Virus Genes (2013) 46:551–553
DOI 10.1007/s11262-013-0879-1

Identification and molecular characterization of *porcine kobuvirus* in U. S. swine

Harsha Verma · Sunil K. Mor · Mostafa Y. Abdel-Glil · Sagar M. Goyal

Received: 5 September 2012 / Accepted: 9 January 2013 / Published online: 20 January 2013

Abstract *Porcine kobuvirus* has been associated with piglet diarrhea in Asia and Europe, but there are no reports of its presence in the U.S. swine farms. We screened intestinal contents from 114 diarrheic pigs and fecal samples from 46 apparently healthy pigs to determine the presence of *kobuvirus* by reverse transcription-polymerase chain reaction using 3D (RNA polymerase) region primers (amplicon size 216 bp). The samples from ill pigs came from 15 different U.S. states, while those from healthy pigs were obtained from three different farms in Minnesota. Twenty-five (21.9 %) pigs with diarrhea and ten (21.7 %) healthy pigs were positive for *kobuvirus*. All strains from diarrheic pigs were further typed by means of VP1 region primers (amplicon size 811 bp). Phylogenetic analysis revealed that all *porcine kobuvirus* strains had 93.1–96.5 % nucleotide identity with NLD45 strain from the Netherlands and BRA24 strain from Brazil in the 3D region. In the VP1 region, only 86.7–88.5 % homology was found with the T247 strain from Japan and 85.8–87.4 % homology with WUH1 strain from China. All 25 *kobuvirus* positive pigs had mixed infection with transmissible gastroenteritis virus and/or rotavirus (groups A, B, or C). Pigs less than 4 weeks of age showed higher prevalence of *kobuvirus* than the older pigs. The results of this preliminary study indicate that *porcine kobuvirus* is present in both healthy and diarrheic pigs in the U.S. and that further studies are needed to determine its role in gastrointestinal infections of pigs.

H. Verma · S. K. Mor · M. Y. Abdel-Glil · S. M. Goyal (✉)
Veterinary Diagnostic Laboratory, Department of Veterinary Population Medicine, University of Minnesota, 1333 Gortner Avenue, St. Paul, MN 55108, USA
e-mail: goyal001@umn.edu

Keywords Diarrhea · *Porcine kobuvirus* · Phylogeny · RT-PCR

Several bacteria and viruses are known to cause gastrointestinal infections in piglets leading to huge economic losses. Recently, a relatively new virus, the *porcine kobuvirus* was found to be associated with swine gastroenteritis [1]. *Porcine kobuviruses* are small, non-enveloped viruses with single-stranded, positive-sense genomic RNA within the family *Picornaviridae,* genus *kobuvirus* [2]. The virus was first detected in 2008 in fecal samples of domestic pigs in Hungary. The complete genome of the S-1-HUN strain is 8,210 nucleotides in length, including a leader (L) protein, three structural (VP0, VP3, and VP1), and seven non-structural (2A, 2B, and 2C; and 3A, 3B, 3C, and 3D) proteins [3]. In this study, we report for the first time the detection of *porcine kobuvirus* in fecal samples of diarrheic and healthy pigs in U.S. swine farms. Phylogenetic relationship of these strains with the reference strains of *kobuviruses* is also presented. Intestinal contents from 114 diarrheic pigs submitted to the Minnesota Veterinary Diagnostic Laboratory (MVDL) for disease diagnosis were included in this study to determine the presence of *porcine kobuvirus*. Before screening for *kobuvirus*, the samples from diarrheic pigs had been tested at the MVDL for the presence of common intestinal pathogens including TGEV (transmissible gastroenteritis virus) and rotavirus groups A, B, and C using quantitative real time reverse transcription-polymerase chain reaction (qRT-PCR) [4]. In addition, fecal samples from 46 apparently healthy pigs from three different farms were also tested.

Viral RNA was extracted from samples using the QIAamp viral RNA easy kit (Qiagen, Valencia, CA). The reverse transcription-polymerase chain reaction (RT-PCR)

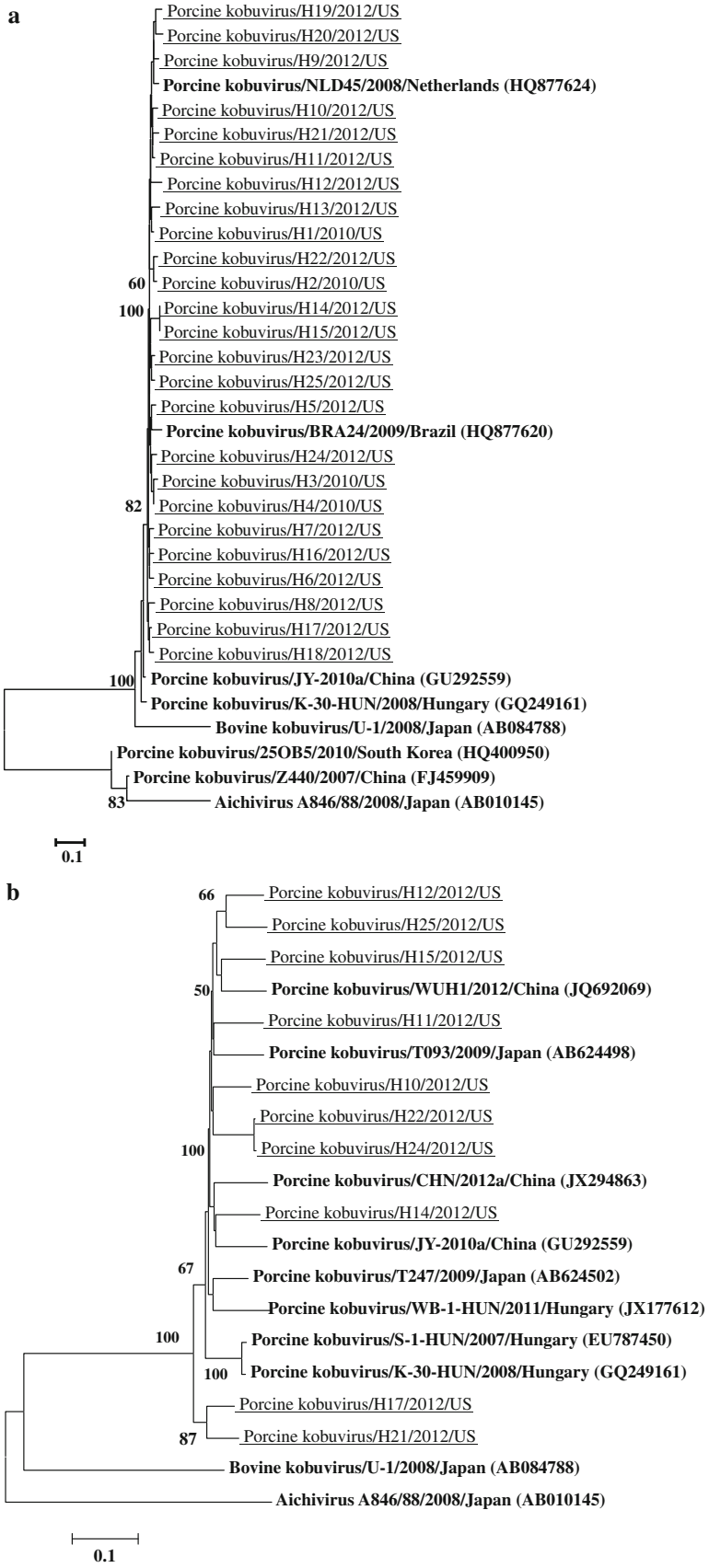

Fig. 1 Phylogenetic trees of *porcine kobuvirus* strains constructed based on **a** partial nucleotide sequences of 3D region (208 bp), **b** VP1 region (780 bp). Our strains are *underlined* and identified by *abbreviations*—specimen number, year of illness, and name of the country. *Scale* indicates genetic distance

was done using Qiagen One Step RT-PCR kit (Qiagen) and universal *kobuvirus* primers of 3D (RNA-dependent RNA polymerase) and VP1 region (capsid protein) [3, 5]. The PCR products were analyzed in 1.2 % agarose gels containing 0.5 μg/mL ethidium bromide and then visualized by ultraviolet illumination. Amplicons of 216 and 811 bp were expected. Of the 114 specimens from ill pigs, 25 were positive for *porcine kobuvirus* with 3D gene primers. The highest positivity rate of 68 % ($p < 0.05$) was observed in piglets under the age of 4 weeks followed by 20 % in pigs up to 8 weeks and 4 % in pigs above 9 weeks. All 25 specimens showed mixed infection with TGEV (8 %) or with rotavirus (72 %). Of the 46 fecal samples from healthy pigs, 10 were found positive.

PCR products were purified using a commercial PCR purification kit (Qiagen) and sequenced at the ACGT (Wheeling, IL). Sequencing was conducted in both directions using the same primers as used in RT-PCR. Forward and reverse sequences were aligned together using Sequencher software (http://genecodes.com/). The obtained sequences were compared with the reference strains available in GenBank and aligned using CLUSTAL W software. Phylogenetic analysis was conducted using MEGA version 5.0 [6]. Neighbor-joining trees were constructed using the Kimura 2 parameter model, and the reliability of different phylogenetic groupings was evaluated by the bootstrap method with 1,000 replications (Fig. 1a, b).

Partial nucleotide sequences of 3D region of 25 *porcine kobuvirus* strains revealed 93.1–96.5 % nucleotide identity with NLD 45 strain from the Netherlands and BRA24 strain from Brazil. The 10 strains from healthy pigs also showed similar nucleotide identity (data not shown). The 25 strains from diarrheic pigs were further typed using primers from VP1 region (capsid protein). Only 10 of 25 (40 %) were typeable with VP1 region primers. In the VP1 region, strains from diarrheic pigs showed only 86.7–88.5 % homology with the T247 strain from Japan and 85.8–87.4 % homology with WUH1 strain from China. The sequences of the *porcine kobuvirus* strains were submitted to GenBank under the following accession numbers: from JX543949 to JX543973 for 3D region sequences and from JX987498 to JX987507 for VP1 region sequences of strains from diarrheic pigs. The accession numbers for sequences of 3D region of strains from healthy pigs are from KC139253 to KC139262.

Porcine kobuvirus has previously been detected in both healthy and diarrheic pigs albeit at different rates. For example, *kobuvirus* was reported in 19.3 % of fecal samples from pigs without diarrhea and 84.5 % from pigs with diarrhea in 2010, and 45.5 and 32.6 % in non-diarrheal and diarrheal samples, respectively, in 2011 from Korea. In Japan, the detection rate of kobuvirus in healthy young pigs was 45 % [7, 8]. In a study in Thailand, 99 % of fecal samples collected from diarrheic pigs were reported as *kobuvirus* positive [9]. In the present study, the prevalence of *kobuvirus* was very similar in healthy (21.7 %) and ill (21.9 %) pigs. These rates are much lower than those observed in Thailand and Korea. However, high positivity rate of *kobuvirus* infection in piglets under the age of 4 weeks in our study is similar to that reported from Shanghai, China [10]. Our results showed, all 25 *kobuvirus* positive pigs had co-infections with other enteric viral pathogens, e.g., rotavirus and/or TGEV. Also, samples from 11 of the 15 states were positive suggesting that *kobuvirus* strains are co-circulating in many different U.S. states.

The role of *porcine kobuvirus* currently is unknown because of its presence in both healthy and ill piglets. Some of the possibilities are that (i) there exist two different pathotypes of *porcine kobuvirus* namely, pathogenic and non-pathogenic; (ii) the virus load is higher in ill pigs versus healthy ones; (iii) *kobuvirus* causes diarrhea only in the presence of other pathogens such as rotavirus or other enteric viruses; and (iv) *kobuvirus* is an endogenous passenger virus and is of no consequence. Nonetheless, our preliminary results indicate the need for further studies on transmission pattern and pathogenesis of *porcine kobuvirus* to determine its etiological role as a viral pathogen in the U.S. swine. Studies are also needed to characterize the circulating strains and their involvement in swine diarrhea. Since kobu-like viruses have been isolated from humans, it is also important to study the zoonotic potential of *porcine kobuviruses*.

Acknowledgments The authors thank Douglas Marthaler and Wendy Wiese for helpful discussions and technical help. The authors also thank Montserrat Torremorell for help in sample collection.

References

1. G. Reuter, A. Boldizsar, I. Kiss, P. Pankovics, Emerg. Infect. Dis. **14**, 1968–1970 (2008)
2. G. Reuter, A. Boros, P. Pankovics, Rev. Med. Virol. **21**, 32–41 (2011)
3. G. Reuter, A. Boldizsar, P. Pankovics, Arch. Virol. **154**, 101–108 (2009)
4. D. Marthaler, K. Rossow, M. Gramer, J. Collins, S. Goyal, H. Tsunemitsu, K. Kuga, T. Suzuki, M. Ciarlet, J. Matthijnssens, Virol. **433**, 85–96 (2012)
5. G. Reuter, L. Egyed, Emerg. Infect. Dis. **15**, 822–823 (2009)
6. K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, Mol. Biol. Evol. **28**, 2731–2739 (2011)
7. S.J. Park, H.K. Kim, H.J. Moon, D.S. Song, S.M. Rho, J.Y. Han, V.G. Nguyen, B.K. Park, Arch. Virol. **155**, 1803–1811 (2010)
8. D.J. An, H.Y. Jeoung, W. Jeong, H.S. Lee, J.Y. Park, B. Kim, Virus Genes **42**, 208–211 (2011)
9. P. Khamrin, N. Maneekarn, A. Kongkaew, S. Kongkaew, S. Okitsu, H. Ushijima, Emerg. Infect. Dis. **15**, 2075–2076 (2009)
10. C. Wang, D. Lan, X. Hua, Virus Genes **43**, 350–352 (2011)