

First Report of *Actinobacillus pleuropneumoniae* Prevalence in Tibetan Pigs in Tibet, China

^{1,2}X.C. Shi, ²N.Z. Zhang, ²D.H. Zhou, ²M.J. Xu, ³C. Danba, ²S.M. Wu,
³G. Ga, ¹Y. Mu and ^{2,4}X.Q. Zhu

¹College of Veterinary Medicine, Northwest A&F University, Yangling,
712100 Shaanxi Province, P.R. China

²State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute,
Chinese Academy of Agricultural Sciences, Lanzhou,
730046 Gansu Province, P.R. China

³College of Animal Science, Tibet College of Agriculture and Animal Husbandry,
Nyingchi, 860000 Tibet Autonomous Region, P.R. China

⁴College of Animal Science and Technology, Yunnan Agricultural University,
Kunming, 650201 Yunnan Province, P.R. China

Abstract: The objective of the present investigation was to examine the seroprevalence of *Actinobacillus Pleuropneumoniae* (APP) in Tibetan pigs in Tibet, China by using an Indirect Hemagglutination Assay (IHA). Serum samples were collected from 332 Tibetan pigs in Nyingchi, Tibet, China between April and December 2010 and were examined for APP antibodies using a commercially available IHA kit. A total of 185 (55.72%) Tibetan pigs were examined seropositive for APP antibodies. The prevalence ranged from 42.68-71.11%, varying in different age groups except breeding boars and sows with higher prevalence in piglets and lower prevalence in slaughter pigs but the difference was not statistically significant ($p>0.05$). The investigation also showed that the prevalence of APP in female animals was 54.70 and 59.72% in male animals. The results indicated a widespread exposure to APP among Tibetan pigs in Tibet, China. This is the first report documenting the infection of APP in Tibetan pigs in Tibet, China.

Key words: *Actinobacillus pleuropneumoniae*, seroprevalence, Indirect Hemagglutination Assay (IHA), Tibetan pigs, Tibet, China

INTRODUCTION

Actinobacillus Pleuropneumoniae (APP) is the causative agent of porcine contagious pleuropneumonia which is responsible for cases of highly contagious respiratory disease in pigs of all ages and serious diseases including high fever, coughing, dyspnea, anorexia, ataxia and chronic persistent disease (Gottschalk and Taylor, 2006; Hoeltig *et al.*, 2009; Meynsa *et al.*, 2011). This organism is an important Gram-negative bacterium which impacts on the pig production varied from peracute mortality to decreased weight gain and feed efficiency (Rohrbach *et al.*, 1993; Willard, 2005).

The epidemiology of APP serotypes is thought complicated. By far, there are 15 characterized APP serotypes (Blackall *et al.*, 2002) with different prevalence in pigs. Under certain conditions, only one dominant serotype is prevalent in a country or simultaneous prevalence of multiple serotypes in the same area or even

in the same farm is also very common (Gottschalk and Taylor, 2006). APP infection has been investigated in many countries and in a numbers of intensive pig farms in China (Assavacheep *et al.*, 2003; Vengust *et al.*, 2006; Fu *et al.*, 2009; He *et al.*, 2009a; Lu *et al.*, 2009; Yu *et al.*, 2009; Zhang *et al.*, 2010) whereas no serological or etiological studies of APP are conducted in Tibetan pigs in Tibet, China. Characterized by cold resistance and crude feed tolerance, Tibetan pigs could easily adapt to the free-range husbandry, the cold climate and the developing management conditions. There are nearly 300 thousand Tibetan pigs in Tibet Plateau (>3000 m above sea level) and this animal is one of the main sources of meat and economy in Tibet (Xin *et al.*, 2011).

The objective of this study was to investigate the seroprevalence of APP in Tibetan pig in Tibet, China which would provide a foundation for taking strategies and measures to control APP infection in Tibetan pigs.

MATERIALS AND METHODS

Serum samples: From April to December 2010, 332 blood samples were collected from Tibetan pigs via the precaval vein by professional veterinary practitioners in Nyingchi Prefecture, Tibet, China. Before sample collection, the general characters of Tibetan pigs were examined thoroughly. All the operations were humane according to the animal welfare. These blood samples were centrifuged at 3000 g for 5 min. The separated sera were stored at -20°C until further analysis.

Determination of antibodies to APP: Antibodies to APP were examined using the Indirect Hemagglutination Assay (IHA) according to the manufacturer's instructions of a commercially available IHA kit purchased from Lanzhou Veterinary Research Institute, Chinese Academy of Agriculture Sciences. In brief, 25 µL of IHA dilution solution was added to 96 well V bottom plates. Then, serial two fold dilutions of test sera were started at 1:2. Each test sera was then screened at dilutions of 1:4, 1:6, 1:16, 1:32, 1:64, 1:128 and 1:256. After addition of 25 µL of sensitized erythrocytes with APP mixed antigen (serovar 1, 3 and 7) to each well, the plates were shaken for 1-2 min and then incubated at 37°C for 2-3 h without shaking. Positive and negative controls were included in each test. The tests were considered positive for APP antibodies when samples reacted above 1:8.

Statistical analysis: Data were statistically analyzed using the procedure of SAS (Statistical Analysis System, Version 8.0). Chi-square analysis was used to analyze the difference in seroprevalence between genders and age groups. The level of significant difference was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Examinations of the 332 sera from Tibetan pigs revealed antibodies against APP in 118 (55.72%) sera by IHA and antibodies titers were 1:256 in three, 1:128 in three, 1:64 in 12, 1:32 in 13, 1:16 in 43 and 1:8 in 111

(Table 1). The 90 (52.02%) out of 173 Tibetan pigs in Nyingchi county and 95 (59.75%) out of 159 in Mainling county were tested positive (Table 2) without significant difference ($p > 0.05$). In male animals, the investigation showed no significant difference in the prevalence of antibodies to APP (59.72%) as compared to females (54.70%, $p > 0.05$).

APP often facilitates the occurrence of other respiratory diseases. In chronically infected herds with no obvious signs of clinical diseases, serological methods can be effective in diagnosing porcine contagious pleuropneumonia (Klausen *et al.*, 2001) because of the difficulty in bacterial isolation (Dubreuil *et al.*, 2000; Wallgren and Persson, 2000). Among epidemiological investigations, IHA is an effective method of serotyping APP (Blackall *et al.*, 1998). In the present study, this method was used in investigation of seroprevalence of APP in Tibetan pigs in Tibet, China.

The overall APP prevalence in Tibetan pigs was higher than other studies performed in pigs from intensive farms in Anhui (He *et al.*, 2009a), Fujian (He *et al.*, 2009b), Guizhou (Yu *et al.*, 2009), Hubei (Gong *et al.*, 2009), Jiangxi (Zhao *et al.*, 2009), Sichuan (Fan *et al.*, 2009) and Henan (Zhang *et al.*, 2010) provinces, Beijing Municipality (Fu *et al.*, 2009) and Ningxia Hui Autonomous Region (Bai *et al.*, 2010) of China. The prevalence is also higher than that in wild boars in Slovenia (Vengust *et al.*, 2006) and Germany (Reiner *et al.*, 2010) but lower than those observed in Qinghai (Lu *et al.*, 2009) province, China, Ontario, Canada and in market-weight pigs in Thailand (Assavacheep *et al.*, 2003). The difference in APP seroprevalence may be due to diagnostic methods, sample sources as well as the environment conditions in which Tibetan pigs are exposed to infectious diseases and predators may increase stress under the free-range system.

Direct contact transmission is considered to be the foremost transmission route of APP because of its limited surviving time in the environment (Nicolet, 1992) and the aerosol and droplet are also the transmission routes (Gottschalk and Taylor, 2006). In Nyingchi, Tibet, moist and warm climate may be beneficial for the survival of

Table 1: Antibody titers to *Actinobacillus pleuropneumoniae* infection in Tibetan pigs determined by an Indirect Hemagglutination Assay (IHA)

Types of pig	No. of examined	IHA titers							Prevalence (%)
		≤1:4	1:8	1:16	1:32	1:64	1:128	1:256	
Breeding boar	4	1	2	1	0	0	0	0	75.00
Breeding sow	5	0	4	0	0	0	1	0	100.00
Slaughter pig	82	47	24	7	2	1	0	1	42.68
Fattening pig	88	35	36	11	5	1	0	0	60.23
Growing pig	108	51	30	15	3	7	1	1	52.78
Piglet	45	13	15	9	3	3	1	1	71.11
Total	332	147	111	43	13	12	3	3	55.72

Table 2: Prevalence of antibodies to *Actinobacillus pleuropneumoniae* in Tibetan pigs by genders and geographical origins in Tibet, China determined by Indirect Hemagglutination Assay (IHA)

Biometric data	Gender			Geographical origin		
	Male	Female	Unknown	Total	Mainling	Nyingchi
No. of examined	144.00	117.00	71.00	332.00	159.00	173.00
No. of positive	86.00	64.00	35.00	185.00	95.00	90.00
Prevalence (%)	59.72	54.70	49.30	55.72	59.75	52.02

APP and the weather also could increase the spread opportunity of both direct and indirect routes. In view of animal husbandry practices in the region (Free-Range Keeping Systems) and animals often become chronic carriers of APP (Radacovici *et al.*, 1994), porcine contagious pleuropneumonia in Tibetan pigs may have already become endemic. All of these may also explain the high seroprevalence of APP in Nyingchi indicating the increasing difficulty in eradicating this bacterium.

Breeding sows in this study were immunized with commercial APP vaccine according to the local veterinary practitioners to prevent or reduce the spread of this etiology from sows to offspring during a 3 weeks nursing period (Vigre *et al.*, 2002). A higher APP prevalence was detected in piglets in comparison with other age groups but the difference were not statistically significant ($p>0.05$). The high seropositivity in piglets can partially indicate the high prevalence of APP in pigs when the level of maternal antibody decreased.

CONCLUSION

The results of the present survey demonstrated a high seroprevalence of APP infection in Tibetan pigs in Tibet, China. Therefore, integrated and improved control strategies and measures become necessary for prevention and control APP infection in Tibetan pigs. To the knowledge, this is the first report of APP infection in Tibetan pigs in Tibet, China.

ACKNOWLEDGEMENTS

Project support was provided, in part, by the Program for Outstanding Scientists in Agricultural Research, the Open Funds of the State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (Grant Nos. SKLVEB2011KFKT004, SKLVEB2011KFKT011, SKLVEB2010KFKT009 and SKLVEB2011KFKT010) and the Yunnan Provincial Program for Introducing High-level Scientists (Grant No. 2009CII125). X.C. Shi and Zhang contributed equally to this research.

REFERENCES

- Assavacheep, P., M. Persson, S. Luengyosluechakul, S. Watanaphansak, D. Laohasinnarong, P. Pungkhun and P. Wallgren, 2003. *Actinobacillus pleuropneumoniae* in Thai pig herds. Prevalence of serum antibodies and relation to performance. J. Vet. Med. B., 50: 390-395.
- Bai, G., Y. He, P. Zhao, Y.F. Chu, P.C. Gao, N.Z. Zhang and Z.X. Lu, 2010. Serologic investigation of porcine infectious pleuropneumonia in parts of Ningxia hui autonomous region. Guizhou Agric. Sci., 38: 125-126.
- Blackall, P.J., H.L.B.M. Klaasen, H. van den Bosch, P. Kuhnert and J. Frey, 2002. Proposal of a new serovar of *Actinobacillus pleuropneumoniae*: Serovar 15. Vet. Microbiol., 84: 47-52.
- Blackall, P.J., R. Bowles, J.L. Pahoff and B.N. Smith, 1998. Serological characterisation of *Actinobacillus pleuropneumoniae* isolated from pigs in 1993 to 1996. Aust. Vet. J., 76: 39-43.
- Dubreuil, J.D., M. Jacques, K.R. Mittal and M. Gottschalk, 2000. *Actinobacillus pleuropneumoniae* surface polysaccharides: Their role in diagnosis and immunogenicity. Anim. Health Res. Rev., 1: 73-93.
- Fan, H.Q., Y.F. Chu, Y. He, P.C. Gao and P. Zhao *et al.*, 2009. Serologic investigation of porcine infectious pleuropneumonia in partial areas of Sichuan province. China Anim. Health Inspect., 9: 49-50.
- Fu, C.X., X.J. Jin, L. Yuan, J. Li and C.H. Yu, 2009. Serological investigation on porcine contagious pleuropneumoniae in some areas of Beijing. Progress Vet. Med., 30: 111-113.
- Gong, Y.D., J.B. Jiang and C.S. Yang, 2009. Serologic investigation on porcine contagious pleuropneumonia in southwestern area of Hubei. Progress Vet. Med., 30: 114-117.
- Gottschalk, M. and D.J. Taylor, 2006. *Actinobacillus pleuropneumoniae*. In: Diseases of Swine, Straw, B.E., J.J. Zimmerman, S. D'Allaire and D.J. Taylor (Eds.). Blackwell Press, Iowa, USA., pp: 563-576.
- He, Y., P. Zhao, Y.F. Chu, P.C. Gao and Z.X. Lu, 2009a. Epidemiological investigation of porcine contagious pleuropneumonia in part area of Fujian province. Hubei Agric. Sci., 48: 1446-1447.
- He, Y., P. Zhao, Y.F. Chu, P.C. Gao and Z.X. Lu, 2009b. Serologic investigation on porcine contagious pleuropneumonia in partial areas of Anhui province. Jiangsu Agric. Sci., 3: 262-263.
- Hoeltig, D., I. Hennig-Pauka, K. Thies, T. Rehm and M. Beyerbach *et al.*, 2009. A novel Respiratory Health Score (RHS) supports a role of acute lung damage and pig breed in the course of an *Actinobacillus pleuropneumoniae* infection. BMC Vet. Res., Vol. 5. 10.1186/1746-6148-5-14.

- Klausen, J., L.O. Andreasen, K. Barfod and V. Sorensen, 2001. Blocking enzyme-linked immunosorbent assay for detection of antibodies against *Actinobacillus pleuropneumoniae* serotype 6 in pig serum. Vet. Microbiol., 79: 11-18.
- Lu, Y., G.P. Wang, Q.G. Cai and L.Q. Ma, 2009. Serologic investigation of porcine infectious pleuropneumonia in Qinghai province. China Anim. Health Inspection, 5: 49-50.
- Meynsa, T., J. Steelant, E. Rolly, J. Dewulf, F. Haesebrouck and D. Maes, 2011. A cross-sectional study of risk factors associated with pulmonary lesions in pigs at slaughter. Vet. J., 187: 388-392.
- Nicolet, J., 1992. *Actinobacillus pleuropneumoniae*. In: Diseases of Pigs, Leman, A.D., B.E. Straw, W.L. Mengeling, S. D'Allaire and D.J. Taylor (Eds.). Wolfe Press, London, pp: 401-407.
- Radacovici, S., J. Gottschalk and J.D. Dubreuil, 1994. Lipopolysaccharides of *Actinobacillus pleuropneumoniae* (serotype 1): A readily obtainable antigen for ELISA serodiagnosis of pig pleuropneumonia. Vet. Microbiol., 39: 219-230.
- Reiner, G., C. Fresen, S. Bronnert, I. Haack and H. Willems, 2010. Prevalence of *Actinobacillus pleuropneumoniae* infection in hunted wild boars (*Sus scrofa*) in Germany. J. Wildl. Dis., 46: 551-555.
- Rohrbach, B.W., R.F. Hall and J.P. Hitchcock, 1993. Effect of subclinical infection with *Actinobacillus pleuropneumoniae* in commingled feeder swine. J. Am. Vet. Med. Assoc., 202: 1095-1098.
- Vengust, G., Z. Valencak and A. Bidovec, 2006. A serological survey of selected pathogens in wild boar in Slovenia. J. Vet. Med. B., 53: 24-27.
- Vigre, H., O. Angen, K. Barfod, D.T. Lavritsen and V. Sorensen, 2002. Transmission of *Actinobacillus pleuropneumoniae* in pigs under field-like conditions: Emphasis on tonsillar colonisation and passively acquired colostral antibodies. Vet. Microbiol., 89: 151-159.
- Wallgren, P. and M. Persson, 2000. Relationship between the amounts of antibodies to *Actinobacillus pleuropneumoniae* serotype 2 detected in blood serum and in fluids collected from muscles of pigs. J. Vet. Med. B., 47: 727-737.
- Willard, C.L., 2005. Economic impacts of reduced pork production associated with the diagnosis of *Actinobacillus pleuropneumoniae* on grower/finisher swine operations in the United States. Prev. Vet. Med., 68: 181-193.
- Xin, S.P., D. Shi and J. Zhang, 2011. Research of protection, development and utilization of genetic resources of Tibetan pig in Tibet Autonomous Region. Sci. Technol. Promotion, 4: 49-51.
- Yu, J., B.J. Zhou and S.X. Zhou, 2009. Serologic investigation on porcine contagious pleuropneumonia in Guizhou. Guizhou J. Anim. Husbandry Vet. Med., 33: 26-27.
- Zhang, G.H., J.H. Jie, J. Chen, W. Li, Y.N. Zhang, H.X. Yi and X.W. Zhang, 2010. Serological investigation on porcine contagious pleuropneumoniae in Henan province. Jiangsu Agric. Sci., 6: 326-327.
- Zhao, P., Y. He, Y.F. Chu, P.C. Gao, Z.X. Lu and H.Y. Zhao, 2009. Epidemiological investigation of porcine contagious pleuropneumonia in part area of Jiangxi province. Jiangxi Agric. Sci., 27: 197-199.