

ISOLATION AND SEROTYPING OF *E. rhusiopathiae* IN INDONESIA

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SUMMARY

Erysipelothrix rhusiopathiae was isolated from tonsils of 77 (39.69%) of 194 apparently healthy slaughter pigs in Indonesia during the period of June to December 1993. A total of 157 out of the *E. rhusiopathiae* isolates could be serotyping within the 22 recognized serotypes. serotype 2 was most prevalent with 31.16% followed by serotype 12 and 6.

INTRODUCTION

Erysipelothrix rhusiopathiae is a causative agent of swine erysipelas, which causes great economic loss in swine-producing area of the world. The clinical signs of swine erysipelas can be divided into three types : acute (septicaemia), subacute (urticaria) and chronic (arthritis, lymphadenitis and endocarditis). At present, strain of *E. rhusiopathiae* are classified into 22 serotypes 1 (consisting of subtypes 1a and 1b) and 2 (Wood, 1984).

Different tissues of apparently healthy pigs have been examined for the presence of *E. rhusiopathiae* by many authors. The preponderance of these isolations has been from the tonsils, although the organisms have been isolated from the intestinal tract, lymph nodes, gall bladder, joints and bone marrow (Connel and Langford, 1953; Roswell, 1958, Murase and Ebi, 1960; Van Damme and Devriese, 1976; Takashi *et al.*, 1987b). Thus, the tonsils are generally recognized as a site of localization by *E. rhusiopathiae* in swine.

In Indonesia, prevalence of swine erysipelas has become a major problem in the aspect of animal hygiene and the commercial erysipelas bacterin is widely used for prevention of the disease. Therefore the main objective of present study is to continue our previous study namely to investigate Indonesian serotype and the distribution of erysipelas in Indonesia.

MATERIALS AND METHODS

Tissues

The tonsils were obtained from 194 pigs, weighing 70-100 kg, selected at slaughter houses in North Sumatra, West Kalimantan, Bali, Riau, Central Java and DKI Jakarta during the period of June to December 1993. Tonsils from each pig were placed in a vinyl specimen bag and transported in ice to the Veterinary Drug Assay Laboratory, Bogor. Tissues were processed for bacteriologic culture immediately upon arrival.

Culture procedures for isolation of *E. rhusiopathiae*

Approximately 1 gram of tonsil tissue was diced into small pieces and inoculated into 10 ml of tryptose phosphate (TP) broth, pH 7.6 (Difco) containing 0.1% Tween 80, 25 µg of gentamycin per ml, and 500 µg of kanamycin per ml. After incubation at 37°C for 24 hours, one loopful of the culture was streaked onto each plate of TP agar pH 7.6 (Difco) containing 0.1% Tween 80, 25 µg of gentamycin per ml, and 500 µg of kanamycin per ml. The agar plates were incubated at 37°C for 24 hours and then examined for the presence of typical *Erysipelothrix* colonies.

Identification of isolates

Representative colonies with typical morphologic characteristics of *E. rhusiopathiae* were picked

from each plate, seeded into tubes of TP broth, and incubated at 37°C for 24 hours. The isolates were identified as *E. rhusiopathiae* on the basis of cellular morphology, typical reactions in triple sugar iron agar slants, and "test-tube brush" growth in gelatin as described by Wood (1970).

Serotyping

Serotyping of *E. rhusiopathiae* isolates was performed by a method described previously (Kucsera, 1973; Takahashi *et al.*, 1984b). Colonies from a 24 h-old agar plate culture of each isolate were inoculated into TP broth containing 0.1% tween 80, incubated at 37°C for 24 hours, and the culture was centrifuged at 12000Xg for 20 min. The bacterial cells were washed three times with physiological saline and suspended in distilled water to 1/30 of the original volume. The bacterial suspension was autoclave at 121°C for 1 hour, cooled, and clarified by centrifugation.

The supernatant fluid was tested in a typing sera (rabbit origin) representing serotypes 1 through 22 of *E. rhusiopathiae*.

RESULT

Bacterial isolation from tonsils of pigs.

E. rhusiopathiae was isolated from the tonsils of 77 (39.69%) of 194 slaughter pig in various port of Indonesia. It can be seen in table 1.

Table 1. Isolated of *E. rhusiopathiae* from the pig tonsils in various port of Indonesia.

No.	Province	No. of Sample examined	No. of Isolates Obtained (%)
1.	North Sumatera	31	7 (22.5%)
2.	West Kalimantan	38	20 (52.6%)
3.	Bali	30	16 (53.3%)
4.	Riau	35	24 (68.5%)
5.	Central Java	30	4 (13.3%)
6.	DKI Jakarta	30	6 (20.0%)

Serotypes of isolates.

The serotype of 77 *E. rhusiopathiae* isolates are shown in table 2.

DISCUSSION

It is well documented that the prevalence of *E. rhusiopathiae* carrier pigs among those examined ranges from 3 to 98%. With most surveys indicating that 20 to 40% pigs are carriers (Langkamp, 1952; Murase and Ebi, 1960; Timoney, 1970; Stephenson *et al.*, 1978; Takahashi *et al.*, 1987b; Takahashi *et al.*, 1989). In this study *E. rhusiopathiae* was isolated from tonsils of 77 (39.69%) of 194 apparently healthy slaughter pigs in Indonesia. Therefore, as in other producing areas world, the organisms is apparently ubiquitous in swine and in the environment as a soil contaminant (Wood and Paker, 1972) and probably constitutes a significant potential pathogen for pigs in Indonesia.

Table 2. Serotype of the 77 *E. rhusiopathiae* isolates from tonsils of apparently healthy slaughter pigs in Indonesia.

No.	Province	S e r o t y p e									untype-able
		1a	2	3	6	9	11	12	13	22	
1.	North Sumatera	-	1	-	-	-	-	4	-	-	2
2.	West Kalimantan	-	11	1	1	-	-	2	-	-	5
3.	Bali	1	5	-	3	-	-	-	-	1	6
4.	Riau	-	6	-	3	1	1	5	1	-	7
5.	Central Java	-	1	-	-	-	-	1	-	-	2
6.	DKI Jakarta	-	1	-	3	-	-	-	-	-	2
Total		1	25	1	10	1	1	12	1	1	24

Previous surveys of *Erysipelothrix rhusiopathiae* in Jakarta and Medan (Takahashi *et al.*, 1989) there were 23 serotype isolated from 245 isolates. It is showed that the distribution of serotype in this investigation area (6 provinces) is smaller than the previous one. Further studies with more isolates are needed to ensure this investigation.

Serotype 1 and 2 are the once most commonly found in cases of septicemia and in tonsils (Hagan and Bruner's, 1989). The present survey showed that serotype 2 was the most prevalent isolate (33.33%).

By using known typing sera representing serotype 1-22 there were 20 isolates (27.7%) could not be determined. In order to know the serotype of this 20 untypeable isolates, further study are needed by testing heat extracted antigen of serotype reference strains with antisera prepared in rabbits againsts the untypeable isolates.

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