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Pandemic A/H1N1/2009 influenza virus in Swine, Cameroon, 2010

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Abstract

Although swine origin A/H1N1/2009 influenza virus (hereafter "pH1N1") has been detected in swine in 20 countries, there has been no published surveillance of the virus in African livestock. The objective of this study was to assess the circulation of influenza A viruses, including pH1N1 in swine in Cameroon, Central Africa. We collected 108 nasal swabs and 98 sera samples from domestic pigs randomly sampled at 11 herds in villages and farms in Cameroon. pH1N1 was isolated from two swine sampled in northern Cameroon in January 2010. Sera from 28% of these herds were positive for influenza A by competitive ELISA and 92.6% of these swine showed cross reactivity with pandemic A/H1N1/2009 influenza virus isolated from humans. These results provide the first evidence of this virus in the animal population in Africa. In light of the

significant role of swine in the ecology of influenza viruses, our results call for greater monitoring and study in Central Africa.

Keywords: swine influenza virus; pandemic A/H1N1/2009 influenza virus; Cameroon; Central Africa; agriculture; zoonotic diseases

Introduction

Although influenza viruses have circulated in swine for at least 80 years, numerous aspects of the virus' ecology in swine hosts remain unknown due to insufficient surveillance efforts (Garten et al., 2009; Shope and Lewis, 1931). These knowledge gaps contributed in part to the emergence and global spread of the swine-origin A/H1N1/2009 influenza virus (hereafter "pH1N1"), which presumably originated in swine in southern Mexico and prompted the World Health Organization to declare a phase 6 pandemic (Vijaykrishna et al., 2010). Although pH1N1 has previously been reported in swine herds in 20 countries, the absence of data on swine influenza viruses in Africa is salient (Girard et al., 2010; World Organisation for Animal Health, 2011). The Influenza Virus Resource and the World Organisation for Animal Health Information Database do not contain a single record of pH1N1 from African swine (Bao et al., 2008; World Organisation for Animal Health, 2011). The objective of this study was to assess the circulation of influenza A viruses, including pH1N1 in swine in Cameroon. Understanding the circulation of pH1N1 in swine is important for human health because swine may serve as a mixing vessel in which influenza strains reassort, creating novel viruses with the potential to infect humans and cause pandemics.

Materials and methods

Domestic pigs were randomly sampled at 11 herds in villages and farms in two Cameroonian regions (Centre and North) from December 2009 through April 2010 (Figure). Nasal swabs and sera were collected and processed following standard protocols (World Organisation for Animal

Health, 2008). Swine from some of these herds showed mild respiratory signs but no swine deaths were recorded during the study period. We collected a total of 104 nasal swabs and 98 sera samples. Nasal swab samples were initially screened for influenza A virus by real-time RT-PCR on an ABI 7300 System with primers targeting a conserved region of the influenza matrix gene (Spackman et al., 2002). Each PCR-positive sample was then tested for pH1N1 by real-time RT-PCR on an ABI 7300 System and by virus isolation on Madin-Darby canine kidney cells and SPF embryonated chicken eggs. The complete hemagglutinin (HA) gene sequence and the partial sequences of the remaining seven genes were obtained from the positive samples (GenBank accession numbers JF707781-JF707788). Hemagglutination-inhibition assays (HAI) were conducted on the sera samples to determine whether they contained antibodies against swine influenza viruses (e.g. Eurasian "avian-like" A/Sw/Italy/5766-15/09 (H1N1), triple-reassortant A/Sw/Italy/716/06 (H3N2), and A/Sw/Italy/4660-3/09 (H1N2)) and human influenza viruses such as pH1N1 (strain A/California/04/2009) and one recent seasonal H1N1 (A/Italy/3983/2009) influenza virus. Serological tests were performed according to international standards (World Organisation for Animal Health, 2008).

Results and discussion

Two nasal swabs tested positive for influenza A virus at two sites in northern Cameroon: a male pig sampled at Ourokessoum Lagdo (N09.03733 E013.64590) on 20 January 2010 and another pig of unknown sex sampled on 25 January 2010 at Malape (N09.22709 E013.1416) (Figure; Table 1). The virus isolates from these two pigs were subtyped as pH1N1 using a specific RT-PCR, HIA, and subsequent sequencing. Nucleotide sequence analysis of the amplicons indicated that each of the eight RNA segments of all the isolates had high nucleotide homology to pH1N1 influenza viruses as determined through BLAST homologies (there was 99.4% sequence identity in the hemagglutinin (HA) gene). Of the 98 pig sera sampled from 11 herds, 27 (28%), all from a

single herd in Vounaloum, were positive for influenza A by a competitive ELISA assay (Table 1) and 24 (88.9 %) of those had high HI titers (≥1,280) specific to pH1N1 A/California/04 (Table 2). The vast majority of the samples from Cameroonian swine showed low reactivity (≤ 160 HI units) against swine influenza A viruses (H3N2 A/Sw/Italy/716/06, H1N2 A/Sw/Italy/4660-3/09, and H1N1 A/Sw/Italy/5766-15/09) and human seasonal H1N1 (92.6-100% of the Cameroonian samples showed low reactivity depending on the antigen tested, Table 2). Although we cannot rule out the event of previous exposure to other influenza viruses, these data clearly indicated specific seroconversion to pH1N1.

Our virological and serological results suggest that pH1N1 is likely widely circulating in swine populations in northern Cameroon. The introduction of pH1N1 to swine in northern Cameroon is noteworthy because the virus has a high transmission rate among swine (Brookes et al., 2010; Song et al., 2010) suggesting that it may become endemic in livestock in the Congo Basin. It is, however, particularly difficult to estimate when the pH1N1 virus was first introduced into northern Cameroon swine herds. The first detection of pH1N1 virus in human occurred in Cameroon in August 2009, from a patient returning from the United States of America and the first confirmation of an indigenous pH1N1case occurred 5 months later in Yaoundé (Njouom R, personal communication). Given that the youngest infected pig was four months old our serological surveillance suggests that pH1N1 was circulating in these herds within the last four months of this survey (December 2009 to March 2010). However, the antibodies against pH1N1 that we detected in piglets could have also arisen from the transplacental transfer of the H1N1 virus or maternal antibodies from sow to fetus (Madec et al., 1989). In light of the significant role of pigs in the ecology of influenza viruses, our results call for greater monitoring and study in Central Africa. No surveillance or any form of challenge studies has been carried out in pigs in Central Africa prior to this study. Whether the virus was newly transmitted to pigs or has been

serially infecting pigs are topics for future studies. We can only infer that following pH1N1 spillover from humans, subsequent infections in swine may have been subclinical since there have been no reports of epizootics prior to this report.

More efforts aimed at confirming the apparent high prevalence of pH1N1 in the swine population of this area should be undertaken. The pH1N1outbreak in Cameroonian swine is an important public health issue because human and avian influenza viruses can reassort in swine, potentially generating novel virions that have increased virulence in humans. The Food and Agricultural Organization (FAO) of the United Nations has recently conducted three regional projects (Southeast Asia, Andean South America, and Central America) to investigate pH1N1 in swine populations, and will soon initiate a similar project in the Caribbean with the goal of cataloging influenza surveillance in swine worldwide (Edie Marshall, personal communication). Nevertheless, there appear to be large geographic gaps in swine influenza surveillance, and to our knowledge, this is the first project to test pigs in Africa for influenza. In order to characterize pH1N1 prevalence worldwide, the results here point toward the need for all countries to implement livestock surveillance to detect the virus and molecular assays to identify possible changes in viral structure that increase pathogenicity or antiviral resistance.

Conflict of interest statement

All authors declare that there are no financial or other relationships that might lead to a conflict of interest. All authors have seen and approved the manuscript and have contributed significantly to the work.

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Table 1 caption

Features of swine samples in Cameroon tested positive for either rt-PCR or influenza type A ELISA serological assay.

Table 2 caption

HI titers for swine influenza subtypes, seasonal human influenza (H1N1), and pandemic H1N1/2009 influenza viruses of influenza type A positive sera collected from swine in Cameroon.

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Figure caption.

Sites in northern Cameroon where pH1N1 was detected in swine and swine density in Central Africa. The pH1N1 virus has isolated from pigs in Malape and Ourokessoum Lagdo. At Vounaloum, 88.9% of influenza-positive pigs had antibodies against pH1N1. The density data are from Wint and Robinson (2007).

Table 1. Features of swine samples in Cameroon tested positive for either rt-PCR or influenza type A ELISA serological assay

ID No	Sex	Location	Rt RT-PCR result in nasal swabs	Type A cELISA* result in serum
1034	M	Lagdo	+	-
1036		Malape	+	-
1056	F	Vounaloum	-	+
1057	F	Vounaloum	-	+
1058	F	Vounaloum	-	+
1059	M	Vounaloum	-	+
1061	F	Vounaloum	-	+
1062	F	Vounaloum	-	+
1063	F	Vounaloum	-	+
1064	F	Vounaloum	-	+
1065	F	Vounaloum	-	+
1066	F	Vounaloum	-	+
1067	F	Vounaloum	-	+
1068	F	Vounaloum	-	+
1069	F	Vounaloum	-	+
1071	F	Vounaloum	-	+
1072	F	Vounaloum	-	+
1073	M	Vounaloum	-	+
1075	F	Vounaloum	-	+**
1076	F	Vounaloum	-	+
1077		Vounaloum	-	+
1078		Vounaloum	-	+**
1079		Vounaloum	-	+
1080		Vounaloum	-	+
1081		Vounaloum	-	+
1082		Vounaloum	-	+
1083		Vounaloum	-	+
1084		Vounaloum	-	+
1085	M	Vounaloum	-	+

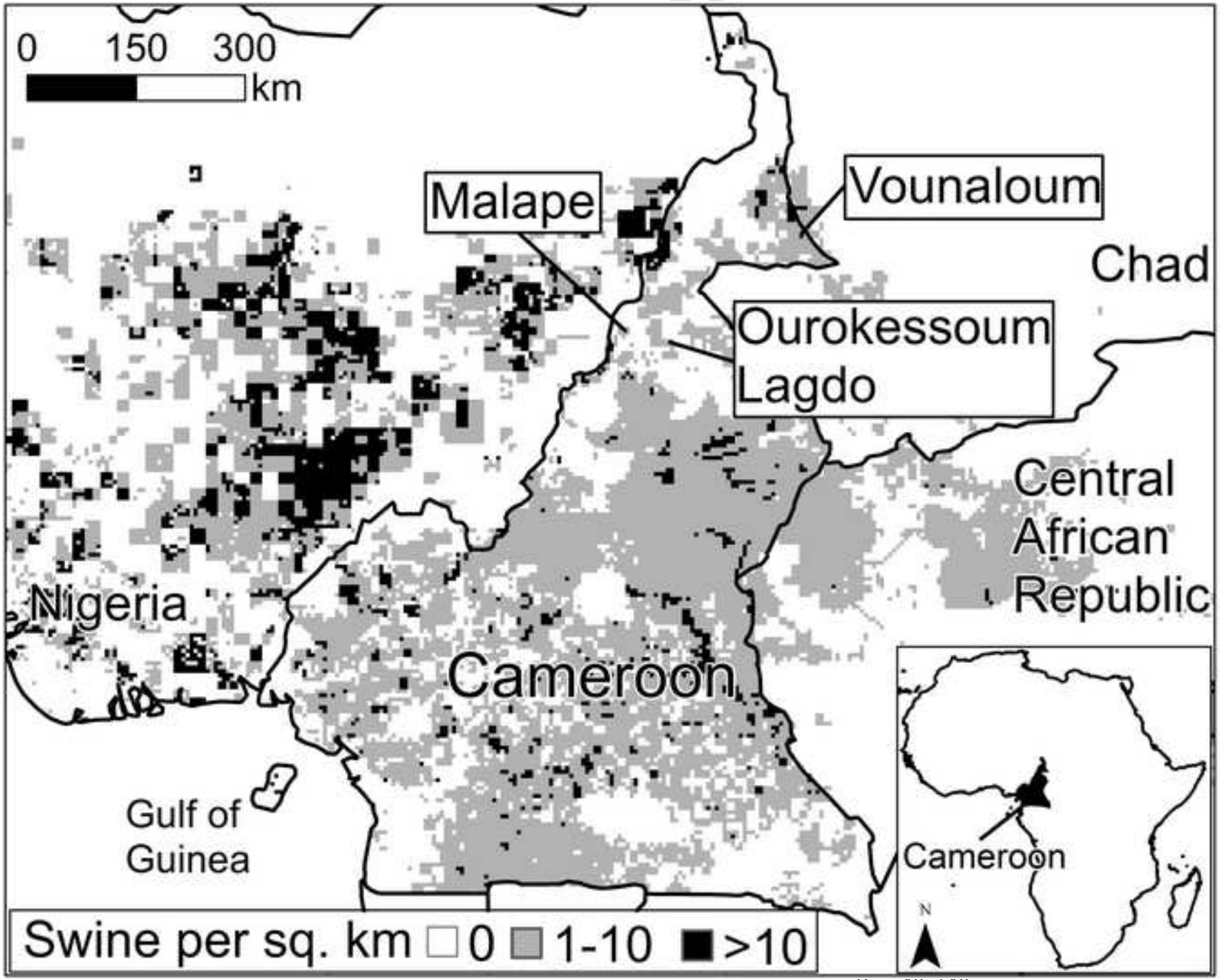
Rt RT-PCR - Real Time Reverse Transcription_Polymerase Chain Reaction
Type A cELISA - competitive ELISA assay for Influenza A antibodies
* competitive ELISA assay with HI titers specific to pH1N1 A/California/04 (see text and Table 2 for other subtypes of swine influenza virus tested)
**HI titers ≤ 1280

Table 2. HI titers for swine influenza subtypes, seasonal human influenza (H1N1) and pandemic H1N1/2009 influenza viruses of influenza type A positive sera collected from swine in Cameroon.

Range of HI titers of positive sera	Number of positive sera for				
	H3N2 A/Sw/Italy/716/06	H1N2 A/Sw/Italy/4660-3/09	H1N1 A/Sw/Italy/5766-15/09	Seasonal H1N1 A/Italy/3983/2009	Pandemic H1N1 A/California/04/2009
20-40	1	20	2	10	0
80-160	24	2	25	17	0
320-640	2	0	0	0	3
1,280-2,560	0	0	0	1	11
5,120-10,240	0	0	0	0	13
20,480- 40,960	0	0	0	0	0
Total	27	22*	27	28	27

*For six samples, titre ≤ 1:10

Figure


0 150 300
km
Malape
Vounaloum
Chad
Ourokessoum
Lagdo
Central
African
Republic
Nigeria
Cameroon
Gulf of
Guinea
Cameroon
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Swine per sq. km 0 1-10 >10