PREVALENCE OF *Erysipelothrix rhusiopathiae* IN HEALTHY SWINE TONSILS AT MATO GROSSO STATE, BRAZIL

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ABSTRACT

The study analyzed 310 swine tonsils collected from slaughterhouse in Mato Grosso State Brazil. The agent Erysipelothrix rhusiopathie was detected by PCR technique and 4.19% animals were positive. Seven out of eleven municipalities have at least one positive animal to Erisipelothrix rhusiopathiae demonstrating that infection is widespread in Mato Grosso pig's farm with low occurrence.

Key words: Erysipelothrix rhusiopathiae, Erysipelothrix tonsillarum, swine

Erysipelothrix rhusiopathiae is a small rod, gram-positive and facultative bacteria[1]. Its distribution is ubiquitous, as commensal or associated to disease in human and animals, being swine an important reservoir. The economic losses due *E. rhusiopathiae* infections in swine production affect principally adult animals worldwide.Three clinical forms are described in humans: the localized cutaneous form (erisipeloid), generalized cutaneous and septic form with endocarditis and arthritis. These infections are generally associated to handling of infected animal tissues and skin lesions. Thus, it is an occupational hazard mainly associated to slaughterhouse employees.

Thirty to fifty percent of healthy swine have *E. rhusiopathiae* in tonsils and lymphoid tissues and they are important source of infection during outbreaks

due to nasal discharges, urine and feces contaminated. Considering the absence of data about this important pathogen in central western of Brazil this study aimed to describe the prevalence of *E. rhusiopathiae* in healthy swine at Mato Grosso State - Brazil.

The number of samples was estimated according to the program EPI-INFO 2008 using 14% prevalence estimated from the media of results of other studies, 6% error and 1.069,301 million animals in the state according to the pigs farmer's association of the State of Mato Grosso (ACRISMAT). Samples were collected from swine tonsils in three slaughterhouses under federal inspection from June 2005 to July 2008, 30 samples in the first year and three collections of 30 samples were collected in each year, 310 samples were collected in total. The collection of batches was carried out according to the sequence of slaughter, collecting only 10 samples per batch. One gram of tonsil tissue were cultivate in 20 mL of try soy broth with violet crystal, tris and tween 80 as a pre-enrichment according to Yamazaki (2006), during 48 hours at 37°Celsius. DNA was extracted from 2 mL, by adding proteinase K followed by phenol-chloroform treatment and isopropanol precipitation. DNA was dissolved in 20 microliters of ultrapure water. To detect *E. tonsillarum*, PCR reaction was performed with 0.16 pmol each primer MO101 and ERS-1S according Yamazaki (2006), 2.4mM $MgCl_2$, 10X of TaqBuffer, 0.2mM of each DNTP, 2 U *Taq* DNA polymerase (Fermentas®) 1,5 microliter of DNA and ultrapure water to 25 microliters final

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volume. Thermal cicling condition was 94°C/4min followed by 35 cycles of 94ºC/1min, 52ºC/1min, 72ºC/2.5min and a final step at 72ºC/5min. performed with 0.16 pmol each primer MO101 and ERS-1S according Yamazaki (2006), 2.4 mM $MgCl_2$, 10X of TaqBuffer, 0.2mM of each DNTP, 2 U *Taq* DNA polymerase (Fermentas®) 1.5 microliter of DNA and ultrapure water to 25 microliters final volume. Thermal cicling condition was 94°C/4min followed by 35 cycles of 94ºC/1min, 52ºC/1min, 72ºC/2.5min and a final step at 72ºC/5min.

Positive samples were submited to *E. rhusiopathiae* identification according Yamazaki (2006). PCR conditions were 0.16 pmol of primers ERY-1F and ERY-2R (16), 5mM $MgSO_4$, 1X buffer PCR, 0.2mM DNTP, 1U of *Taq* DNA polimerase high fidelity (Platinum®) in final reaction volume of 25L. Thermal cycling condition were initial step at 94°C/4min followed by 35 cycles of 94ºC/1min, 58°C/40sec, 68°C/2.5min and a final step at 72ºC/5 min. All PCR products were separated by electrophoresis in gel agarose (2%), stained by ethidium bromide and analyzed in transilluminator.

From 310 samples, 71 (22.9%) were positive to *E. tonsillarum* and 13 (4.19%) to *E. rhusiopathiae* as shown in table 1. All municipalities had positive animals to *E. tonsillarum* in Mato Grosso State but only 63.63% (7/11) had positive to *E. rhusiopathiae*. Prevalence studies about *E. rhusiopathiae* and *E. tonsillarum* shows great differences in many studies probably because diagnostic test used and local areas of study. Our results based on PCR show a high prevalence of *E. tonsillarum* (22.9%) compared to other countries, such Thailand countries (8.14%). In similar study realized in south and southeast Brazil it was find 4.7 to 43% of prevalence of *E. rhusiopathiae* but these results could have been underestimated because the low sensibility of technique employed or the immunization herd status.

Herein we found a lower prevalence of *E. rhusiopathie* comparing to others Brazilian regions,

Table 1 : Occurrence of *E. rhusiopathiae* and *E. tonsillarum* in swine tonsils during June 2005 to July 2008 at Mato Grosso State, Brazil.

Region	City	Samples (n)	Positive samples (%)	
			Erysipelothrix tonsillarum	*Erysipelothrix rhusiopathiae*
Middle northern	Diamantino	61	11 (3,54)	3 (0,97)
	Nova Mutum	50	5 (1,61)	1 (0,32)
	Sinop	33	12 (3,88)	1 (0,32)
	Sorriso	29	9 (2,9)	2 (0,65)
	Tapurah	52	9 (2,9)	1 (0,32)
	Lucas do Rio Verde	10	4 (1,29)	0 (0)
	Santa Rita do Trivelato	10	1 (0,33)	0 (0)
Middle southern	Poconé	8	3 (0,97)	0 (0)
	Santo Antônio do Leverger	10	6 (1,93)	1 (0,32)
Southeast	Itiquira	10	1 (0,33)	0 (0)
	Pedra Preta	37	10 (3,22)	4 (1,29)
	Total	310	71 (22,9)	13 (4,19)

19.41% of positive animal which 86.87% were *E. rhusiopathiae*. This low prevalence of *E. rhusiopathiae* when compared to *E. tonsillarum* could be result from the vaccination scheme used in

our region where just one animal was found unvaccinated to *E. rhusiopathiae*. *E. tonsillarum* was been detected widespread in swine of Mato Grosso State and was demonstrated that have a low prevalence of *E. rhusiopatiae.*

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REFERENCES

Associação dos Criadores de Suínos do Mato Grosso (ACRISMAT). Avaliação de Pedro de Camargo Neto, presidente da ABIPECS, sobre o ano de 2008. Accessed dez./11/2008. Online. Avaible in http://www.acrismat.com.br/mostrar_noticias.asp?id =4679.

Eamens GJ, Forbes WA, Djordjevic SP. Characterization of *Erysipelothrix rhusiopathiae* isolates from pigs associated with vaccines breakdowns. *Vet. Microbiol.* 2006; **115**: 329-338.

Fidalgo SG, Wang Q, Riley TV. Comparison of methods for detection of *Erysipelothrix* spp and their distribuition in some Australasian seafoods. *Appl. Environ. Microbiol.* 2000; **66**: 2066-2070.

Makino S, Okada Y, Maruyama T, Ishikawa K, Takahashi T, Nakamura M, Ezaki T, Morita H. Direct and rapid detection of *Erysipelothrix rhusiopathiae* DNA in animals by PCR. *J. Clin. Microbiol.* 1994; **31**: 1526-153.

Oliveira SJ, Rodrigues PC, Okatani AT, Lunge VR. Monitoria da Erisipela suína por análises bacteriológicas e moleculares em suínos de abate de granjas do Rio Grande do Sul. *Arq. Inst. Biol.* 2009; **76**: 689-692.

Opriessing T, Hoffman LJ, Harris DL, Gaul SB, Halbur PG. *Erysipelothrix rhusiopathiae*: genetic characterization of midwest US isolates and live commercial vaccines using pulsed-field gel electrophoresis. *J. Vet. Diagn. Invest.* 2004; **16**: 101-107.

Pal N, Bender JS, Opriessnig T. Rapid detection and differentiation of *Erysipelothrix* spp by a novel multiplex real-time PCR assay. *J. Appl Microbiol.* 2010; **108**: 1083-1093.

Romney M, Cheung S, Montessori V. *Erysipelothrix rhusiopathiae* endocarditis and presumed

osteomyelitis. *Can. J. Infect. Dis.* 2001; **12**:254-256.

Ruiz ME, Richards JS, Kerr GS, Kan VL. *Erysipelothrix rhusiopathiae* septic arthritis. *Arthritis Rheum.* 2003; **48**:1156-1157.

Shimoji Y, Mori Y, Hyakutake K, Sekizaki T, Yokomizo Y. Use of an enrichment broth cultivation-PCR combination assay for rapid diagnosis of swine erysipelas. *J. Clin. Microbiol.* 1998; **36**:86-89.

Shimoji Y. Patogenicity of *Erysipelothrix rhusiopathiae:* Virulences Factors e protective immunity. *Microbes. Infect. J.* 2000; **2**:965-972.

Stephenson EH, Berman DT. Isolation of *Erysipelothrix rhusiopathiae* from tonsils of apparently normal swine by two methods. *Am. J. Vet. Res.* 1978; **39**: 187-188.

Takahashi T, Sunama P, Satra J, Cholsindhu N, Kongthon S, Jitnupong W, Yamamoto K, Kijima M, Furuuchi S. Serotyping and Patogenicity of *Erysipelothrix* Strains

Isolated from Tonsils of Slaughter Pigs in Thailand. *J. Vet. Med. Sci.* 1999; **61**: 1007-1011.

Takahashi T, Takaji M, Yamaoka R, Ohishi K, Norimatsu M, Tamura T, Nakamura M. Comparison of the patogenicity for chickens of *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum. Avian Pathol.* 2004; **23**: 237-245.

Wang Q, Chang BJ, Riley TV. Review *Erysipelothrix rhusiopathiae*. *Vet. Microbiol.* 2010; **140**: 405-417.

Wang Q, Fidalgo S, Chang BJ, Mee BJ, Riley TV. The detection and recovery of *Erysipelothrix* spp in meat and abattoir samples in Western Australia. *J. Appl. Microbiol.* 2002; **92**: 844 850.

Yamazaki, Y. A multiplex pcr polymerase chain reaction for discriminating *Erysipelothrix rhusiopathiae* from *Erysipelothrix tonsillarum. J. Vet. Diagn. Invest.* 2006; **18**: 384-387.