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Molecular identification and subtype analysis of *Blastocystis* in pigs, cattle and goats in Hainan, China

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Objective: To determine the infection rates and subtype distributions of *Blastocystis* in pigs, cattle, and goats in Hainan Province, China.

Methods: From September 2018 to May 2019, a total of 659 fresh fecal samples were collected from 188 pigs, 341 goats, and 130 cattles farmed in cities of Hainan Province. All the samples were analyzed for the presence of *Blastocystis* by amplifying the SSU rRNA gene. Phylogenetic analysis was performed by constructing a neighborjoining tree using the program Mega X.

Results: Thirty-one percent (204/659) of the animals were confirmed to be infected with Blastocystis, with 42.2% (144/341) of the goats, 30.8% (40/130) of the cattle, and 10.6% (20/188) of the pigs. Twelve subtypes of Blastocystis were identified, including ST10 (n=114), ST5 (n=37), ST26 (n=16), ST24 (n=8), ST14 (n=8), ST21 (n=8), ST23 (n=4), ST25 (n=4), ST1 (n=2), ST2 (n=1), ST4 (n=1), and ST12 (n=1). There were 11 (ST1, ST2, ST4, ST5, ST10, ST12, ST14, ST21, ST23, ST24, and ST26) and six subtypes (ST10, ST21, and STs23-ST26) in goats and cattle, respectively. All the pig-derived Blastocystis isolates belonged to ST5. A total of 37 representative sequences were obtained from the 12 subtypes of Blastocystis, including 14 sequences that have been described previously and 23 novel sequences were identified which were composed of ST10 (n=9), ST5 (n=8), ST21 (n=3), ST26 (n=2), and ST14 (n=1).

Conclusions: This is the first study to identify the presence of *Blastocystis* in pigs, cattle, and goats from Hainan Province, China, and provides baseline data useful for controlling and preventing *Blastocystis* infection in farm communities. Pigs and goats appear to be the major reservoirs of potential zoonotic infections with ST1, ST2, ST4, ST5, and ST12 and zoonotic infections in pigs and goats with those subtypes should be considered potential public health threats. The new ST sequences of *Blastocystis* identified here provide novel insights into the genotypic variation in *Blastocystis*.

Keywords: Blastocystis; Pig; Goat; Cattle, Genotypic variation

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