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Research article

Prevalence of Avian Origin H5 and H7 Influenza Virus Antibodies in Dogs in Ibadan and Sagamu, Southwestern Nigeria

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ABSTRACT: Highly pathogenic avian influenza H5N1 subtype was recently reported in some states of Southwestern Nigeria including Oyo and Ogun states. As part of ongoing influenza surveillance efforts in livestock and companion animals in Nigeria, a study was conducted to investigate the prevalence of avian H5 and H7 influenza virus antibodies in exotic and Nigerian village dogs in Ibadan and Sagamu, two cities in Oyo and Ogun states respectively. One hundred and sixty two (162) dogs comprising 85 exotic dogs from Ibadan and 77 Nigerian village dogs from Ibadan and Sagamu were screened for the presence of avian H5 and H7 influenza virus antibodies. Using the hemagglutination inhibition (HI) test, none of the samples from exotic dogs had HI antibodies to both virus strains while all 77 Nigerian village dog samples were negative for H5 antibodies but two (2.6%) were positive for H7 antibodies at a titre of 1:32. The presence of H7 influenza virus antibodies in Nigerian village dogs, although at a low rate, suggests that these dogs had naturally been infected with the virus. It is possible that the dogs acquired the infection through consumption of dead chickens or internal organs of animals killed during hunting. The close contact between these dogs and their owners, domestic poultry and wildlife underscores their importance in the epidemiology of influenza in Nigeria.

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Keywords: Avian influenza, Serosurvey, Dogs, Southwestern Nigeria

INTRODUCTION

Influenza A viruses are negative sense, single-stranded, segmented RNA viruses belonging to the genus *Influenzavirus A* of the family *Orthomyxoviridae*. They are highly contagious pathogens known to cause acute respiratory disease in humans, horses, pigs, and domestic poultry (Webster et al., 1992). Based on their antigenic properties, influenza A viruses are classified into 16 hemagglutinin (H) and 9 neuraminidase (N) subtypes (Fouchier et al., 2005). Additionally, they are grouped on the basis of their pathogenicity for chickens into highly pathogenic avian influenza (HPAI) viruses, in which mortality may be as high as 100%, and low pathogenic avian influenza (LPAI) viruses that cause much milder respiratory disease (Alexander, 2000). HPAI viruses have been restricted to H5 and H7 subtypes, although not all viruses of these subtypes are highly pathogenic for chickens.

The interspecies transmission of influenza A virus is a crucial feature of its ecology and epidemiology. Since HPAI virus subtype H5N1 was first detected in 1996 in domestic geese in China, it has crossed the species barrier and is now known to have infected humans, ferrets, mice, stone martens, civets, pigs, cynomolgus monkeys, tigers, leopards, domestic cats and dogs (Marschall & Hartmann, 2008). In particular, it caused fatal infection in a domestic dog (Songserm et

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al., 2006) and 25% seroprevalence in village dogs (Butler, 2006) in Thailand. Moreover, there have been reports of bird-to-human transmission of HPAI H7N7 virus (Fouchier et al., 2004) as well as interspecies transmission of H7N3 virus from wild birds to intensively reared domestic poultry (Campitelli et al., 2004). These reports indicate an increasing need for surveillance of different animal species, especially those that enjoy a close relationship with humans such as dogs, cats, domestic poultry and horses so as to better define their role in the epidemiology and ecology of influenza A viruses. Considering the close relationship between humans and dogs, the emergence and interspecies transmission of avian influenza virus to dogs raises concerns about their possible role in the transmission and adaptation of influenza viruses to mammals.

In Nigeria, there has been a recent upsurge in the keeping of exotic dogs as pets, especially by the middle class. This has led to a growing awareness among pet dog owners of the need for veterinary care for their dogs and the establishment of close bonds between these dogs and their owners. Similarly, Nigerian village dogs which are mostly used for hunting purposes enjoy a close relationship with the hunters. However, infectious diseases continue to be a threat to the health and survival of these animals. Some viral infections that have been reported to affect dogs in Nigeria include rabies (Aghomo et al., 1989) and canine distemper (Oyedele et al., 2004). However, there have been no reports of influenza virus infections in dogs in Nigeria although outbreaks of avian influenza H5N1 virus infections in Nigerian poultry have recently been recorded (Joannis et al., 2006; Owoade et al., 2008).

As part of ongoing influenza surveillance efforts in livestock and companion animals in Nigeria, a preliminary study was conducted to investigate the prevalence of avian H5 and H7 influenza virus antibodies in exotic and Nigerian village dogs in Ibadan and Sagamu, two cities located in Oyo and Ogun states of southwestern Nigeria respectively. HPAI H5N1 had recently been reported in these two states (Owoade et al., 2008).

MATERIALS AND METHODS

Study locations

The study was conducted using five veterinary clinics located in Ibadan, a major urban centre in Oyo state, southwestern Nigeria. The city is known for influx of people and dogs from all parts of the country since trade in dogs across the country is common and unregulated. Village dogs sampled were from Ajibode community, a peri-urban settlement in Ibadan, and from a rural community in Sagamu, Ogun State of Nigeria. Southwestern Nigeria is regarded as the main hub of the poultry industry in the country.

Viruses

Due to unavailability of a complete influenza A identification panel, the study was limited to the use of H5 and H7 influenza A viruses for screening purposes. HI test using standardized avian H5 and H7 antigens and antisera (National Veterinary Research Institute, Vom, Nigeria) were conducted on all sera.

Survey animals

A total of 162 dogs made up of 85 exotic breeds and 77 Nigerian village dogs were sampled. The exotic breeds were of both sexes and included 47 Alsatians, 25 Rottweilers, 5 Boerboels, 2 Pit-Bull Terriers, 3 Bull Mastiffs, 2 Samoyeds and one Rhodesian Ridgeback. Dogs presented to the clinic with any complaint were eligible for inclusion in the study, but they were excluded if their owners declined to give consent or if blood collection would have posed undue stress to the animal based on its clinical condition. The clinic records showed that most of them had been vaccinated against rabies, canine distemper, canine parvovirus, infectious canine hepatitis, leptospirosis and parainfluenza. The 77 village dogs, which are used mostly for hunting purposes, were also of both sexes and none of them had been vaccinated against any known disease of dogs. All the 162 dogs sampled were between 4 months and 8 years of age.

Sample collection

Blood samples were collected from the cephalic vein into dry, sterile bottles without anticoagulant. They were allowed to clot at room temperature for about 5-6 hours. Separated sera were inactivated at 56^{o}C for 30 minutes and preserved at -20^{o}C until required for analysis.

Hemagglutination inhibition test

Hemagglutination inhibition (HI) test was performed using the World Organisation for Animal Health (OIE) protocol (OIE, 2009) with slight modifications. Briefly, 25µl of serial two-fold dilutions of the serum samples in phosphate-buffered saline were mixed with 25µl of 4 hemagglutinating (HA) units of virus in U-bottom microtiter plates and incubated at room temperature for 30 min. Then, 25µl of 1% chicken red blood cells was added to each well and incubated at room temperature for 40 min. The HI titre was expressed as the highest

serum dilution that completely inhibited 4 HA units of the virus.

RESULTS

HI titres were regarded as being positive if there is inhibition at a serum dilution of 1:16 or more against 4 HAU of antigen. None of the samples from the exotic breed dogs had HI antibodies to both the H5 and H7 virus strains. However, all 77 samples from Nigerian village dogs were negative for H5 antibodies but two (2.6%) were positive for H7 antibodies at a titre of 1:32 (Table 1). The two positive samples were from a 5-month old male puppy and a 3-year old bitch in Sagamu.

Table 1:
Results of Haemagglutination Inhibition (HI) Testing of Exotic and Nigerian Village Dog Sera for H5 and H7 Influenza Virus Antibodies

Avian influenza virus subtype	Exotic dogs	Nigerian village dogs
	No. positive/ No. tested (%)	No. positive/ No. tested (%)
H5	0/85 (0)	0/77 (0)
H7	0/85 (0)	2/77 (2.6)

DISCUSSION

Following an outbreak of avian influenza, rapid diagnosis is critical not only for the control of HPAI but also for human health. The detection of avian influenza virus antibodies in domestic animals that enjoy a close relationship with humans, and which can thus serve as sentinels, is a good early warning system for the detection and subsequent control of possible outbreaks in humans. Following the recent reports of avian influenza H5N1 outbreaks in Nigeria, we embarked on a survey of exotic and village dogs, which enjoy close relationships with their owners, as a first step towards determining the role of dogs in the epidemiology of avian influenza in Nigeria.

All the exotic dogs sampled were negative for H5 and H7 avian influenza virus antibodies. While this shows that they had not been naturally exposed to these viruses, it does not preclude the fact that they are susceptible to influenza infections especially those caused by viruses belonging to other H and N subtypes that may be circulating in Nigeria. Dogs have previously been shown to be susceptible to influenza viruses (Nikitin et al., 1972) and recently, fatal infection with HPAI H5N1 was documented in a domestic dog in Thailand following ingestion of an H5N1-infected duck carcass (Songserm et al., 2008). In Nigeria, risk factors that can predispose exotic dogs to influenza virus infections include feeding them with carcasses of dead birds from poultry houses and annual dog shows. In particular, dog shows which are gatherings of exotic dogs are gradually becoming fashionable in Nigeria. Routine screening of dogs that visit veterinary clinics and those paraded at dog shows for influenza virus antibodies is advocated as part of necessary surveillance activities to aid in early detection of infection.

The low level (2.6%) detection of H7 influenza virus antibodies in Nigerian village dogs which are typically found in close association with wild animals, backyard poultry and humans, suggests natural infection with the virus since they had no history of previous vaccination. Moreover, the fact that the two dogs positive for H7 influenza virus antibodies were 5 months and 3 years old respectively indicates that there is no age predisposition with the infection. In a previous work (Butler, 2006), approximately 25% seroprevalence of H5N1 influenza virus was reported among 629 village dogs in Thailand. Song et al. (2008) suggested that the use of untreated meats of ducks or chickens, including internal organs and heads, to feed dogs for fattening in local canine farms or kennels in South Korea is a likely means of transmission of avian influenza virus to dogs. It is possible that the Nigerian village dogs acquired the infection through being fed dead chickens or internal organs of animals killed during hunting. The implications of this finding for the epidemiology of influenza virus infections among humans and wildlife needs to be further investigated. Furthermore, there is likelihood that the exotic and village dogs tested might be infected with the LPAI virus strains which were not screened for in this study.

Despite the non-detection of influenza virus antibodies in exotic dogs and the low level detection in Nigerian village dogs in this study, we conclude that the possibility of humans acquiring influenza virus infection from direct contact with infected dogs and, perhaps other domestic animals, warrants concern and underscores the need for monitoring domestic animals during future avian influenza outbreaks in Nigeria. Moreover, continuous surveillance efforts aimed at screening larger populations of exotic and Nigerian village dogs, especially those presenting with respiratory signs, using all the H subtypes of influenza virus should be deployed in order to identify the influenza virus strains circulating among them and establish the true status of this disease in Nigeria.

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