Veterinary Microbiology 156 (2012) 189–192

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

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

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ARTICLE INFO

Article history:
Received 5 April 2011
Received in revised form 30 August 2011
Accepted 5 September 2011

Keywords:
Swine influenza virus
Pandemic A/H1N1/2009 influenza virus
Cameroon
Central Africa
Agriculture
Zoonotic diseases

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.

1. 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.

2. 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 (Fig. 1). Nasal swabs and sera were collected and processed following standard protocols (World Organisation for

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doi:10.1016/j.vetmic.2011.09.003

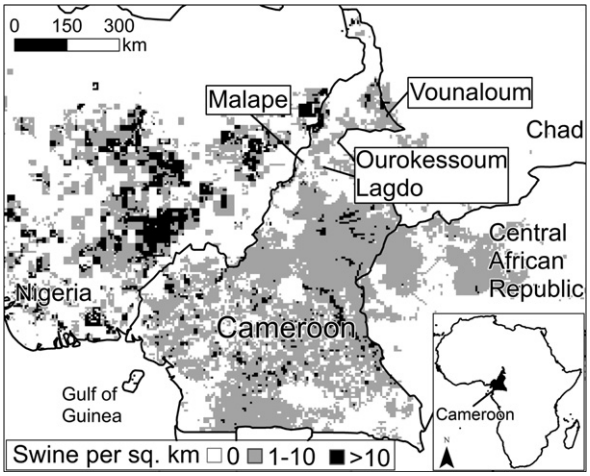

Fig. 1. Sites in northern Cameroon where pH1N1 was detected in swine and swine density in Central Africa. The pH1N1 virus has isolated from pigs in Malape and Ourokessoum Lagdo. At Vounaloum, 88.9% of influenza-positive pigs had antibodies against pH1N1. The density data are from Wint and Robinson (2007).

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).

3. 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) (Fig. 1; 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

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[a] 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	–	+[b]
1076	F	Vounaloum	–	+
1077		Vounaloum	–	+
1078		Vounaloum	–	+[b]
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.
[a] 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).
[b] HI titers ≤1280.