The Journal of Veterinary Medical Science

NOTE

Bacteriology

Differences in phenotypic and genetic characteristics of *Trueperella pyogenes* detected in slaughtered cattle and pigs with septicemia

Hideki FUJIMOTO[1)], Natsuki SHIMOJI[1)], Tatsumi SUNAGAWA[1)], Kaoru KUBOZONO[1)], Chikage NAKAJIMA[1)] and Takehisa CHUMA[2)]*

[1)]Shibushi Meat Inspection Center, Kagoshima Prefecture, Anraku 5972-10, Shibushi, Kagoshima 899-7104, Japan
[2)]Laboratory of Veterinary Public Health, Joint Faculty of Veterinary Medicine, Kagoshima University, Korimoto 1-21-24, Kagoshima-shi, Kagoshima 890-0065, Japan

J. Vet. Med. Sci.
82(5): 626–631, 2020
doi: 10.1292/jvms.19-0370

Received: 8 July 2019
Accepted: 27 February 2020
Advanced Epub:
24 March 2020

ABSTRACT. We investigated the hemolytic properties, biochemical properties, and possession of virulence factor genes of *Trueperella pyogenes* isolated from cattle and pigs with septicemia. The porcine strains showed significantly stronger hemolyticity than the bovine strains. In addition, *T. pyogenes* from cattle and pigs also differed in biochemical properties. Virulence factor genes (*nanP*, *cbpA, fimC*, and *fimE*) were more prevalent in bovine strains, whereas other virulence factor genes (*nanH* and *fimG*) were more prevalent in porcine strains. *T. pyogenes* isolated from pig and cattle with septis cases in Japanese meat inspection showed variability in biochemical and genetic properties. Differences were observed between porcine and bovine strain in term of the hemolytic strength and possession of genes for factors promoting adhesions which are considered pathogenic.

KEY WORDS: cattle, pig, septicemia, *Trueperella pyogenes*, virulence factor gene

Trueperella pyogenes is a gram-positive, small, irregular, non-motile, non-spore-forming, β-hemolytic bacterium that grows under aerobic condition [10]. The organism is a common inhabitant of the upper respiratory and urogenital tracts of many domestic animals [9, 10]. *T. pyogenes* expresses several known and putative virulence factors, including pyolysin (*plo*), neuraminidase (*nanH* and *nanP*) and collagen-binding protein (*cbpA*), which may contribute to its pathogenicity [3, 5, 6, 8]. Although this species has been known for a long time, many questions regarding its infection's pathogenesis, as well as reservoirs and routes of transmission, remain unanswered [13]. *T. pyogenes* is frequently detected during slaughter inspection from purulent diseases and has become a major cause of cattle septicemia endocarditis [16]. Additionally, in the pig industry, these diseases can cause significant economic losses related to mortality, respiratory infections, bleeder disposal, and even abandoned carcasses in the slaughterhouse [10]. Although few common bacteria are isolated from cattle and pig with septicemia, *T. pyogenes* is commonly observed in both animals. The differences in the properties of *T. pyogenes* between bovine and porcine strains remain unknown. During post-mortem inspection, septicemia is suspected if verrucous endocarditis is confirmed in the heart and abnormalities in the lungs, kidneys, liver, or spleen. In the event where septicemia is suspected, samples from the heart, liver, kidney, and spleen are tested for bacteria, and septicemia is diagnosed if the same bacteria is detected in multiple organs. To date, many studies of *T. pyogenes* strains isolated from livestock have been reported [1–3, 7, 12, 14, 15, 17], but there are no report on *T. pyogenes* derived from animals with septicemia in slaughterhouses. To our best knowledge, there have been no reports of phenotypic and genetic traits of *T. pyogenes* isolated in Japan. Studies involving virulence factors are important in investigating molecular epidemiology, pathogenicity and hypothetical differences in virulence between *T. pyogenes* strains from different geographic areas [11]. In addition, as far as we know, there is only one report comparing virulence factor genes of a strain derived from different animals [12]. The purpose of this study was to investigate the characteristics of bovine and porcine strains to help control the disease. *T. pyogenes* strains from either cattle or pigs were compared with respect to their known and putative virulence factor genes, such as *plo* which ecodes hemolytic exotoxin pyolysin, Plo, and genes for factors promoting adhesion to host cells, with the latter consisting of neuraminidase genes (*nanH* and *nanP*), collagen-binding protein (*cbpA*), and 4 fimbrial genes (*fimA*, *fimC*, *fimE,* and *fimG*).

T. pyogenes strains were isolated from the heart, liver, kidney, spleen, and verruca of individuals with endocarditis suspected of having septicemia. The strains were collected from 2006 to 2017 from 139 farms (cattle: 88, pigs: 51) located in the

*Correspondence to: Chuma, T.: chuma@vet.kagoshima-u.ac.jp

Kagoshima, Miyazaki, and Kumamoto prefectures of Japan. We used *T. pyogenes* strains from 167 domestic animals. In addition, the macroscopic findings compiled using data from a total of 75 animals (43 bovine, 32 porcine) from April 2012 to March 2017 that were recorded. These specimens were cultured on 5% sheep blood agar plates (Nissui plate sheep blood agar: Nissui Pharmaceutical, Tokyo, Japan) at 37°C for 48 hr in 5% CO_2. The *T. pyogenes* strains were identified based on their hemolytic activity, gram staining, catalase test, oxidase test, and a biochemical identification kit, API Coryne (bioMérieux, Marcy-l'Étoile, France). After identification, *T. pyogenes* isolates were stored at −80°C in Mueller-Hinton broth (BD Biosciences, Franklin Lakes, NJ, USA) containing 20% glycerol until use. *T. pyogenes* ATCC 19411 (pig origin) and *T. pyogenes* ATCC 49698 (unknown origin) were used as reference strains (American Type Culture Collection, Manassas, VA, USA). To compare hemolytic ability by animal species, Nissui plate sheep blood agar and Nissui plate horse blood agar (Nissui Pharmaceutical, Tokyo, Japan) were used. We inoculated each strain into the agar plates using a needle, followed by culturing at 37°C in 5% CO_2 for 48 hr, until hemolysis spots were significantly stabilized. Strains with a hemolysis diameter wider than the average were judged as "strong", and strains with a hemolysis diameter narrower than the mean were judged as "weak". To prepare a PCR template, a frozen solution containing *T. pyogenes* was added dropwise to 1 m*l* of Mueller-Hinton broth and incubated at 37°C for 24–48 hr to obtain bacterial cultures. Genomic DNA was isolated using InstaGene™ Matrix (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. As previously reported, PCR was used to detect the presence of virulence factor genes (*plo, nanH*, *nanP, cbpA, fimA, fimC, fimE*, and *fimG*) [7, 15, 17]. The PCR conditions were the same as the previous reports [7, 15]. The reaction mixtures, in a final volume of 50 *µl*, contained 0.1 mM of each primer, 0.2 mM of each deoxynucleotide triphosphate (Takara, Kusatsu, Japan), 1.5 mM of $MgCl_2$ (Promega, Madison, WI, USA), 10X Ex Taq Buffer, 1.0 U of TaKaRa Ex Taq®DNA Polymerase (Takara), and 1 *µl* of DNA template. PCR was performed in a TaKaRa PCR Thermal Cycler Dice® Gradient. Two reference strains of *T. pyogenes* were used as positive controls for the PCR for *plo, nanH, nanP, fimA, fimC, fimE,* and *fimG* genes. Distilled water was used as a negative control. Amplification products were electrophoresed through a 1.5% (w/v) agarose gel stained with 0.5 mg/m*l* ethidium bromide and visualized with a 2UV High Performance Transilluminator (Analytik Jena, Jena, Germany).

The statistical significance of the results was established using Fisher's exact test, and the levels of significance were set at $P<0.05$ and $P<0.01$.

All 167 strains were identified as *T. pyogenes* by API Coryne. The results of biochemical property analysis using an API Coryne with strains derived from bovine and porcine are shown in Table 1. The positive rates of pyrrolidonyl arylamidase, alkaline phosphatase reaction, fermentation of sucrose, and fermentation of glycogen of porcine strains were significantly higher than those of bovine strains. This suggests that biochemical differences exist between bovine and porcine-derived *T. pyogenes*. In addition, forty different Api-codes were obtained from the strains indicating that the biochemical properties of *T. pyogenes* were variable. From these cases, the following symptoms were observed. Macroscopic findings were verrucous endocarditis (56/75: 74.7%), pneumonia (35/75: 46.7%), hepatitis (27/75: 36.0%), splenomegaly (16/75: 21.3%), kidney bleeding (36/75: 48.0%), abscess (31/75: 41.3%) and enteritis (11/75: 14.7%).

Table 1. Biochemical properties of *Trueperella pyogenes* isolated from bovine and porcine

	% (n) of isolates from		Reference strain	
	Bovine (n=100)	Porcine (n=67)	ATCC 19411	ATCC 49698
Nitrate reduction	0.0 (0)	0.0 (0)	-	-
Pyrazinamidase	0.0 (0)	0.0 (0)	-	-
Pyrrolidonyl arylamidase[a)]	75.0 (75)	94.0 (63)	+	+
Alkaline phosphatase[b)]	62.0 (62)	79.1 (53)	+	+
β-Glucuronidase	99.0 (99)	100.0 (67)	+	+
β -Galactosidase	92.0 (92)	94.0 (63)	+	+
α-Glucosidase	95.0 (95)	98.5 (66)	+	+
N-Acetyl-β-glucosaminidase	88.0 (88)	89.6 (60)	+	+
Esculin hydrolysis	3.0 (3)	1.5 (1)	-	-
Urease	0.0 (0)	0.0 (0)	-	-
Gelatin hydrolysis	99.0 (99)	100.0 (67)	+	+
Fermentation of:				
Glucose	100.0 (100)	100.0 (67)	+	+
Ribose	100.0 (100)	100.0 (67)	+	+
Xylose	100.0 (100)	100.0 (67)	+	+
Mannitol	1.0 (1)	0.0 (0)	-	-
Maltose	99.0 (99)	98.5 (66)	+	+
Lactose	96.0 (96)	98.5 (66)	+	+
Sucrose[a)]	43.0 (43)	67.2 (45)	-	-
Glycogen[a)]	15.0 (15)	38.8 (26)	-	-

a) $P<0.01$, b) $P<0.05$, significantly different between bovine and porcine strains by Fisher's exact test.

The hemolytic activity was averaged by measuring hemolysis rings on horse and sheep blood agar. The measured average values were 3.49 ± 0.94 mm on 5% horse blood agar and 2.46 ± 1.22 mm on 5% sheep blood agar (Fig. 1). Examination of hemolytic properties on 5% horse blood agar plates showed that 61 of 67 (91.0%) porcine strains had strong hemolytic activity, whereas 25 of 100 (25.0%) bovine strains had strong hemolytic activity. At the same time, examination of hemolytic properties on 5% sheep blood agar plates showed that 64 of 67 (95.5%) porcine strains had strong hemolytic activity, whereas only 7 of 100 (7.0%) bovine strains had strong hemolytic activity (Table 2). The number of strains showing strong hemolytic activity was significantly greater in porcine than in bovine. *T. pyogenes* strains with strong hemolytic activity on sheep and horse blood agar plates may have been present in the environment around the pigs. This result suggests that the biological environment of the host may have an effect on hemolysis, even with the same bacterial species.

Subsequently, we searched for the presence of eight virulence genes in 167 *T. pyogenes* strains (100 strains from bovine and 67 from porcine) by PCR. All strains possessed the *plo* gene. Of the 167 investigated strains, 163 (97.6%) were positive for *fimA*, 150 (89.8%) were positive for *fimE*, 95 (56.0%) were positive for *fimC*, and 39 (23.4%) were positive for *fimG*. *nanH*, *nanP*, and *cbpA* were present in 119 (71.3%), 90 (53.9%), and 12 (7.2%) strains, respectively. Comparison of each virulence gene in bovine or porcine strains revealed that *fimA* was present in 98.0% and 97.0%, *fimE* in 95.0% and 82.1%, *fimC* in 80.0% and 22.4%, *fimG* in 11.0% and 41.8%, *nanH* in 56.0% and 94.0%, *nanP* in 62.0% and 41.8%, and *cpbA* in 11.0% and 1.5% of bovine or porcine strains, respectively (Table 3). *nanP*, *cbpA*, *fimC*, and *fimE* were significantly present in more bovine strains than in porcine strains. In contrast, *nanH* and *fimG* were significantly present in more porcine strains than in bovine strains. We detected 8 virulence factor genes in the *T. pyogenes* strains tested. The positive rates varied with each pathogenic gene. The positive rate differed between bovine and porcine strains. *T. pyogenes* virulence is determined by the cytolytic activity of PLO and presence of factors associated with adhesion to host cells and tissue colonization [12]. In the present study, the expression of the genes was not confirmed, necessitating future confirmation studies. We confirmed that all *T. pyogenes* strains harbored *plo*, but we did not clearly determine explicitly which of the known putative virulence factors play a crucial role in the pathogenesis of the disease caused by the bacteria. *T. pyogenes* belongs to a narrow group of gram-positive bacteria that produce fimbriae [17]. All previously reported strains possess *fimA* [2, 12, 15, 17]. In this study, this gene was also detected in as high as 97.6% of the strains isolated from septicemia cases in both cattle and pigs. Therefore, this gene appear to be closely related to pathogenicity of septicemia, although we did not investigate its expression. *fimE* was previously detected in the uterus of cows with clinical mastitis (98%) [13]. The positive rate of this gene was high in both bovine (95.0%) and porcine (82.1%) strains isolated from septicemia cases. The bovine strains showed a significantly higher positive rate than porcine strains. Since the *fimE* detection rate for cattle vary from literature to literature [2, 4, 11, 15, 17], it seems likely to be region-specific. In previous studies, *fimC* was detected more frequently in isolates with bovine mastitis origin (82% and 88%) than in those with bovine metritis origin (69.2% and 67%) [1, 2, 10, 15, 17]. Rzewuska *et al.* reported that 80.6% of bovine strains and 65.4% of porcine strains harbored the *fimC* gene [12]. In the present study, only 56.9% of the strains from septicemia cases contained *fimC*, with a rate of 80.0% in bovine strains and 22.4% in porcine strains, showing a clear difference. The prevalence of *fimC* gene in bovine strains was almost the same as that in previous reports [1, 12, 17]. This gene may not be closely related to adhesion of pig vascular endothelial cells. In contrast, *fimG* was detected in 11.0% of bovine strains and 41.8% of porcine strains. The detection rate of *fimG* varies widely among past studies [1, 2, 4, 11, 12, 14, 15, 17]. Therefore, it was not possible to find any particular characteristics. Rzewuska *et al.*, reported that 58.1% of bovine strains and 88.5% of porcine strains possessed the *fimG* gene [12]. The same was observed in this study where the number of porcine strains harboring this gene was higher than that of bovine strains. This gene is thought to be involved in adhesion to vascular endothelial cells of pigs rather than cattle. Prevalence of *fimC* in bovine strains was higher than that in porcine strains; conversely, the prevalence of *fimG* in porcine strains was higher than that in bovine strains. The high prevalence of these four fimbrial genes

Tp-20200312

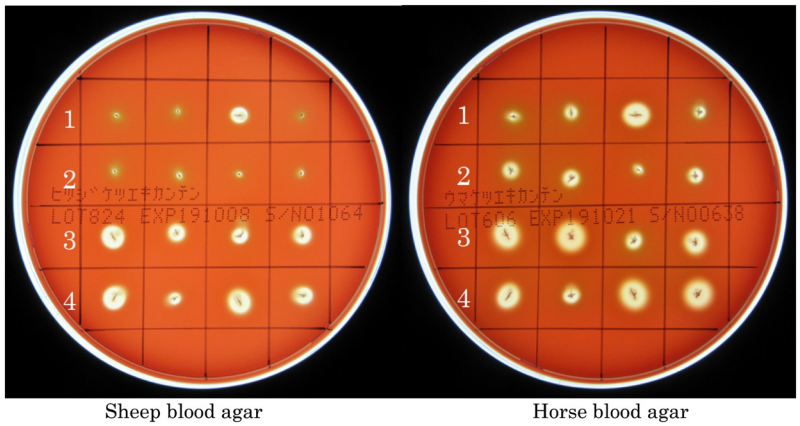

Fig. 1. Hemolytic activity on 5% sheep blood agar plate (36°C, 48 hr culture), lines 1 and 2 are bovine strains and lines 3 and 4 are porcine strains (on both plates).

Table 2. Number of isolates that showed "strong" hemolytic activity

Origin of the strain	Agar plate used	
	Sheep blood	Horse blood
Bovine (n=100)	7 (7.0%)*	25 (25.0%)#
Porcine (n=67)	64 (95.5%)*	61 (91.0%)#

Significant difference between the same symbols ($P<0.01$).

indicates that they are necessary for cattle and pig colonization and play important roles as virulence factor genes.

T. pyogenes strains can produce neuraminidases H and P, encoded by the *nanH* and *nanP* genes, respectively [6, 8, 13]. We detected the *nanH* and *nanP* genes, which are also involved in the adhesion to host epithelial cells in *T. pyogenes*. Previous studies showed that all investigated *T. pyogenes* isolates recovered from different types of infections were positive for *nanH* activity, and that 64.2% of the isolates harbored *nanP* [6, 8]. Both *nanH* and *nanP* genes were detected in all isolates recovered from the uterus of dairy cows [15]. In this study, among the bovine and porcine strains isolated from septicemia cases, 71.3% and 53.9% possessed *nanH* and *nanP*, respectively. *nanH* was detected significantly more frequently in porcine (94.0%) than in bovine (56.0%) strains. *nanP* was detected significantly more often in bovine (62.0%) than in porcine (41.8%) in contrast to Rzewska's report [12]. This indicates that there was a difference in the positive rate of neuraminidase genes depending on the livestock species. We also thought that the possession of the *nanH* and *nanP* genes would vary depending on the strain's origin and disease. In our result, *nanH* was detected at a higher rate in porcine strains than in bovine strains in contrast to the previous report [12]. A previous report on abscesses [8] and the result from our septicemia cases showed a similar trend with higher *nanP* detection rate in bovine strains than in porcine strains. Although, the prevalence of the *nanP* and *nanH* genes between animal species shows various according to the above cited references, our result indicate that *nanH* gene was more abundant in pigs and *nanP* gene was more common in cattle.

Additionally, *cbpA*, which encodes a collagen binding protein, was detected only in 7.2% (12/167) of isolates from clinical septicemia cases. This protein is involved in the adherence of bacteria to epithelial and fibroblast cell lines [3]. It may be supposed that *T. pyogenes* strains that produce CbpA have a higher potential to colonize collagen-rich tissues [9], although the *cbpA* gene was found in many isolates of various origins, with different frequencies (1.4–100%) [1–4, 11, 12, 14, 15, 17]. The adherence of *T. pyogenes* to host tissues may also be determined by the ability to bind collagen, fibrinogen, and fibronectin [3, 7]. However, our results confirmed the low frequency of *cbpA* in bovine and porcine *T. pyogenes* isolates from septicemia cases (Table 3). CbpA, a protein attaching factor, did not appear to be involved in the pathogenesis of *T. pyogenes* induced septicemia. We compared the prevalence of virulence factor genes in *T. pyogenes* strains by origin (Table 4). There were no significant difference in the prevalence of the virulence factor genes among the organs of origin. In addition, the prevalence of virulence factor genes in *T. pyogenes* strains was compared for each macroscopic finding (Table 5). There was no significant difference in the prevalence of virulence factor genes in *T. pyogenes* strains, even by macroscopic findings. Therefore, we could not find any association between

Table 3. Prevalence of virulence factor genes in the *Trueperella pyogenes* isolated from bovine and porcine

Virulence factor genes	% (n) of isolates from			Reference strains	
	Bovine (n=100)	Porcine (n=67)	Total (n=167)	ATCC 19411	ATCC 49698
plo	100.0 (100)	100.0 (67)	100.0 (167)	+	+
nanH a)	56.0 (56)	94.0 (63)	71.3 (119)	+	+
nanP b)	62.0 (62)	41.8 (28)	53.9 (90)	+	+
cbpA b)	11.0 (11)	1.5 (1)	7.2 (12)	-	-
fimA	98.0 (98)	97.0 (65)	97.6 (163)	+	+
fimC a)	80.0 (80)	22.4 (15)	56.9 (95)	+	+
fimE	95.0 (95)b)	82.1 (55)	89.8 (150)	+	+
fimG a)	11.0 (11)	41.8 (28)	23.4 (39)	+	+

a) $P<0.01$, b) $P<0.05$, significantly different between bovine and porcine strains by Fisher's exact test.

Table 4. Prevalence of virulence factor genes in the *Trueperella pyogenes* isolated from each organ

Virulence factor genes	% (n) of isolates from			
	Heart (n=113)	Liver (n=27)	Spleen (n=10)	Kidney (n=17)
plo	100.0 (113)	100.0 (27)	100.0 (10)	100.0 (17)
nanH	70.8 (80)	74.1 (20)	80.0 (8)	64.7 (11)
nanP	54.0 (61)	44.4 (12)	50.0 (5)	70.6 (12)
cbpA	6.2 (7)	7.4 (2)	10.0 (1)	11.8 (2)
fimA	99.1 (112)	92.6 (25)	100.0 (10)	94.1 (16)
fimC	54.9 (62)	63.0 (17)	70.0 (7)	52.9 (9)
fimE	88.5 (100)	92.6 (25)	80.0 (8)	100.0 (17)
fimG	23.9 (27)	22.2 (6)	20.0 (2)	23.5 (4)

Not significant difference in all items.

Table 5. Distribution of selected virulence factor genes in the *Trueperella pyogenes* strains from various types of macroscopic findings

Virulence factor genes	% (n) of macroscopic finding from						
	Endocarditis (n=56)	Pneumonia (n=35)	Hepatitis (n=27)	Splenomegaly (n=16)	Kidney bleeding (n=36)	Abscess (n=31)	Enteritis (n=11)
plo	100.0 (56)	100.0 (35)	100.0 (27)	100.0 (16)	100.0 (36)	100.0 (31)	100.0 (11)
nanH	71.4 (40)	88.6 (31)	63.0 (17)	75.0 (12)	80.6 (29)	71.0 (22)	72.7 (8)
nanP	46.4 (26)	48.5 (17)	55.6 (15)	31.3 (5)	52.8 (19)	61.3 (19)	45.5 (5)
cbpA	1.8 (1)	0.0 (0)	3.7 (1)	0.0 (0)	2.8 (1)	0.0 (0)	9.1 (1)
fimA	100.0 (56)	97.1 (34)	92.6 (25)	93.8 (15)	97.2 (35)	96.8 (30)	100.0 (11)
fimC	50.0 (28)	40.0 (14)	63.0 (17)	31.3 (5)	55.6 (20)	48.4 (15)	36.4 (4)
fimE	89.3 (50)	88.6 (31)	92.6 (25)	93.8 (15)	91.6 (33)	96.8 (30)	90.9 (10)
fimG	32.1 (18)	28.6 (10)	14.8 (4)	37.5 (6)	25.0 (9)	32.3 (10)	27.3 (3)

Not significant difference in all items.

the organ of the strain or macroscopic findings and the virulence factor genes.

Twenty-nine gene patterns were detected among the bovine and porcine strains (Table 6). The pattern, *plo/nanH/nanP/fimA/fimC/fimE*, was the most frequent (15.0%), followed by *plo/nanH/fimA/fimE/fimG* (13.2%), and *plo/nanP/fimA/fimC/fimE* (10.2%). Twenty-four gene patterns were detected in bovine strains, with *plo/nanH/nanP/fimA/fimC/fimE* being the most frequent (20.0%), followed by *plo/nanP/fimA/fimC/fimE* (15.0%) and *plo/fimA/fimC/fimE* (15.0%). These trends agreed with previously recorded trends in dairy cows with clinical mastitis [17]. On the other hand, 16 gene patterns were detected in porcine strains, with *plo/nanH/fimA/fimE/fimG* being the most frequent (32.8%), followed by *plo/nanH/fimA/fimE* (13.4%) and *plo/nanH/nanP/fimA* (11.9%). There have been few reports showing gene patterns in porcine strains [11], and comparison with past cases was difficult. From this result, porcine strains showed very different gene patterns from bovine strains. The gene pattern detected from the bovine septicemia-derived strain was similar to that of the strain detected from bovine mastitis [17], and similar to that of the bovine uteritis-derived strain except for the *cbpA* gene [2]. However, the frequently detected patterns differed between the bovine and porcine strains. Thus, low genetic similarity was observed between the strains from cattle and pigs. The main difference was that 20% of bovine strains did not have *nanH* and *nanP* genes either, but all strains of porcine strains possessed both *nanH* and/or *nanP*. This suggests that gene pattern differences exist between bovine and porcine-derived *T. pyogenes*.

We found that the positive rates of pyrrolidonyl arylamidase, alkaline phosphatase reaction, and fermentation of sucrose and glycogen in porcine strains were higher than those in bovine strains. In septicemia, the same bacteria spread throughout the body, and symptoms appear in each organ. The causes of septicemia vary and thus it is difficult to identify the specific cause. Moreover, diversity was observed in the biochemical properties of *T. pyogenes*. Also, the patterns of possession of virulence factor gene were diverse. We confirmed that a high percentage of septicemia-derived strains harboring the *nanH* or *nanP* gene, and that all strains harbor either of the *fim* genes. In this study, there was no clear conclusion as to whether a virulence factor gene involved in epithelial cell adhesion is essential in sepsis-divided strains. Further, there was a difference in the hemolytic properties on sheep and horse blood agar, some biochemical properties, and virulence gene statuses between bovine and porcine strains. It was confirmed that *T. pyogenes* detected in cases of septicemia has different characteristics between bovine and porcine strains. This study could help control *T. pyogenes* infection in cattle and pigs.

Table 6. Genotypes of *Trueperella pyogenes* isolated from bovine and porcine (167strains)

Genotype								% (n) of isolates from		
plo	*nanH*	*nanP*	*cbpA*	*fimA*	*fimC*	*fimE*	*fimG*	Bovine (n=100)	Porcine (n=67)	Total (n=167)
+	+	+	+	+	+	+	-	1.0 (1)	1.5 (1)	1.2 (2)
+	+	+	+	+	-	+	+	1.0 (1)	ND	0.6 (1)
+	+	+	-	+	+	+	+	2.0 (2)	ND	1.2 (2)
+	+	+	-	+	+	+	-	20.0 (20)	7.5 (5)	15.0 (25)
+	+	+	-	+	-	+	+	1.0 (1)	3.0 (2)	1.8 (3)
+	+	+	-	+	+	-	-	4.0 (4)	1.5 (1)	3.0 (5)
+	+	+	-	+	-	+	-	7.0 (7)	10.4 (7)	8.4 (14)
+	+	+	-	-	+	+	-	1.0 (1)	ND	0.6 (1)
+	+	+	-	+	-	-	-	1.0 (1)	11.9 (8)	5.4 (9)
+	+	-	+	+	-	+	-	3.0 (3)	ND	1.8 (3)
+	+	-	-	+	+	+	+	1.0 (1)	3.0 (2)	1.8 (3)
+	+	-	-	+	+	+	-	11.0 (11)	4.5 (3)	8.4 (14)
+	+	-	-	+	-	+	+	ND	32.8 (22)	13.2 (22)
+	+	-	-	+	-	+	-	2.0 (2)	13.4 (9)	6.6 (11)
+	+	-	-	-	+	+	-	1.0 (1)	ND	0.6 (1)
+	+	-	-	+	-	-	+	ND	1.5 (1)	0.6 (1)
+	+	-	-	-	-	+	+	ND	1.5 (1)	0.6 (1)
+	+	-	-	+	-	-	-	ND	1.5 (1)	0.6 (1)
+	-	+	+	+	+	+	-	3.0 (3)	ND	1.8 (3)
+	-	+	-	+	+	+	+	2.0 (2)	ND	1.2 (2)
+	-	+	-	+	+	+	-	15.0 (15)	3.0 (2)	10.2 (17)
+	-	+	-	+	-	+	+	1.0 (1)	ND	0.6 (1)
+	-	+	-	+	-	+	-	3.0 (3)	1.5 (1)	2.4 (4)
+	-	+	-	-	+	-	-	ND	1.5 (1)	0.6 (1)
+	-	-	+	+	+	+	+	1.0 (1)	ND	0.6 (1)
+	-	-	+	+	+	+	-	2.0 (2)	ND	1.2 (2)
+	-	-	-	+	+	+	+	2.0 (2)	ND	1.2 (2)
+	-	-	-	+	+	+	-	14.0 (14)	ND	8.4 (14)
+	-	-	-	+	-	+	-	1.0 (1)	ND	0.6 (1)

ND: Not detected.

REFERENCES

1. Alkasir, R., Wang, J., Gao, J., Ali, T., Zhang, L., Szenci, O., Bajcsy, A. C. and Han, B. 2016. Properties and antimicrobial susceptibility of *Trueperella pyogenes* isolated from bovine mastitis in China. *Acta Vet. Hung.* **64**: 1–12. [Medline] [CrossRef]
2. Ashrafi Tamai, I., Mohammadzadeh, A., Zahraei Salehi, T. and Mahmoodi, P. 2018. Genomic characterisation, detection of genes encoding virulence factors and evaluation of antibiotic resistance of *Trueperella pyogenes* isolated from cattle with clinical metritis. *Antonie van Leeuwenhoek* **111**: 2441–2453. [Medline] [CrossRef]
3. Esmay, P. A., Billington, S. J., Link, M. A., Songer, J. G. and Jost, B. H. 2003. The *Arcanobacterium pyogenes* collagen-binding protein, CbpA, promotes adhesion to host cells. *Infect. Immun.* **71**: 4368–4374. [Medline] [CrossRef]
4. Hadimli, H. H. and Kav, K. 2011. The molecular characterization of *Arcanobacterium pyogenes* strains isolated from samples of sheep and cattle. *Kafkas Univ. Vet. Fak. Derg.* **17**: 893–899.
5. Jost, B. H., Songer, J. G. and Billington, S. J. 1999. An *Arcanobacterium (Actinomyces) pyogenes* mutant deficient in production of the pore-forming cytolysin pyolysin has reduced virulence. *Infect. Immun.* **67**: 1723–1728. [Medline] [CrossRef]
6. Jost, B. H., Songer, J. G. and Billington, S. J. 2001. Cloning, expression, and characterization of a neuraminidase gene from *Arcanobacterium pyogenes*. *Infect. Immun.* **69**: 4430–4437. [Medline] [CrossRef]
7. Jost, B. H., Post, K. W., Songer, J. G. and Billington, S. J. 2002. Isolation of *Arcanobacterium pyogenes* from the porcine gastric mucosa. *Vet. Res. Commun.* **26**: 419–425. [Medline] [CrossRef]
8. Jost, B. H., Post, K. W., Songer, J. G. and Billington, S. J. 2002. Identification of a second *Arcanobacterium pyogenes* neuraminidase and involvement of neuraminidase activity in host cell adhesion. *Infect. Immun.* **70**: 1106–1112. [Medline] [CrossRef]
9. Jost, B. H. and Billington, S. J. 2005. *Arcanobacterium pyogenes*: molecular pathogenesis of an animal opportunist. *Antonie van Leeuwenhoek* **88**: 87–102. [Medline] [CrossRef]
10. Moreno, L. Z., Matajira, C. E. C., da Costa, B. L. P., Ferreira, T. S. P., Silva, G. F. R., Dutra, M. C., Gomes, V. T. M., Silva, A. P. S., Christ, A. P. G., Sato, M. I. Z. and Moreno, A. M. 2017. Characterization of porcine *Trueperella pyogenes* by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), molecular typing and antimicrobial susceptibility profiling in Sao Paulo State. *Comp. Immunol. Microbiol. Infect. Dis.* **51**: 49–53. [Medline] [CrossRef]
11. Risseti, R. M., Zastempowska, E., Twarużek, M., Lassa, H., Pantoja, J. C. F., de Vargas, A. P. C., Guerra, S. T., Bolaños, C. A. D., de Paula, C. L., Alves, A. C., Colhado, B. S., Portilho, F. V. R., Tasca, C., Lara, G. H. B. and Ribeiro, M. G. 2017. Virulence markers associated with *Trueperella pyogenes* infections in livestock and companion animals. *Lett. Appl. Microbiol.* **65**: 125–132. [Medline] [CrossRef]
12. Rzewuska, M., Czopowicz, M., Gawryś, M., Markowska-Daniel, I. and Bielecki, W. 2016. Relationships between antimicrobial resistance, distribution of virulence factor genes and the origin of *Trueperella pyogenes* isolated from domestic animals and European bison (*Bison bonasus*). *Microb. Pathog.* **96**: 35–41. [Medline] [CrossRef]
13. Rzewuska, M., Kwiecień, E., Chrobak-Chmiel, D., Kizerwetter-Świda, M., Stefańska, I. and Gieryńska, M. 2019. Pathogenicity and virulence of *Trueperella pyogenes*. *Int. J. Mol. Sci.* **20**: 2737–2769. [Medline] [CrossRef]
14. Santos, T. M. A., Caixeta, L. S., Machado, V. S., Rauf, A. K., Gilbert, R. O. and Bicalho, R. C. 2010. Antimicrobial resistance and presence of virulence factor genes in *Arcanobacterium pyogenes* isolated from the uterus of postpartum dairy cows. *Vet. Microbiol.* **145**: 84–89. [Medline] [CrossRef]
15. Silva, E., Gaivão, M., Leitão, S., Jost, B. H., Carneiro, C., Vilela, C. L., Lopes da Costa, L. and Mateus, L. 2008. Genomic characterization of *Arcanobacterium pyogenes* isolates recovered from the uterus of dairy cows with normal puerperium or clinical metritis. *Vet. Microbiol.* **132**: 111–118. [Medline] [CrossRef]
16. Une, Y. 2010. Endocarditis, Animal Pathology. 2nd ed., pp. 11–13, Japan Veterinary Pathology Society, Buneido Publishing, Tokyo (in Japanese).
17. Zastempowska, E. and Lassa, H. 2012. Genotypic characterization and evaluation of an antibiotic resistance of *Trueperella pyogenes* (*Arcanobacterium pyogenes*) isolated from milk of dairy cows with clinical mastitis. *Vet. Microbiol.* **161**: 153–158. [Medline] [CrossRef]