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Cross-species transmission resulted in the emergence and establishment of circovirus in pig

To the Editor,

Emerging infectious diseases (EIDs) are a great threat to public health worldwide. Around 60%–80% of human EIDs originate from wildlife, the typical examples being influenza, coronavirus and henipavirus-related lethal neurologic and respiratory diseases that originated from wild animals and bats, respectively(Hu et al., 2017; Su et al., 2017, 2016). Pigs act as "mixing vessels" since they are susceptible to viral infections of wild animal species and can transmit zoonotic diseases to humans (Zhou et al., 2019). In addition, the high-density farming and international trade within the pig industry increases the risk of transmission and global spread (He et al., 2019b; Morilla GonzáLez et al., 2003). Indeed, contact between pigs and humans was an important factor for the emergence of pandemic influenza virus and Ebola-Reston virus, among others (Horimoto and Kawaoka, 2005; Morris, 2009). Considering the broad spectrum of viruses to which pigs are susceptible and the number of pathogens currently identified in pig, it is essential to understand the factors determining the emergence and evolution of these pathogens.

Circoviruses (family *Circoviridae,* genus *Circovirus*) are the smallest known autonomously replicating viruses. Their genomes consist of a circular, single stranded DNA molecule with two open reading frames (ORFs) encoding the *REP* and *CAP* proteins using an ambisense strategy (Cao et al., 2015; He et al., 2013; Lin et al., 2018; Palinski et al., 2016). Although they were originally described in avian species, numerous members have been characterized in fish, insects and mammals (Garigliany et al., 2015; Li et al., 2010). Until now, three species of circovirus are known to infect pigs including, porcine circovirus (PCV) 1, PCV2 and the novel PCV3 (Li et al., 2018a, 2018b, 2018c; Palinski et al., 2017). The diversity of *circoviruses* and its association with multiple vertebrate hosts represents an important model for understanding how viruses emerge and establish themselves in pig hosts.

1. The study

It has been reported that PCV2 entered wild boar from avian species, and subsequently transmitted to pigs(Firth et al., 2009). Despite an increasing number of available sequences, the origin and transmission patterns of PCVs remain obscure. At the same time, there is controversy regarding the evolutionary relationship between *circoviruses* and their hosts. While particular studies have suggested co-speciation between circovirus and host (Johne et al., 2006), other studies indicate that these viruses may be introduced to new hosts through cross-species transmission (Firth et al., 2009; He et al., 2019a). The increasing availability of sampling and sequences data offers an important opportunity to reconstruct the origin and transmission patterns of PCVs. Here, we analysed 95 circovirus *Rep* from different hosts retrieved from GenBank (https://www.ncbi.nlm.nih.gov/) to trace the origin and evolutionary history of PCV (Table S1). Based on a maximum likelihood (ML) tree of the conserved *Rep* sequences from multiple hosts inferred using RAxML (v4.8.10), we found that PCV1 and PCV2 cluster together, while PCV3 represents a more divergent lineage from PCV1 and PCV2 (Fig. 1a) (Stamatakis, 2014). PCV3 clusters with BatCV in line with our previous report (Li et al., 2018c). Additionally, PCV1 and PCV2 may have originated from bat circovirus (BatCV), however not from the bat clade 2 because they are related to a novel bat genotype (Wu et al., 2016). These results are also confirmed by a Bayesian tree reconstructed using MrBayes (v3.8.2) with a mixed model and a sample size of 2,000,000 and a sample frequency of 200 (Fig. 1b). In addition, we performed selection analysis based on the ML tree and we identified positive selection in some branches (Fig. 1). In particular, we found positive selection in the branch (red branch) of the diversification of BatCV and PCV (Fig. 1) using DATAMONKEY (http://www.datamonkey.org/), suggesting that the transmission of circovirus between bats and the porcine host resulted in adaptation to pigs. Then PCV diverged into PCV1 and PCV2, moreover causing huge economic losses to the pig industry.

We also detected recombination between BatCV and PCV using RDP4 (p value < .05) and Simplot (similarity plot) (Fig. 2a) (Lole et al., 1999; Martin et al., 2015). After RDP4 analysis, one bat circovirus was detected by a potential recombinant between BatCV and PCV2. To confirm this, we observed the sequences characteristic and reconstructed a phylogenetic tree using recombined and non-recombined regions separated by the potential recombination breakpoint (the 777th nucleotide) (Fig. 2b). The position of the BatCV (YN/ZQ924) sequence varies in the two trees. The non-recombined region of the BatCV (YN/

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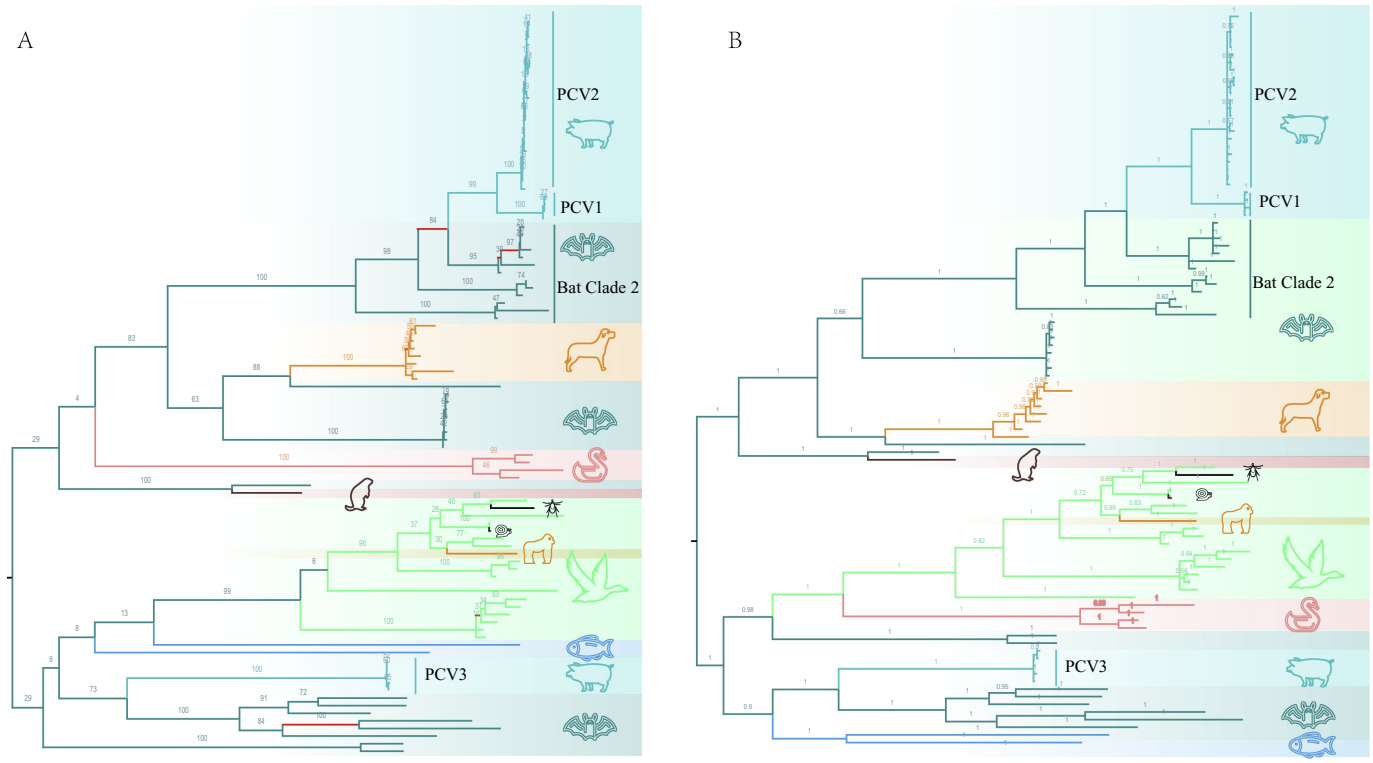

Fig. 1. Origin analysis of PCV. a) ML tree reconstructed using 95 circovirus *REP* sequences using RAxML (4.8.10) based on the PROTCATLG model and 1000 bootstraps. Different hosts are depicted in different colours. Bootstraps values are shown in each node. b) Bayesian tree constructed using MrBayes with a mixed model, a sample size of 2,000,000 and a sample frequency of 200. The posterior probability is shown in each node. The red branch means positive selected branch. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ZQ924) sequence clusters with other BatCVs. However, the recombined region clusters with PCV2. This indicates that BatCV recombined with PCV2 in the *REP* gene, suggesting that bats might be co-infected with PCV to form a new BatCV. Although it is not clear if this recombination facilitated the cross-species transmission of the virus, it suggests that virus transmission and recombination occurred between bats and pigs (Zhou et al., 2018). We can speculate that bat-originated DNA viruses can recombine and cross-species transmission concomitant with the migration, co-roosting and intra- or inter-species contact of other hosts. Our result suggests that the emergence and host range expansion of *circoviruses* could be the result of the close contact of domestic and wild pigs. Indeed, PCVs are highly prevalent in China while closely-related bat CVs have been sequenced from Southern China (Fig. 1 and Table S1) where there is a large number of pig slaughterhouses and a wide distribution and diversity of bat species displaying unique behaviour including characteristic flight patterns, mobility, diet, and roosting. This, together with the constant interaction of pig and humans, presents a potential threat to the pig industry and public health.

2. Conclusion

In summary, we provide evidence suggesting that PCV1 and PCV2 might originated from bats and the occurrence of recombination with PCV2. Overall, we provide new insights into the evolution of CVs in different hosts and unravel transmission/recombination events between pigs and other hosts. Due to the special bat migration ecology and pig farming methods, the transmission models and instances of evolutionary adaptation need to be meticulously researched in the future. Given the fact that bats carry a variety of viruses with human infection potential and that pigs can act as adaptive intermediates for bat pathogens, it is important to monitor these animal species in China for the potential of the emergence of novel zoonosis.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.103973.

Declaration of Competing Interest

The authors declare not conflict of interest.

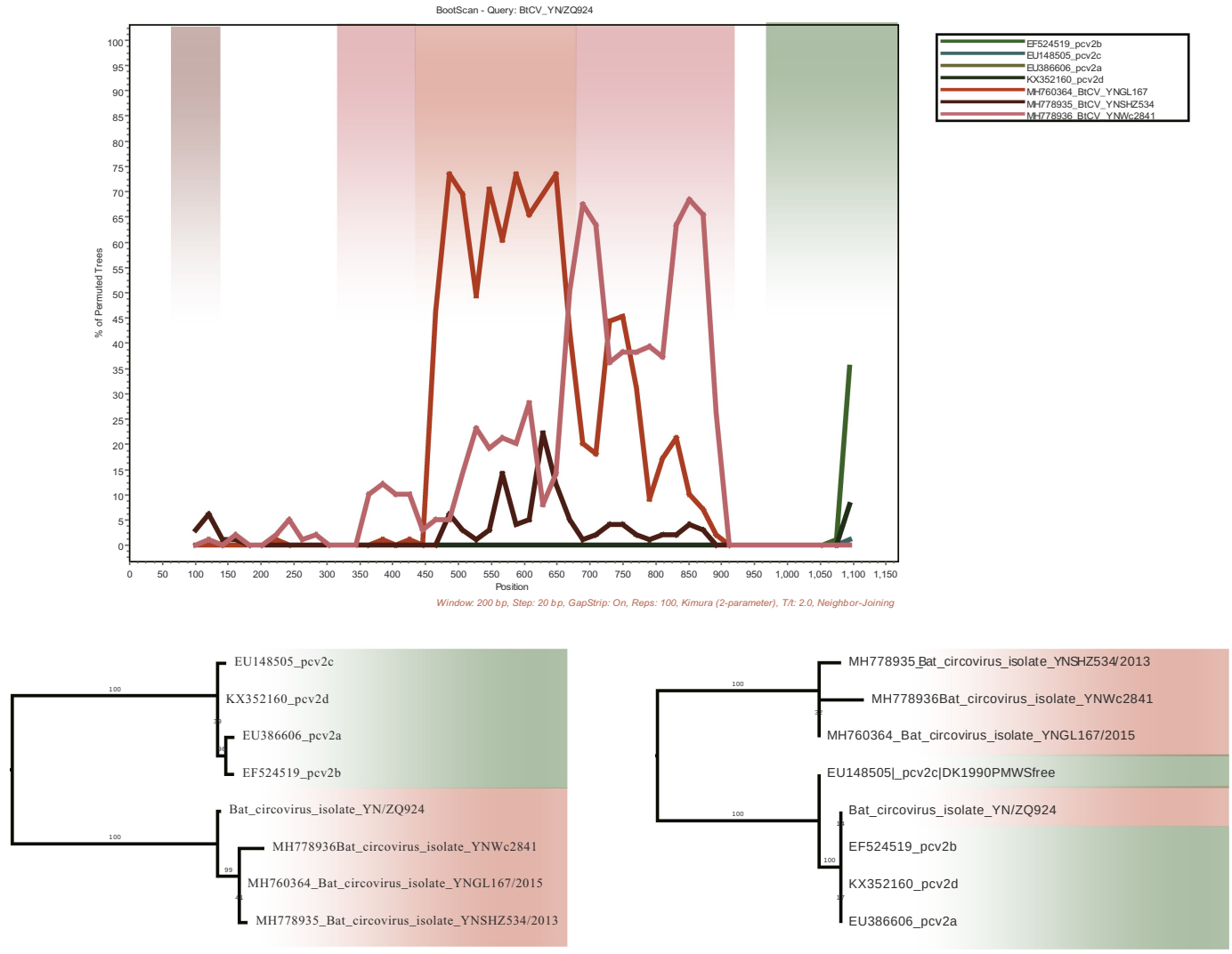

Fig. 2. Detection of recombination in the BatCV(YN/ZQ924). a) Bootscan analysis was conducted using a window size of 200 bp, a step size of 20 bp, and the Kimura (2-parameter) model. In green are indicated PCVs isolates while in red BatCV. The coloured regions suggest potential areas of recombination. b) ML trees reconstructed according to the recombined regions. The left tree was reconstructed using non-recombined regions (nucleotides 1 to 777) while the right tree using recombined regions (nucleotides 777 to 942) using RAxML (4.8.10). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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