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First serological evidence of H9 low pathogenic avian influenza virus exposure among rural population in Sidi Kacem province in Morocco: a cross-sectional study

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Abstract

Introduction: *H9 Avian influenza viruses are a threat to public health. In Morocco, the first outbreaks of the H9N2 influenza virus were reported in 2016, affecting different types of poultry production. This study aims to identify the potential existence of H9 avian influenza infections in farmworkers and habitants in Sidi Kacem province in Morocco.* ***Methods:*** *we conducted a cross-sectional study performed on sera samples collected between November 1, 2017 and May 30, 2018 on 36 farms in the province of Sidi Kacem, where there is traditional breeding of poultry.* ***Results:*** *a total of 185 sera were received and analyzed at the Avian Pathology Unit of the Hassan II Agronomic and Veterinary Institute, of which 62.2% (115/185) were positive for H9. Anti-H9 was found in all municipalities, with positivity rates ranging from 38.9% in the municipality of Bir Taleb to 100% in the municipality of Sidi Azouz. The mean age was 45.0 + 14.9. The positivity rate ranged from 66.7% among farmers to 52.5% among livestock keepers. It also ranged from 46.9% among those with primary school education to 77.8% among those who had attended only preschool level. This rate was 60.0% among those with secondary school level, 67.4% among those who had never attended school and 75.0% among those with high education level.* ***Conclusion:*** *this study highlighted the H9 low pathogenic avian influenza virus exposure among the rural population in Sidi Kacem province in Morocco. Therefore, there is a need to monitor this subtype as part of the influenza sentinel surveillance system.*

Introduction

Avian influenza (AI) viruses are highly contagious as a zoonotic pathogen [1], highly variable and widespread in birds [2]. Low Pathogenic Avian Influenza (LPAI) causes a mild infection in birds and generally does not pose a significant threat to human health. However, strains of Highly Pathogenic Avian Influenza (HPAI) are often lethal to birds and easily transmitted between susceptible species [3]. In deed, HPAI viruses that naturally cause acute clinical signs in broilers, turkeys, and other farmed birds have been associated only with H5 and H7 subtypes [4]. Rarely, AI viruses can adapt its characteristics to circulate in mammals. Over the past century, these viruses have contributed to pandemics in humans and influenza virus diversity in pigs and were the source of the currently circulating canine influenza in dogs [5].

In addition, the emergence of pandemic influenza viruses envisages a gene flow from the aquatic avian reservoir to humans via reassortment in pigs. The study of the recent outbreaks of H5N1 in Hong Kong since 1997 [6] and the isolation of the H9N2 avian virus in humans raise hypotheses about the risk of the emergence of a new pandemic virus. These H9N2 viruses have amino acids in their hemagglutinin, indicating their potential to infect humans directly [7]. In a study comparing the HA sequence of 16 H9N2 viruses to the first strain isolated in Morocco, all the Moroccan H9N2 viruses carried 158N, H183 amino acid substitutions (H3 numbering) in their receptor binding site related to human virus like receptor specificity [8]. In addition, some of H9N2 viruses contain gene segments closely related to those of A/Hong Kong/156/97 (H5N1/97, H5N1) or A/Quail/Hong Kong/G1/97 (G1-like, H9N2) [9]. Thus, avian influenza H9 is a severe health hazard [7].

A small number of influenza virus subtypes circulate in humans, such as H1N1 and H3N2, resulting in seasonal infections or occasional

pandemics [10]. Other AI subtypes, such as H5N1, H5N6, H6N1, H7N2, H7N3, H7N4, H7N7, H7N9, H9N2, H10N7 and H10N8, may occasionally cause sporadic infections and/or deaths [11-17] without sustainable human-to-human transmission [18]. The adaptability of influenza viruses to circulate between different species raises the possibility of a highly pathogenic variant that could lead to a devastating pandemic and devastating economic losses. Therefore, the control of this disease at the human-animal interface is an important part of the control and preparedness strategies against Influenza pandemics [19,20].

In Morocco, the first outbreaks of H9N2 influenza virus were recorded in early 2016, affecting different types of poultry production. The virus has spread rapidly throughout the country and is now endemic in poultry [8,21]. Multiple mutations associated with the mammalian host have been detected which would promote transmission of the virus from avian hosts to mammals, including humans, other virulence-related mutations have also been identified [21]. Therefore, vigilance is required in the human health sector to detect any possible human contamination by this subtype, especially since human cases of H9N2 have been reported by the WHO, including 17 cases in China [18,22]. This study aims to identify the potential circulation of H9 avian influenza in farmworkers and habitants with poultry contact in Sidi Kacem province in Morocco.

Methods

Study design: we conducted a cross-sectional study.

Study period and setting: this study was conducted on sera collected between November 1, 2017 and May 30, 2018, in 36 farms of 11 municipalities in the province of Sidi Kacem in the northwest of Morocco. This province has a total area of 4.060 km^2(of which, 3024.25 km^2 is rural) and an estimated population of 514 000 (67% rural). It is composed of 29 municipalities (5 urban and 24 rural) whose economy is based primarily on agriculture. Livestock is one of the concerns of many farmers in the province of Sidi Kacem. This province encompasses 22 679 breeding farms for all types of livestock. There is traditional breeding of a limited amount of poultry on these farms. The province is also included in one of the paths of migratory birds.

Participants: we used a convenience sample. Our study was carried out on 185 samples taken, as part of a seroprevalence study of brucellosis in Morocco [23], from a population of breeders and their entourage living in farms in 11 communes of Sidi Kacem (Figure 1). The farm residents raise various animals around their houses, including poultry. The sera were shared with an anonymous database.

Laboratory analysis: the samples were analyzed in the laboratory of the Avian Pathology Unit at the Hassan II Agronomic and Veterinary Institute in Rabat. H9 avian influenza virus antibodies in the sera samples were detected by using the haemagglutination inhibition (HI) test. The HI is the standard method for serological detection of influenza virus infection in humans. The sera obtained were treated with RDE (Receptor Destroying Enzyme) by diluting one part of the serum with three parts of enzyme and incubated overnight in a water bath at 37°C. Then, the enzyme was inactivated by incubating for 30 minutes at 56°C, followed by adding six parts of 0.85% saline to obtain a final dilution of 1/10. Hemagglutination inhibition (HI) assays were performed in 96-well U-bottom microtiter plates with 0.5% poultry erythrocytes [24]. To assess the levels of influenza H9 antibody titers, we used the HI assay using H9 antigens. Twice serial dilutions of the collected serum (25 µL) were prepared, and then 4 HA units of influenza H9 virus were added to each dilution. Then, after 30 minutes of incubation, 25 µl of 0.1% washed chicken red blood cells were added to each well, and the mixture was incubated for 30 minutes at room temperature. The endpoint of HI (the highest dilution of serum causing complete inhibition) was

noted and recorded as common log2 values of the highest dilution of HI.

Statistical analysis: a statistical analysis was performed using Epi-Info7 and Excel software. It concerned demographic, epidemiological characteristics such as age, gender and profession; then the H9 status and the antibody titer.

Ethical aspects: the sera samples used in this study were collected for another study granted, under number 34/16 dated 07/01/2016, by the Ethics Committee for Biomedical Research of Mohammed V University of Rabat (Faculty of Medicine and Pharmacy of Rabat).

Results

A total of 185 sera were received and analyzed at the Avian Pathology Unit of IAV, of which 37.8% (70) were negative for H9, and 62.2% (115) were positive. H9 antibody titers ranged from (3 $log_2$to 6 log_2) with 25.9% having a titer equal to 4 log_2, cases with a titer less than or equal to 2 log 2 (37.8%) were considered negative (Table 1). Anti-H9 were found in all municipalities, with positivity rates ranging from 38.9% in the Bir Taleb municipality to 100% in the Sidi Azouz municipality (Figure 2). Among the 115 positive cases, 65 (56.5%) were female. The study series ranged in age from 18 to 83 years. The mean age was 45.0 + 14.9 and 46.0 + 16.7 years old for the positive and negative cases, respectively, without any statistically significant difference (P=0.68). Indeed, 65.5% of the seropositive cases were in the 25-64 age group. The highest positivity rate (68%) was recorded in the 25-40 age group (Figure 3).

The majority of those sampled were housewives (42.4%), while farmers, livestock keepers and students accounted for 33.2%, 21.7% and 1.6%, respectively, and 1.1% were in other occupations, namely, a driver transporting livestock and an Imam. The latter two were H9 positive, while the positivity rate ranged from 66.7% among farmers to 52.5% among livestock keepers (Figure 4). Regarding educational level, 48% of the sera tested belonged to persons who had never attended school, 10% had preschool level, 26% and 14% had attended primary and secondary school respectively, and only 2% had a higher education level. The positivity rate ranged from 46.9% among those with primary school education to 77.8% among those who had attended only preschool level. This rate was 60.0% among those with secondary school level, 67.4% among those who had never attended school and 75.0% among those with high education level.

Discussion

Avian Influenza (AI) viruses circulating in birds are a serious issue to public health, in fact, they could be transmitted from avian species to mammals and humans, thus causing public health threats [25]. Avian Influenza viruses are divided on the Low Pathogenic and High Pathogenic AI, according to their potential to kill chickens and/or induce severe symptoms in commercial flocks [26]. H9N2 is considered one of the most critical subtypes for avian sector, responsible for low pathogenic avian influenza. H9N2 is considered as an endemic virus within the poultry population of Eurasia and Africa. In fact, in the Eurasian region, the H9N2 subtype has caused enormous economic losses to the poultry industry due to decreased egg production and high mortality combined with other infections [27]. The same symptoms and economic issues were reported in Morocco, since the first detection of H9N2 in January 2016 [8,28]. To control and to prevent infection caused by H9N2 AIV, inactivated vaccines have been used in chickens, these inactivated vaccines have protected against clinical signs [29], unfortunately, all vaccination strategies implemented in Morocco since 2016 were not been able to eradicate completely the virus in chicken [30].

Therefore, H9N2 AIVs still circulated in farmed chickens during the past decade [31], with the influenza virus subtype evolving rapidly and may acquire mutations that would facilitate its

multiplication in mammals, including humans [32]. In fact, our study revealed for the first time the presence of H9 antibodies on samples collected from a breeder and their entourages within the rural population of 11 municipalities in Sidi Kacem. These results might be explained by the contact and interaction with animals, including poultry and wild birds, and confirm the genomic finding and the adaptability of H9 to human receptors, therefore inducing an immune response measured by HA [8]. The positivity rate in this study was 62.2%, close to that recorded at the farm level in Pakistan (45.5%) [7], lower than that found by Hadipour (87%) among chicken farmers [33] and significantly higher than the general population in central China (22.72%) [34]. In other studies, serological investigations of AIV antibodies in poultry were conducted, the seroprevalence was variable, indeed Bangladesh have reported (9.82%) [35], Bosnia-Herzegovina (2.87%) [36], Nigeria (4.4%) [37] and Pakistan (14.3%) [38]. The difference between those results might be explained by the size and the characteristics of the sampled population, the used tests, but confirm the circulation of H9 virus among poultry.

The current study was based on the search for anti-influenza H9 antibodies in asymptomatic people, the detection of viral RNA in those showing signs in favor of influenza would be a better way to confirm their infection by this type of virus. In addition, we could not estimate the seroprevalence because we conducted this study on 185 sera collected as part of another brucellosis seroprevalence study which has a different prevalence from influenza A H9, thus, our sample is not representative. Moreover, as this virus includes a human-like receptor specificity [39], it has the potential to transmit to humans. Furthermore, it has recently been recognized that AI subtype H9 has shared gene segments with highly zoonotic viruses such as H7N9 that would contribute to the emergence of the next influenza pandemic [40,41]. In fact, Bennani et al. have reported that H9N2 isolates from broiler flocks in Morocco, carried 158N, H183 amino acid substitutions (H3 numbering) in their receptor binding site related to human virus like receptor specificity [8]. Actually, all H9 duck isolates, except for two H9N1 and one wild-duck H9N2 (WDk/ST/4808) virus lineages, had amino acid residue leucine (L) at position 226 (H3 numbering) and glycine (G) at position 228 at the receptor-binding site.

It is important to highlight that since 1999, several cases of human infection with H9N2 virus have been reported in Asia and are generally associated with mild disease [42], and the transmission of H9N2 virus appears to be exclusively from birds to humans, including highly pathogenic strains. Recently, many H9N2 cases have been found in China, most likely due to the ongoing screening for zoonotic H7N9, and in Egypt and Bangladesh due to ongoing screening for zoonotic H5N1 infections; however, no human-to-human transmission by H9N2 virus has been reported [43,44], although reassortment of H9N2 with other highly pathogenic viruses of type H5 and H7 is possible [44], because H9 could donate its gene segments to other emerging avian influenza viruses, including H5N2, H6N1, H7N7, H7N9 and H10N8 [25,45-50].

In 2013, an H5N1 virus detected in a patient in Canada after a stay in China contained 2 polymerase genes from an H9N2 virus [28]. The H5N2 viruses expressing the HA gene have been isolated with internal genes from both H5N1 viruses and H9N2 viruses [51]. Actually, as part of the ONE HEALTH approach, the detection of antibodies anti-H9 in human population, and the notification of H9 human cases, in many countries, encourages us to strengthen the epidemiological surveillance taking into account the risk of a new pandemic for which this subtype may be responsible, especially since avian influenza A(H9N2) virus infections in humans are usually mild or even subclinical [33]. This study had certain limitations. It is based on the search for anti-influenza H9 antibodies in asymptomatic people, the detection of viral RNA in those showing signs in favor of influenza would be a

better way to confirm their infection by this type of virus. In addition, we could not estimate the seroprevalence because we conducted this study was not representative, a study with a representative random sample would have made it possible to estimate the seroprevalence of this infection in the province of Sidi Kacem.

Conclusion

This study demonstrate that subtype H9 has been circulating in human population in the province of Sidi Kacem in Morocco, after its detection in poultry flocks among the country. It is therefore necessary to monitor this subtype through the human influenza sentinel surveillance system. Finally, our finding reinforces the worth value of the ONE HEALTH approach in public health and multisectoral collaboration.

What is known about this topic

- *The subtypes of the influenza virus AH1N1 and A H3N2 and the virus type B circulate in humans in Morocco;*
- *In 2016, the H9N2 subtype was detected in poultry for the first time in Morocco.*

What this study adds

- *First serological evidence of H9 low pathogenic avian influenza virus exposure among the rural population at Sidi Kacem province in Morocco;*
- *Anti H9 positivity rate in farms in Sidi Kacem province was 62.2% during the study period.*

Competing interests

The authors declare no competing interests.

Authors' contributions

Hind Ezzine and Mariam Laatifi designed the study and performed data analysis and interpretation of results. Hind Ezzine, Mohamed Elazhari and KF helped with data collection. Hind Ezzine, Mohamed Elazhari, Kaoutar Faddane, Sara Rakani, Saadia Nassik and Mariam Naciri assisted with laboratory analysis and interpretation of laboratory results. Hind Ezzine and Mariam Laatifi wrote the paper. Saadia Nassik and Mariam Naciri provided pedagogic support, corrected the manuscript, and approved its final version to be published. All the authors have read and agreed to the final manuscript.

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Tables and figures

Table 1: anti-H9 antibody titers in Sidi Kacem farms, Morocco, November 2017-May 2018

Figure 1: Sidi Kacem municipalities where the sera were collected, Morocco, November 2017-May 2018

Figure 2: distribution of cases (positive and negative) and positivity rate by municipality, Sidi Kacem, Morocco, November 2017-May 2018

Figure 3: distribution of anti-H9 positivity rate by age group at the studied farms, Sidi Kacem province, Morocco, November 2017-May 2018

Figure 4: positivity rate by occupation at studied farms, Sidi Kacem, Morocco November 2017-May 2018

References

1. World Health Organization WHO. **Influenza Avian and other zoonotic, 2018**. Accessed Dec 14, 2022.

2. Swayne DE. Animal influenza. John Wiley & Sons. 2016. **Google Scholar**
3. Plague F, Aviaire G. Avian influenza. bmc. 2006;(5): 54. **Google Scholar**
4. De Bruin AC, Funk M, Spronken MI, Gultyaev AP, Fouchier RA, Richard M. Hemagglutinin Subtype Specificity and Mechanisms of Highly Pathogenic Avian Influenza Virus Genesis. Viruses. 2022;14(7): 1566. **PubMed**| **Google Scholar**
5. Taubenberger JK, Morens DM. H5Nx panzootic bird flu-influenza´s newest worldwide evolutionary tour. Emerging infectious diseases. 2017;23(2): 340. **Google Scholar**
6. Sims LD, Ellis TM, Liu KK, Dyrting K, Wong H, Peiris M *et al*. Avian influenza in Hong Kong 1997-2002. Avian diseases. 2003;47(s3): 832-838. **PubMed**| **Google Scholar**
7. Tahir MF, Abbas MA, Ghafoor T, Dil S, Shahid MA, Bullo MM *et al*. Seroprevalence and risk factors of avian influenza H9 virus among poultry professionals in Rawalpindi, Pakistan. Journal of Infection and Public Health. 2019;12(4): 482-485. **PubMed**| **Google Scholar**
8. Amal EB, Saâdi N, Asma F, Moncef B, Ouafae FF. Characterization and Phylogenetic Analysis of the Hemagglutinin Gene in H9 Influenza Viruses from Chickens in Morocco from 2017 to 2019. Avian Diseases. 2020;64(3): 310-314. **PubMed**| **Google Scholar**
9. Guan Y, Shortridge KF, Krauss S, Chin PS, Dyrting KC, Ellis TM *et al*. H9N2 influenza viruses possessing H5N1-like internal genomes continue to circulate in poultry in southeastern China. Journal of virology. 2000;74(20): 9372-9380. **PubMed**| **Google Scholar**
10. Gatherer D. The 2009 H1N1 influenza outbreak in its historical context. Journal of Clinical Virology. 2009;45(3): 174-178. **PubMed**| **Google Scholar**
11. Parry J. H7N9 avian flu infects humans for the first time. BMJ. 2013 Apr 4;346: f2151. **PubMed**| **Google Scholar**
12. To KK, Tsang AK, Chan JF, Cheng VC, Chen H, Yuen KY *et al*. Emergence in China of human disease due to avian influenza A(H10N8)-cause for concern. Journal of Infection. 2014;68(3): 205-215. **PubMed**| **Google Scholar**
13. Yuen KY, Chan PK, Peiris M, Tsang DN, Que TL, Shortridge KF. H5N1 Study Group. Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. The Lancet. 1998;351(9101): 467-471. **PubMed**| **Google Scholar**
14. Shi W, Shi Y, Wu Y, Liu D, Gao GF. Origin and molecular characterization of the human-infecting H6N1 influenza virus in Taiwan. Protein & cell. 2013;4(11): 846-853. **PubMed**| **Google Scholar**
15. Wong SS, Yuen KY. Avian influenza virus infections in humans. Chest. 2006 Jan;129(1): 156-68. **PubMed**| **Google Scholar**
16. Tong XC, Weng SS, Xue F, Wu X, Xu TM, Zhang WH. First human infection by a novel avian influenza A (H7N4) virus. Journal of infection. 2018;77(3): 249-257. **PubMed**| **Google Scholar**
17. Chen H, Yuan H, Gao R, Zhang J, Wang D, Xiong Y *et al*. Clinical and epidemiological characteristics of a fatal case of avian influenza A H10N8 virus infection: a descriptive study. The Lancet. 2014;383(9918): 714-721. **PubMed**| **Google Scholar**
18. World Health Organization WHO. Antigenic and genetic characteristics of zoonotic influenza viruses and development of candidate vaccine viruses for pandemic preparedness. Wkly Epidemiol Rec. 2013 Oct 18;88(42): 449-63. **PubMed**| **Google Scholar**

19. Cauldwell AV, Long JS, Moncorge O, Barclay WS. Viral determinants of influenza A virus host range. Journal of General Virology. 2014;95(6): 1193-1210. **PubMed**| **Google Scholar**
20. Short KR, Richard M, Verhagen JH, van Riel D, Schrauwen EJ, van den Brand JM *et al*. One health, multiple challenges: The inter-species transmission of influenza A virus. One health. 2015;1: 1-13. **PubMed**| **Google Scholar**
21. Rahmatallah N, Nassik S, El Rhaffouli H, Amine IL, El houadfi M. Détection de souches multi-résistantes d´Escherichia coli d´origine aviaire dans la région de Rabat-Salé-Zemmour-Zaer. Revue Marocaine des Sciences Agronomiques et Vétérinaires. 2017;5(2). **Google Scholar**
22. Rowe T, Abernathy RA, Hu-Primmer J, Thompson WW, Lu X, Lim W *et al*. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. Journal of clinical microbiology. 1999;37(4): 937-943. **PubMed**| **Google Scholar**
23. Faddane K, Moumni H, Cherkaoui I. Seroprevalence of brucellosis among farmers in Morocco. Moroccan Journal Of Public Health. 2022;4(1): 49-58. **Google Scholar**
24. Rowe T, Abernathy RA, Hu-Primmer J, Thompson WW, Lu X, Lim W *et al*. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. Journal of clinical microbiology. 1999;37(4): 937-943. **PubMed**| **Google Scholar**
25. Peiris JM, De Jong MD, Guan Y. Avian influenza virus (H5N1): a threat to human health. Clinical microbiology reviews. 2007;20(2): 243-267. **PubMed**| **Google Scholar**
26. Guo YJ, Krauss S, Senne DA, Mo IP, Lo KS, Xiong XP *et al*. Characterization of the pathogenicity of members of the newly established H9N2 influenza virus lineages in Asia. Virology. 2000;267(2): 279-288. **PubMed**| **Google Scholar**
27. El Houadfi M, Fellahi S, Nassik S, Guérin JL, Ducatez MF. First outbreaks and phylogenetic analyses of avian influenza H9N2 viruses isolated from poultry flocks in Morocco. Virology Journal. 2016;13(1): 1-7. **PubMed**| **Google Scholar**
28. Dong J, Zhou Y, Pu J, Liu L. Status and challenges for vaccination against avian H9N2 influenza virus in China. Life. 2022;12(9): 1326. **PubMed**| **Google Scholar**
29. El Mellouli F, Mouahid M, Fusaro A, Zecchin B, Zekhnini H, El Khantour *et al*. Spatiotemporal Dynamics, Evolutionary History and Zoonotic Potential of Moroccan H9N2 Avian Influenza Viruses from 2016 to 2021. Viruses. 2022;14(3): 509. **PubMed**| **Google Scholar**
30. Liu L, Wang T, Wang M, Tong Q, Sun Y, Pu J *et al*. Recombinant turkey herpesvirus expressing H9 hemagglutinin providing protection against H9N2 avian influenza. Virology. 2019;529: 7-15. **PubMed**| **Google Scholar**
31. Elderfield RA, Watson SJ, Godlee A, Adamson WE, Thompson CI, Dunning J *et al*. Accumulation of human-adapting mutations during circulation of A (H1N1) pdm09 influenza virus in humans in the United Kingdom. Journal of virology. 2014;88(22): 13269-13283. **PubMed**| **Google Scholar**
32. Hadipour MM. H9N2 avian influenza virus antibody titers in human population in fars province, Iran. Brazilian Journal of Poultry Science. 2010;12: 160-164. **Google Scholar**

33. Qi Y, Guo W, Liu C, Li W, Gu Y, Li S *et al*. Seroprevalence of influenza A (H9N2) virus infection among humans in China: A meta-analysis. Microbial Pathogenesis. 2021;155: 104881. **PubMed**| **Google Scholar**
34. Nooruddin GM, Hossain MT, Mohammad M, Rahman MM. Sero-epidemiology of avian influenza virus in native chicken in Bangladesh. Int J Poult Sci. 2006;5: 1029-1033. **Google Scholar**
35. Golic B, Stevanovic O, Nedic DN, Maric J. Serological survey of avian influenza virus infection of backyard chickens in Bosnia and Herzegovina. Bulgarian Journal of Veterinary Medicine. 2016;19(4): 328-333. **Google Scholar**
36. Oluwayelu DO, Aiki-Raji CO, Adigun OT, Olofintuyi OK, Adebiyi AI. Serological survey for avian influenza in turkeys in three states of Southwest Nigeria. Influenza Res Treat. 2015;2015: 787890. **PubMed**| **Google Scholar**
37. Arif M, Rind RU, Shah MG, Nisha AR, Umer M, Kaka U *et al*. Seroprevalence of avian influenza in broilers of district Quetta, Balochistan, Pakistan. Journal of Chemical and Pharmaceutical Research. 2015;7(4): 1378-1384. **Google Scholar**
38. Matrosovich MN, Krauss S, Webster R G. H9N2 influenza A viruses from poultry in Asia have human virus-like receptor specificity. Virology. 2001;281(2): 156-162. **PubMed**| **Google Scholar**
39. Di Liu WS, Gao GF. Poultry carrying H9N2 act as incubators for novel human avian influenza viruses. Lancet. 2014 Mar 8;383(9920): 869. **PubMed**| **Google Scholar**
40. Carnaccini S, Perez DR. H9 influenza viruses: an emerging challenge. Cold Spring Harbor perspectives in medicine. 2020;10(6): a038588. **PubMed**| **Google Scholar**
41. Peiris M, Yuen KY, Leung CW, Chan KH, Ip PL, Lai RW *et al*. Human infection with influenza H9N2. The Lancet. 1999;354(9182): 916-917. **PubMed**| **Google Scholar**
42. Monne I, Hussein HA, Fusaro A, Valastro V, Hamoud MM, Khalefa RA *et al*. H9N2 influenza A virus circulates in H5N1 endemically infected poultry population in Egypt. Influenza and other respiratory viruses. 2013;7(3): 240-243. **PubMed**| **Google Scholar**
43. Peacock THP, James J, Sealy JE, Iqbal M. A global perspective on H9N2 avian influenza virus. Viruses. 2019;11(7): 620. **PubMed**| **Google Scholar**
44. Iqbal M, Yaqub T, Reddy K, McCauley JW. Novel genotypes of H9N2 influenza A viruses isolated from poultry in Pakistan containing NS genes similar to highly pathogenic H7N3 and H5N1 viruses. PloS one. 2009;4(6): e5788. **PubMed**| **Google Scholar**
45. Gu M, Chen H, Li Q, Huang J, Zhao M, Gu X *et al*. Enzootic genotype S of H9N2 avian influenza viruses donates internal genes to emerging zoonotic influenza viruses in China. Veterinary microbiology. 2014;174(3-4): 309-315. **PubMed**| **Google Scholar**
46. Bi Y, Xiao H, Chen Q, Wu Y, Fu L, Quan C *et al*. Changes in the length of the neuraminidase stalk region impact H7N9 virulence in mice. Journal of Virology. 2016;90(4): 2142-2149. **PubMed**| **Google Scholar**
47. Liu D, Shi W, Shi Y, Wang D, Xiao H, Li W *et al*. Origin and diversity of novel avian influenza A H7N9 viruses causing human infection: phylogenetic, structural, and coalescent analyses. The Lancet. 2013;381(9881): 1926-1932. **PubMed**| **Google Scholar**

48. Gao R, Cao B, Hu Y, Feng Z, Wang D, Hu W *et al*. Human infection with a novel avian-origin influenza A (H7N9) virus. New England Journal of Medicine. 2013;368(20): 1888-1897. **PubMed**| **Google Scholar**
49. Cheng X, Zengel JR, Suguitan Jr, Xu Q, Wang W, Lin J *et al*. Evaluation of the humoral and cellular immune responses elicited by the live attenuated and inactivated influenza vaccines and their roles in heterologous protection in ferrets. The Journal of infectious diseases. 2013;208(4): 594-602. **PubMed**| **Google Scholar**
50. Ip HS, Torchetti MK, Crespo R, Kohrs P, DeBruyn P, Mansfield KG *et al*. Novel Eurasian highly pathogenic avian influenza A H5 viruses in wild birds, Washington, USA, 2014. Emerging infectious diseases. 2015;21(5);886. **PubMed**| **Google Scholar**
51. Pasick J, Berhane Y, Joseph T, Bowes V, Hisanaga T, Handel K *et al*. Reassortant highly pathogenic influenza A H5N2 virus containing gene segments related to Eurasian H5N8 in British Columbia, Canada, 2014. Sci Rep. 2015 Mar 25;5: 9484. **PubMed**| **Google Scholar**

Table 1: anti-H9 antibody titers in Sidi Kacem farms, Morocco, November 2017-May 2018

Anti H9 antibodies	**Number of cases**	**Proportion**
< 2 log2	70	37.8%
3 log2	39	21.1%
4 log2	48	25.9%
5 log2	24	13.0%
6 log2	4	2.2%
Total	**185**	**100.0%**

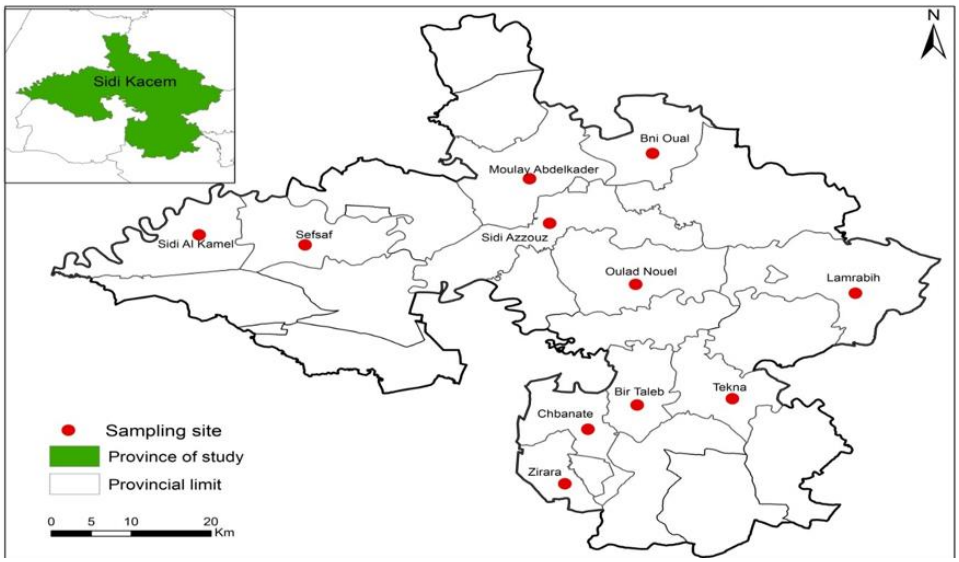

Figure 1: Sidi Kacem municipalities where the sera were collected, Morocco, November 2017-May 2018

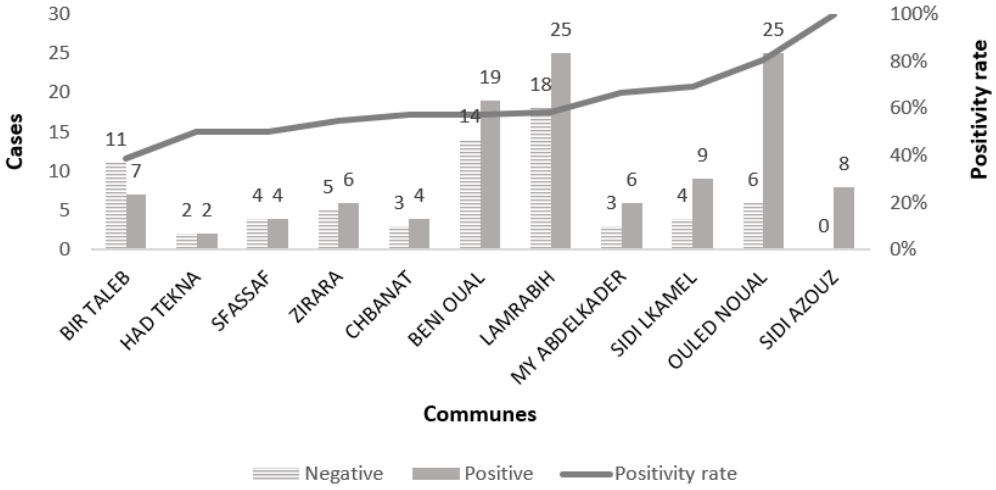

Figure 2: distribution of cases (positive and negative) and positivity rate by municipality, Sidi Kacem, Morocco, November 2017-May 2018

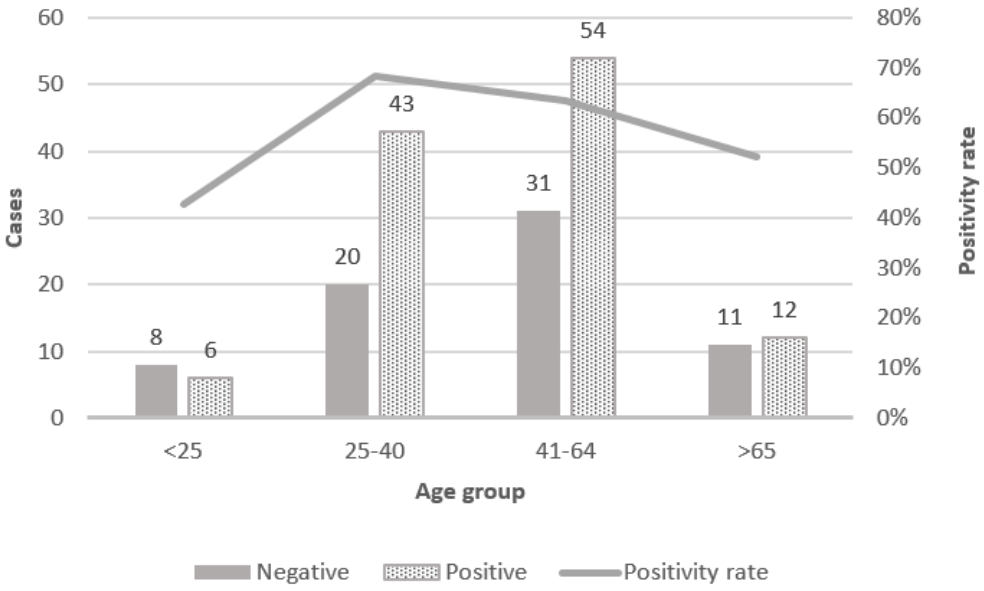

Figure 3: distribution of anti-H9 positivity rate by age group at the studied farms, Sidi Kacem province, Morocco, November 2017-May 2018.

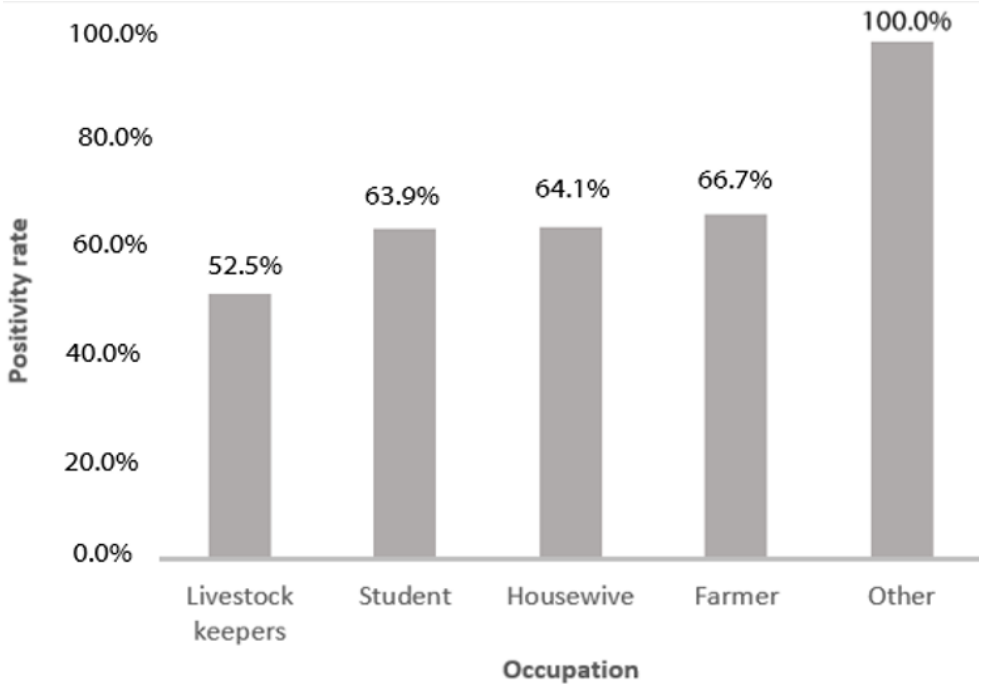

Figure 4: positivity rate by occupation at studied farms, Sidi Kacem, Morocco November 2017-May 2018