, to detect the infection in live pigs there was no other methodology. Although, the diagnosis by sign and symptoms only not easy in the last decennary while a part of the individuals has been infected with the influenza and infections have been recognized sub clinically due to indistinct immunological protection (Böttcher et al. 2006). Currently to detect influenza infections rapid diagnostic tests are available. Information on the seroprevalence and distribution of swine influenza viruses remains limited in different countries globally including Bangladesh. Understanding the swine influenza epidemiology is required for the improvement of gainful influenza surveillance and control plan to limit the spread of these viruses in swine. Epidemiology of Swine influenza varies throughout the countries due to several factors such as season, swine population, farming and management practices.

There are no available data of seroprevalence of influenza A in swine population of Hill tracts area of Bangladesh. To generate a new idea this research was undertaken to explore the seroprevalence status of influenza a in swine population of Rangamati and Khagracchari districts of Bangladesh by using indirect ELISA and Identification of associated risk factors of Influenza A virus Epidemiology.

MATERIALS AND METHODS

Study design: We conducted this cross sectional study from May to November of 2013. Backyard pigs of the pig-rearing village of Rangamati and Khagracchari districts of Bangladesh are our target population. We chosen the earliest house at random and a house that was placed 2–3 house far from the first house in the same route was chosen for sampling, and this procedure was continued for next house selection.

Study area: The study was conducted in pig-raising villages of Rangamati and Khagracchari districts of Bangladesh.

Questionnaire: Data collection was performed by using a pretested questionnaire through in person interview. First author carried out the interviews of pig owner in study area. In general, the information covered in the questionnaire were demographic characteristics, age grou

p, contact with other species, housing system of pig, flulike sign, pigs were sick compared to the total in last month, ili symptoms, coughing, nasal discharge, lethargy, increased temperature and biosecurity practices. A total of 180 farmers have given consent to be interviewed across 2 districts.Information gathered in the questionnaire was entered in the Microsoft Excel worksheet, 2007 and then exported to STATA 13 for statistical analysis.

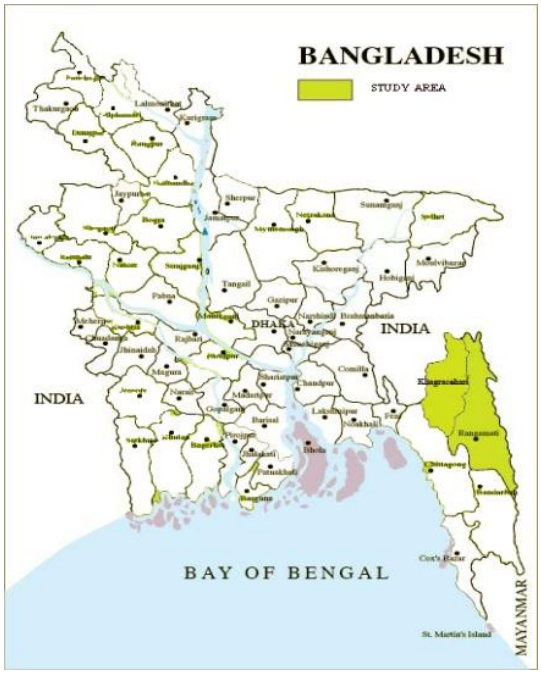

Figure: Study area for Influenza A in Bangladesh.

Sample collection and testing: In every selected household, we perform blood sampling in single pig. Samples of 3-5 mL of blood were collected from cranial vena cava/external jugular vein of individual pigs. Sample containing syringes are positioned at on a 45 degree slant direction for two hours at ambient room temperature for serum separation. All the collected serum samples in cryotube and shifted to the Veterinary Medicine Laboratory, HSTU maintaining cool chain at 4°C and then store at -20°C until testing. IDEXX Influenza A Ab test kit (IDEXX Laboratories, Westbrook, ME, USA), is used to test the samples for the presence of antibodies to Influenza A virus. This test is a blocking ELISA (IDEXX, 2013), 95.4% sensitivity and 99.7% Specifically with results being expressed as sample to negative control (S/N) ratios.

Approval: Approval was taken from ethical committee of Faculty of Veterinary and Animal Sciences, HSTU for using animals by describing the protocols. After explaining the objectives of this study, consent was taken from all participating backyard pig raisers. Collection of blood samples was done by following standard operating procedure and questionnaire responses were given on a voluntary basis.

Statistical analysis: Individual household questionnaire data were entered into Microsoft Excel file. This was then exported to STATA 13.0 (Stata Corporation., College Station, TX, USA) for data cleaning and data management. . Stata were used for descriptive and statistical analyses. In addition, to assess risk factors associated with Influenza A in backyard pigs, we estimated odds ratios and confidence interval performing Chi square test and bivariate logistic regression using Stata software.

RESULTS

The cases of seropositive animals for Influenza A from each district are given in **Table 1**. Total number of samples were180 and number of positive cases were 22.Then the overall seroprevalence between the aforesaid districts was found to be 12.22% (**Table 1**). Results of the investigation revealed that the seroprevalence of influenza A was 15% in Rangamati district, 10% in Khagracchari district (**Table 1**).

Table 1. Prevalence of Influenza A in Rangamati and Khagracchari districts.

Samples	Sample (N)	Positive cases (n)	Negative cases (n)	Prevalence (%)
Total Sample	180	22	158	12.22
Rangamati district	80	12	68	15
Khagracchari district	100	10	90	10
Male	98	14	84	14.28
Female	82	8	74	9.76
Grower	52	6	46	11.54
Fattening	64	8	56	12.5
Adult	64	8	56	12.5

The highest seroprevalence was found in Rangamati district (15%) and the lowest seroprevalence was (10%) found in Khagracchari district. On the basis of sex, seroprevalence rate of influenza A was found 14.29% in male pigs and 9·76% in female pigs (**Table 1**).The seroprevalence of influenza A was studied based on age group and presented on **Table 1**. It was observed that 11·5% seroprevalence found in grower pigs, 12·5% in fattening pigs and 12·5% in adult pigs. The highest (12·5%) prevalence was found in fattening and adult pigs in comparison to grower pigs.

Table 2 show factors that were significantly associated with Influenza A in pig , using chi-squared test. The significance level was considered at 5 % ($P<0.05$). The result of the analysis revealed that Contact with other Species, Pigs were sick compared to the total in last month, Sneezing, Coughing, Nasal Discharge, Increased temperature were significantly associated with the likelihood for Influenza A in pigs. Pig reared in confined outdoor housing system showed to have a significantly greater risk for Influenza A infection compared to pigs reared under confined-indoor, tethered-outdoor and scavenging system. The odds of Influenza A of pigs reared in these housing systems were around four times higher than scavenging system. If the sick Pigs compared to the total in last month is less than 10 % were fifteen times less likely (OR=0.56) seropositive to Influenza A compared to number of sick Pigs compared to the total in last month is 10-50 %.

The presence of sneezing significantly increased the probability of infection. Pigs with sneezing were about 6 times more likely (OR=5.88) to be seropositive for Influenza A, compared to pigs without sneezing. Coughing in pigs significantly increased the probability of infection. Pigs with coughing were 10 times more likely (OR=10.13) to be seropositive for Influenza A, compared to pigs without coughing. The disease odds increased by five times when body temperature of pig increased compared to Normal body temperature of pig. Nasal discharge in Pigs make 5.75 times more likely (OR=5.75) to test seropositive for Influenza A as compared to pigs without nasal discharge.

Table1. Prevalance and associated risk factors of influenza A in swine population of Rangamati and Khagracchari Districts

Characteristics	N=180(%)	OR(95% CI)	*P*-value
Sex			
Male	98(54.4)	1.54(0.61- 3.88)	0.358
Female	82(45.6)	1	
Age group			
Fattening	64(35.6)	1.10(0.35-3.38)	0.87
Adult	64(35.6)	1.10(0.35-3.38)	0.87
Grower	52(28.9)	1	
Contact with other Species			
Chicken	78(49.3)	6.90(.82- 58.21)	0.076
Neighboring pig		1	
Housing system of pig			
Confined-indoor	12(6.7)	4.8(.60-38.22)	0.138
Confined-outdoor	78(40)	4.8(1.02- 22.49)	0.047
Tethered-outdoor	46(25.6)	3.6(0.69-18.83)	0.129
Scavenging	50(27.8)	1	
Flulike sign			
Yes	128(71.1)	4.63(1.04-20.58)	0.044
No		1	
Pigs were sick compared to the total in last 6 months			
10%	142(78.9)	.56(.133- 2.33)	0.424
50%	34(18.9)	8.4(2.46- 28.66)	0.001
>50%	4(2.2)	1	
Ili symptoms			
Sneezing			
Yes	22(12.2)	5.88(2.10- 16.42)	0.001
No		1	
Reduced feed intake	24(13.3)	NA	
Coughing			
Yes	22(12.2)	10.13(3.64-28.26)	0.000
No		1	
Nasal discharge			
Yes	30(16.7)	5.75(2.19-15.04)	0.000
No		1	
Lethargy			
Yes	64(35.6)	2.45(1.00-6.03)	0.052
No		1	
Poor growth	94(52.2)		
Increased temperature			
Yes	54(30)	5.16(2.02-13.21)	0.015
No		1	
Biosecurity			
Wild birds visible on property	130(72.2)	1.21(0.25- 5.84)	0.806
Water pond on farm or nearby	36(20)	1.8(0.73-4.44)	0.202
Farm located within 500 meters of main road	32(17.8)	1.91(0.71-5.15)	0.199

DISCUSSION

This is the initial seroprevalence study of influenza A in the pig population ever conducted in Rangamati and Khagracchari districts of Bangladesh. The study demonstrated the presence of antibodies to influenza A with a high degree of certainty. Transmission of the influenza virus in local pigs in Rangamati and Khagracchari districts brought about occurrences of respiratory disease with high morbidity except low mortality. Although, it is unsure whether or not the dissemination of this agent assisted to the expression of signs and symptoms of other respiratory illnesses and appeared to increase severity during this same time period. In most countries influenza A Infections of backyard pigs discovered in rigorously rearing pigs however there are few documents revealed, regarding the circulation of illnesses in pigs (Olsen et al., 2000). By ELISA, approximately 12.22 % of pigs serum tested in this study were positive serologically for Influenza A. In Belgium and France serologic study conducted by (Van Reeth et al., 2004) of swine of finishing stage revealed that prevalences of influenza A are upper (55.4% and 28.9%) compared to this study findings. (Jung et al., 2007) found elevated seropositive cases within pigs for pandemic influenza (51.2%) and (Liu et al., 2011) found 31.1% seropositivity of influenza A in the pig population in China. Results of the investigation revealed that the seroprevalence of influenza A was 15% in Rangamati district, 10% in Khagracchari district.

The highest seroprevalence was found in Rangamati district (15%) in contrast to Khagracchari district (10%). Highest seroprevalence in Rangamati is due to presence of kaptai lake where different water lives in all seasons and foreighner waterfowls accumulated during winter season. Since, pig acts as mixing vessels for reassortment of influenza A virus so seroprevalence of influenza A in rangamati districts higher than that of khagracchari due to presence of lake where waterfowls live their lives. The finding of the study is similar to the (Webster et al., 1978; Hinshaw et al., 1980; Serratosa et al., 2007).

In the study areas, native pigs were reared under confined-outdoor, tethering system and also allowed to scavenge with backyard chickens and ducks in the yard, in the crop field near to water sources where domestic ducks, wild ducks and migratory ducks used to scavenge. These factors may contribute in natural infection to the native pigs (van der Vries et al., 2013). On the basis of sex, seroprevalence rate of influenza A was found 14.29% in male pigs and 9·76% in female pigs. This might be due to difference of immune status at various sex of pigs.It was observed that 11·5% seroprevalence was found in grower pigs, 12·5% in fattening pigs and 12·5% in adult pigs.

The highest (12·5%) prevalence was found in fattening and adult pigs and the lowest prevalence was found in grower pigs. The result showed similarity with the study conducted by (Loeffen et al., 2003; Jeong et al., 2004). The nearly all commonly Influenza infection occurs at about 10 weeks of age. Colostrum antibodies in pigs against influenza virus endure for two to four months, relying on the primary level (Candotti et al., 2003). Weaning pigs may be safe from serious disease with elevated amount of MDA but cannot give protection against diseases and multiplication of the viral agent (Loeffen et al., 2003). This study found that Pigs moves in a farms where ducks and chickens were reared in close distance, they appear to be more likely to be seropositive for influenza A. which has similarity with the findings of (Ayora-Talavera, Cadavieco-Burgos et al. 2005). As result of reduced MDA swine could get infection, release viral particles, show characteristics signs , and hold innate immune reaction (Easterday and Van Reeth, 1999). The sampled pigs of this study previously not vaccinated against Influenza A virus. The animals seropositive to influenza may have been exposed to the virus from other infected pigs at any point of their lifetime. This study clearly demonstrated that influenza viruses were able to spread throughout the hill tract pig population.

LIMITATIONS

Our study had a number of limitations. First, it is possible that results may be confounded by serologic cross-reactivity between different viral subtypes. In our study, the possibility of cross-reaction between subtypes was not determined. Second, pigs from slaughterhouses or commercial farms are not included in these districts.

CONCLUSION

The study findings reveals that influenza A virus is circulating in the pig populations of Rangamati and Khagracchari districts which is interesting. To know the type and clade of the virus furtherr epidemiological investigations should be carried out throughout the country with molecular characterization.

ACKNOWLEDGEMENT

This work was supported by faculty of postgraduate studies of the Hajee Mohammad Danesh Science and Technology University (HSTU). The authors would like

to thanks to the all the staff, research fellows and teachers of the Medicine, Surgery and Obstetrics department under Veterinary and Animal Sciences faculty of HSTU for their contribution to this research.

CONFLICT OF INTEREST

There is no conflict of interest to declare.

AUTHORS' CONTRIBUTION

MKU and MFH designed the study. MFH supervised and MSA co-supervise the overall research work and provided valuable suggestions throughout the experiment. MMI assist MKU in the collection of samples from pigs and conducted laboratory test of serum. MKU performs statistical analysis. In writing and reviewing the manuscript and approved the final manuscript all the authors contributed.

REFERENCES

1. Ayora-Talavera G, Cadavieco-Burgos JM, Canul-Armas AB. Serologic evidence of human and swine influenza in Mayan persons. Emerging Infectious Diseases. 2005; 11(1):158. https://doi.org/10.3201/eid1101.040554
2. Böttcher E, Matrosovich T, Beyerle M, Klenk H-D, Garten W, Matrosovich M. Proteolytic activation of influenza viruses by serine proteases TMPRSS2 and HAT from human airway epithelium. Journal of Virology. 2006; 80(19):9896–9898. https://doi.org/10.1128/JVI.01118-06
3. Brown IH. The epidemiology and evolution of influenza viruses in pigs. Veterinary Microbiology. 2000; 74(1):29–46. https://doi.org/10.1016/S0378-1135(00)00164-4
4. Candotti P, Foni E, Leotti G, Joisel F, Longo S, Rota Nodari S, editors. Serological prevalence for swine influenza virus in pigs between 3 and 15 weeks of age in Italian farms: evaluation of a maternal antibody decay curve. Proc 4th International Symposium on Emerging and reemerging pig disease, Rome, Italy. 2003.
5. De Vleeschauwer AR, Van Poucke SG, Karasin AI, Olsen CW, Van Reeth K. Cross-protection between antigenically distinct H1N1 swine influenza viruses from Europe and North America. Influenza and other Respiratory Viruses. 2011; 5(2):115–122. https://doi.org/10.1111/j.1750-2659.2010.00164.x
6. Deblanc C, Robert F, Pinard T, Gorin S, Quéguiner S, Gautier-Bouchardon AV, Ferré S, Garraud JM, Cariolet R, Brack M, Simon G. Pre-infection of pigs with Mycoplasma hyopneumoniae induces oxidative stress that influences outcomes of a subsequent infection with a swine influenza virus of H1N1 subtype. Veterinary Microbiology. 2013; 162(2–4):643–651. https://doi.org/10.1016/j.vetmic.2012.11.028
7. Easterday B, Van Reeth K. Diseases of Swine/Eds BE Straw, S D'Allaire, WL Mengeling, DJ Taylor-8th Ed-Blackwell Sciences. 1999; p. 277–290.
8. Fablet C, Simon G, Dorenlor V, Eono F, Eveno E, Gorin S, Quéguiner S, Madec F, Rose N. Different herd level factors associated with H1N1 or H1N2 influenza virus infections in fattening pigs. Preventive Veterinary Medicine. 2013; 112(3-4):257–265. https://doi.org/10.1016/j.prevetmed.2013.07.006
9. Hinshaw V, Webster R, Turner B. The perpetuation of orthomyxoviruses and paramyxoviruses in Canadian waterfowl. Canadian Journal of Microbiology. 1980; 26(5):622–629. https://doi.org/10.1139/m80-108
10. Imai M, Kawaoka Y. The role of receptor binding specificity in interspecies transmission of influenza viruses. Current Opinion in Virology. 2012; 2(2):160-167. https://doi.org/10.1016/j.coviro.2012.03.003
11. Ito T, Couceiro JN, Kelm S, Baum LG, Krauss S, Castrucci MR, Donatelli I, Kida H, Paulson JC, Webster RG, Kawaoka Y. Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. Journal of Virology. 1998; 72(9):7367–7373.
12. Jeong K, Park Y, Jin W, Han J, Jeong H, Kim H. Sero-prevalence of swine influenza virus in Korea. Proc 18th International Pig Veterinary Society Congress, Hamburg, Germany. 2004.
13. Jung K, Song D-S, Kang B-K, Oh J-S, Park B-K. Serologic surveillance of swine H1 and H3 and avian H5 and H9 influenza A virus infections in swine population in Korea. Preventive Veterinary Medicine. 2007; 79(2-4):294–303. https://doi.org/10.1016/j.prevetmed.2006.12.005
14. Khatri M, Dwivedi V, Krakowka S, Manickam C, Ali A, Wang L, Qin Z, Renukaradhya GJ, Lee CW. Swine influenza H1N1 virus induces acute inflammatory immune responses in pig lungs: a potential animal model for human H1N1 influenza virus. Journal of Virology. 2010; 84(21):11210–11218. https://doi.org/10.1128/JVI.01211-10
15. Kyriakis CS, Olsen CW, Carman S, Brown IH, Brookes SM, Doorsselaere JV, Reeth KV. Serologic cross-reactivity with pandemic (H_1N_1) 2009 virus in

pigs, Europe. Emerging Infectious Diseases. 2010; 16(1):96. https://doi.org/10.3201/eid1601.091190
16. Lamb RA. Orthomyxoviridae: the viruses and their replication. Fields virology. 2001.
17. Liu W, Wei M-T, Tong Y, Tang F, Zhang L, Fang L, Yang H, Cao W-C. Seroprevalence and genetic characteristics of five subtypes of influenza A viruses in the Chinese pig population: a pooled data analysis. The Veterinary Journal. 2011; 187(2):200–206. https://doi.org/10.1016/j.tvjl.2009.10.026
18. Loeffen W, Heinen P, Bianchi A, Hunneman W, Verheijden J. Effect of maternally derived antibodies on the clinical signs and immune response in pigs after primary and secondary infection with an influenza H1N1 virus. Veterinary Immunology and Immunopathology. 2003; 92(1):23–35. https://doi.org/10.1016/S0165-2427(03)00019-9
19. Nelli RK, Kuchipudi SV, White GA, Perez BB, Dunham SP, Chang K-C. Comparative distribution of human and avian type sialic acid influenza receptors in the pig. BMC veterinary Research. 2010; 6(1):4. https://doi.org/10.1186/1746-6148-6-4
20. Olsen C, Brown I, Easterday B, Van Reeth K. Swine influenza. Diseases of swine. 2006; 9:469–482.
21. Olsen C, Carey S, Hinshaw L, Karasin A. Virologic and serologic surveillance for human, swine and avian influenza virus infections among pigs in the north-central United States. Archives of Virology. 2000; 145(7):1399–1419. https://doi.org/10.1007/s007050070098
22. Serratosa J, Ribo O, Correia S, Pittman M. EFSA scientific risk assessment on animal health and welfare aspects of avian influenza (EFSA-Q-2004-075). Avian Diseases. 2007; 51(s1):501–503. https://doi.org/10.1637/7574-040106R.1
23. Smith GJ, Vijaykrishna D, Bahl J, Lycett SJ, Worobey M, Pybus OG, Ma SK, Cheung CL, Raghwani J, Bhatt S, Peiris JS, Guan Y, Rambaut A. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. Nature. 2009; 459(7250):1122. https://doi.org/10.1038/nature08182
24. Thomas JC, Weber DJ. Epidemiologic methods for the study of infectious diseases: Oxford University Press; 2001; p. 70–125.
25. Trebbien R, Larsen LE, Viuff BM. Distribution of sialic acid receptors and influenza A virus of avian and swine origin in experimentally infected pigs. Virology Journal. 2011; 8(1):434. https://doi.org/10.1186/1743-422X-8-434
26. van der Vries E, Anber J, van der Linden A, Wu Y, Maaskant J, Stadhouders R, van Beek R, Rimmelzwaan G, Osterhaus A, Boucher C, Schutten M. Molecular assays for quantitative and qualitative detection of influenza virus and oseltamivir resistance mutations. The Journal of Molecular Diagnostics. 2013; 15(3):347–354. https://doi.org/10.1016/j.jmoldx.2012.11.007
27. Van Reeth K, Brown IH, Olsen CW. Influenza virus. Diseases of swine. 2012; 10:557–571.
28. Van Reeth K, Brown I, Essen S, Pensaert M. Genetic relationships, serological cross-reaction and cross-protection between H1N2 and other influenza A virus subtypes endemic in European pigs. Virus Research. 2004; 103(1-2):115–124. https://doi.org/10.1016/j.virusres.2004.02.023
29. Webster RG, Yakhno M, Hinshaw VS, Bean WJ, Murti KC. Intestinal influenza: replication and characterization of influenza viruses in ducks. Virology. 1978; 84(2):268–278. https://doi.org/10.1016/0042-6822(78)90247-7

****