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Full Length Research Paper

Prevalence of bacterial infection responsible for bovine mastitis

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Mastitis continues to be the most economically important disease of dairy cattle, and current data on mastitis is even less readily available. To investigate into the prevalence of the pathogens responsible for bovine mastitis, 100 raw milk samples were obtained from the dairy cows with clinical or sub-clinical bovine mastitis in 5 farms in Jiangsu Province, China. All the samples were submitted to bacterial isolation and identification by morphologic examination and biotyping. The data revealed that *Escherichia coli* was the commonest organism in mastitis cases, being implicated in 82% cases, and *Streptococcus uberis* (53%) continues to be a prevalent pathogen closely followed by *Staphylococcus aureus* (41%), *Streptococcus dysgalactiae* (29%) and *Streptococcus agalactiae* (27%). In addition, *Str. uberis* and *S. aureus* were more frequently associated with clinical mastitis than sub-clinical case, while the infection rates of other bacteria were similar. Further more, *Staphylococcus epidermids* (15%) and *Staphylococcus saprophyticus* (10%), previously considered as naught pathogenic bacteria, were also detected in the diseased mammary gland of the problem cows.

Key words: Mastitis, bovine, bacteria, infection, prevalence.

INTRODUCTION

Bovine mastitis, defined as “inflammation of the mammary gland”, is the most economically important disease in dairy milk production worldwide (Bradley, 2002; Gruet et al., 2001; Viguier et al., 2009). This disease can have an infectious or noninfectious etiology, and the infectious pathogeny is the most important ones that frequently due to infection by one and/or the other pathogens, such as bacteria, viruses, mycoplasma, yeasts and algae (Chaneton et al., 2008; Malinowski et al., 2006; Osumi et al., 2008; Watts, 1988; Wellenberg et al., 2002). Fortunately the vast majority of mastitis is of bacterial origin and just a few of species of bacteria account for most cases, such as *E. coli*, *S. aureus*, *Str. uberis*, *Str. dysgalactiae* and *Str. agalactiae* (Aarestrup et al., 1995; Annemüller et al., 1999; Aouay et al., 2008; from the previous study on mastitis etiology revealed that Chaneton et al., 2008; Dogan et al., 2006; Kuang et al., 2009; Leigh, 1999; Varella Coelho et al., 2007). The data Enterobacteriacae were the commonest cause responsible for 40.9% of all mastitis, and *S. aureus*, *Str*. *dysgalactiae*, *Str*. *agalactiae* accounted for only 10% of clinical cases (Bradleyl, 2002). But there is a dearth of recent information on the incidence and aetiology of mastitis in China, although the impact of the implementation of mastitis control strategies has been optimistically controlled. To give an insight into the current profile of mastitis organisms and a true reflection of the problem herds and cows situation, we need to focus our efforts on investigating into the prevalence of the pathogens that are more frequently associated with clinical and sub-clinical infections.

The aim of this research was to characterize the bacteria isolates from bovine mastitis from China by means of morphologic examining and biotyping, and to investigate into the possible infection types within

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problem herds.

MATERIALS AND METHODS

Collection of diseased sample

From April to May, 2009, a total of 100 raw milk samples were obtained from the dairy cows with clinical or sub-clinical bovine mastitis in 5 farms in the Jiangsu Province, China. Prior to sampling, teat ends were swabbed with 70% ethylalcohol. The initial milk stripped from each udder (one udder each cow) was discarded and the next 10 ml were collected in a sterile container. Separate samples were chilled to 4°C and transported to the laboratory (Boynukara et al., 2008; Ericsson Unnerstad et al., 2009; White et al., 1999). The more detailed information was illustrated in Table 1.

Bacterial pre-culturing

Transfer each raw milk (500 l) into separate tubes containing 5 ml of nutrient broth (10 g tryptone, 5 g beef extract, 10 g NaCl, 0.5 g K_2HPO_3, H_2O to 1 liter, pH 7.4) with 10% heat - inactivated fetal calves serum (FCS) (Hangzhou Sijiqing Biological Engineering Materials Co., Ltd. China) and grow the liquid cultures with vigorous agitation at 37°C for 18 h.

Isolation and identification of E. coli

From each pre - cultured sample, a loopfull of bacteria suspension was streaked on Mackonkey agar plate (Shanghai China Academy of Sciences Shanghai Hexapod Technology Development Co., Ltd.) and then incubated at 37°C for 24 h under aerobic conditions. Ten red colored colonies were randomly selected and transferred to individual plate of nutrient agar (1000 ml nutrient broth, 20 g agar) to make pure culture of bacteria isolates. After 24 h incubation under the same condition, each presumptive bacteria isolate was characterized on the basis of gram staining and conventional biochemical tests, including indole test, methyl red test, Voges - Prokauer test, citrate test and sugar (glucose, lactose, maltose, mannose and sucrose) fermentation test (Cao, 1991).

Isolation and identification of Staphylococcus

From each pre - cultured sample, a loopfull of bacteria suspension was streaked on mannitol salt agar (MSA) (10 g tryptone, 1 g beef extract, 75 g NaCl, 10 g mannitol, 20 g agar, 6 ml 0.4% phenol red solution, H_2O to 1 litre, pH 7.4) that was widely used to cultivate *Staphylococcus* from clinical specimens and then incubated at 37°C for 48 h under aerobic conditions (Boynukara et al., 2008; Cenci-Goga et al., 2003). Ten presumptive colonies were randomly selected and transferred to individual plate of nutrient agar to make pure culture of bacteria isolates. Following incubation for 24 h under the same condition, a single colony of bacteria was streaked on MSA plate. Yellow colored colonies were mannitol - positive and suspected as *S. aureus* or *S. saprophyticus*, while red colored colonies were mannitol - negative and suspected as *S. epidermids* or *S. saprophyticus* (Boynukara et al., 2008). Subsequently, gram staining, pigment producing, maltose fermentation test, alkaline phosphatase test, catalase test, polymyxin B susceptibility test, coagulase test using fresh rabbit plasma (tube method) and DNase test (determine DNase production and activity) were used for the presumptive identification of all isolates (Gundogan et al., 2006; Monsen et al., 1998).

Isolation and identification of *Streptococcus*

From each pre - cultured sample, a loopfull of bacteria suspension was streaked on Colistin - oxolinic acid blood agar (COBA) plate (Guangzhou Huikang Biotech Co., Ltd. China) and then incubated at 37°C for 48 h under aerobic conditions. Ten pinpoint and dewdrop - like colonies were randomly selected and transferred to individual plate of nutrient agar with 10% heat - inactivated FCS to make pure culture of bacteria isolates. Following incubation for 24 h under the same condition, each presumptive bacteria isolate was characterized by gram staining and conventional biochemical tests, including catalase assay, esculin hydrolysis test, sodium hippurate hydrolysis test, sugar (lactose, synanthrin, mannitol and sorbitol) fermentation test (Cao, 1991; Ericsson et al., 2009).

RESULTS

Prevalence of bacterial infection as causes of bovine mastitis in five dairy herds

The presumptive bacteria, including 10 *E. coli*, 10 *Staphylococcus* and 10 *Streptococcus* isolates, were randomly selected from each raw milk sample, and characterized by gram staining and conventional biochemical tests. The data was figured in Table 2. It revealed that *E. coli* was the commonest organism in mastitis cases, being implicated in 82% cases, and 11% cases were only infected with *E. coli*. *Str. uberis* (53%) continues to be a prevalent pathogen closely followed by *S. aureus* (41%), *Str. dysgalactiae* (29%) and *Str. agalactiae* (27%). Fortunately, *S. epidermids* (15%) and *S. saprophyticus* (10%), previously considered as environmental organisms live in the environment and contaminate the teats, were also detected in the problem cows.

In addition, multiple microbial infections (82%) were more prevalent in bovine mastitis in five dairy herds. *Staphylococcus* and *Streptococcus*, considered being the major contagious pathogens of bovine mastitis, frequently combined and mixed infection with *E. coli*. No single case infected with *S. epidermids* or *S. saprophyticus* was found. Further more, there are 7% cases were no *E. coli*, *Staphylococcus* and *Streptococcus* was identified, which be may caused by other pathogens, such as viruses, mycoplasma, yeasts and algae.

Different bacterial infection between clinical and sub - clinical mastitis

In summary, all the seven species of bacteria (*E. coli*, *Str. uberis*, *S. aureus*, *Str. dysgalactiae*; *Str. agalactiae*, *S. epidermids* and *S. saprophyticus*) could be identified both in clinical and sub - clinical bovine mastitis (Table 3). At the same time, *Str. uberis* and *S. aureus* were more frequently found in clinical mastitis than sub-clinical case, while the infection rates of *E. coli*, *Str. dysgalactiae*; *Str. agalactiae*, *S. epidermids* and *S. saprophyticus* were similar in statistics between clinical and sub - clinical

Table 1. The detailed information of the five dairy cow farms.

Fram	No. of dairy cows	Incidence of clinical mastitis (%)	Incidence of sub-clinical mastitis (%)	No. of raw milk samples
A	240	10.0	32.5	20 (7^{a}+13^{b})
B	200	10.0	28.0	15 (5^{a}+10^{b})
C	200	12.0	30.5	15 (6^{a}+ 9^{b})
D	400	5.8	20.0	20 (8^{a}+12^{b})
E	700	5.0	22.3	30 (13^{a}+17^{b})

a. Raw milk samples from clinical mastitis; b. raw milk samples from sub-clinical mastitis.

Table 2. The infection type of bovine mastitis in the 5 dairy cow farms.

Farm	Infection type	*E. coli*	*Str. uberis*	*S. aureus*	*Str. dysgalactiae*	*Str. agalactiae*	*S. epidermids*	*S. saprophyticus*	Number
A		+							5
		+	+			+			2
		+	+						2
		+		+					2
		+		+	+	+			1
		+	+		+		+	+	1
		+	+						1
		+			+				1
				+	+				1
			+		+		+	+	1
			+			+			1
									2*
B		+							3
		+	+		+				2
		+	+	+	+		+		2
		+		+					1
		+	+	+		+			1
		+		+		+		+	1
		+	+			+			1
				+					1
			+	+					1
			+						1
									1*
C		+	+	+					3
		+		+		+			2
		+				+			2
		+	+	+		+			1
		+		+					1
		+	+			+			1
		+	+						1
		+				+		+	1
		+			+				1
				+		+			1
									1*

Table 2. Contd.

	+	+		+				6
	+	+	+					3
	+							2
	+		+	+				1
	+	+	+		+		+	1
	+		+					1
D	+		+	+	+			1
	+	+		+			+	1
	+	+				+	+	1
	+			+	+		+	1
			+		+			1
								1*
	+							3
	+	+	+					3
	+		+	+		+		2
	+		+					2
	+	+			+			2
	+	+				+		2
	+	+	+					1
	+	+	+	+	+			1
	+	+	+	+				1
	+	+	+		+	+		1
E	+	+	+		+			1
	+		+		+	+	+	1
	+	+		+	+	+		1
	+	+		+		+		1
	+	+		+		+	+	1
	+	+						1
	+			+		+		1
		+	+	+				1
		+		+	+			1
		+						1
								2*
Total	82	53	41	29	27	15	10	100

*, No *E. coli*, *Staphylococcus* and *Streptococcus* were identified.

bovine mastitis (Figure 1).

DISCUSSION

Mastitis is the most important disease in dairy milk production worldwide (Kossaibati et al., 1997), and it is notoriously difficult to estimate the losses associated with clinical and sub - clinical mastitis, which arise from the costs of treatment, culling, death and decreased milk production and constituent quality (Bradley, 2002).

Classically, mastitis pathogens have been classified as either “contagious” or “environmental” (Bradley, 2002). In essence, the contagious pathogens can be considered as organisms adapted to survive within the mammary gland, and can establish infections to trigger inflammatory response, which are typically manifest as an elevation in the somatic cell count of milk from the affected quarter (Bradley, 2002). In contrast, the environmental pathogens are best described as opportunistic invaders of the mammary gland, not adapted to survival within the host; typically they “invade” the udder when the teat orifice is open, e.g. at or soon after milking or after teat damage. The major contagious pathogens comprise *S. aureus*, *Str. dysgalactiae* and *Str. agalactiae*, while the major environmental pathogens comprise the Enterobacteriacae

Table 3. Comparison of infection type between clinical and sub-clinical bovine mastitis.

Mastitis type	*E. coli*	*Str. uberis*	*S. aureus*	*Str. dysgalactiae*	*Str. agalactiae*	*S. epidermids*	*S. saprophyticus*	Number
Clinical	+	+	+	+				1
mastitis	+	+	+	+	+			1
	+	+	+	+		+		2
	+	+	+		+		+	1
	+	+	+		+			2
	+	+	+					5
	+	+		+				3
	+	+		+		+		1
	+	+			+			3
	+	+				+	+	1
	+		+					4
	+		+	+		+		2
	+		+		+		+	1
	+		+		+			2
	+				+			1
	+							3
		+		+		+	+	1
		+			+			1
		+	+					1
			+		+			1
		+						1
								3*
Sub-clinical	+	+	+		+	+		1
mastitis	+	+	+		+			1
	+	+	+					5
	+	+		+				5
	+	+		+			+	1
	+	+		+		+	+	2
	+	+		+	+	+		1
	+	+			+			3
	+	+				+		2
	+	+						5
	+		+					3
	+		+	+	+			2
	+		+	+				1
	+		+		+	+	+	1
	+			+				2
	+			+		+		1
	+			+	+		+	1
	+				+		+	1
	+				+			1
	+							10
		+						1
		+		+	+			1
		+	+	+				1
			+	+				1
			+		+			1
			+					1
								4*

*, No *E. coli*, *Staphylococcus* and *Streptococcus* were identified.

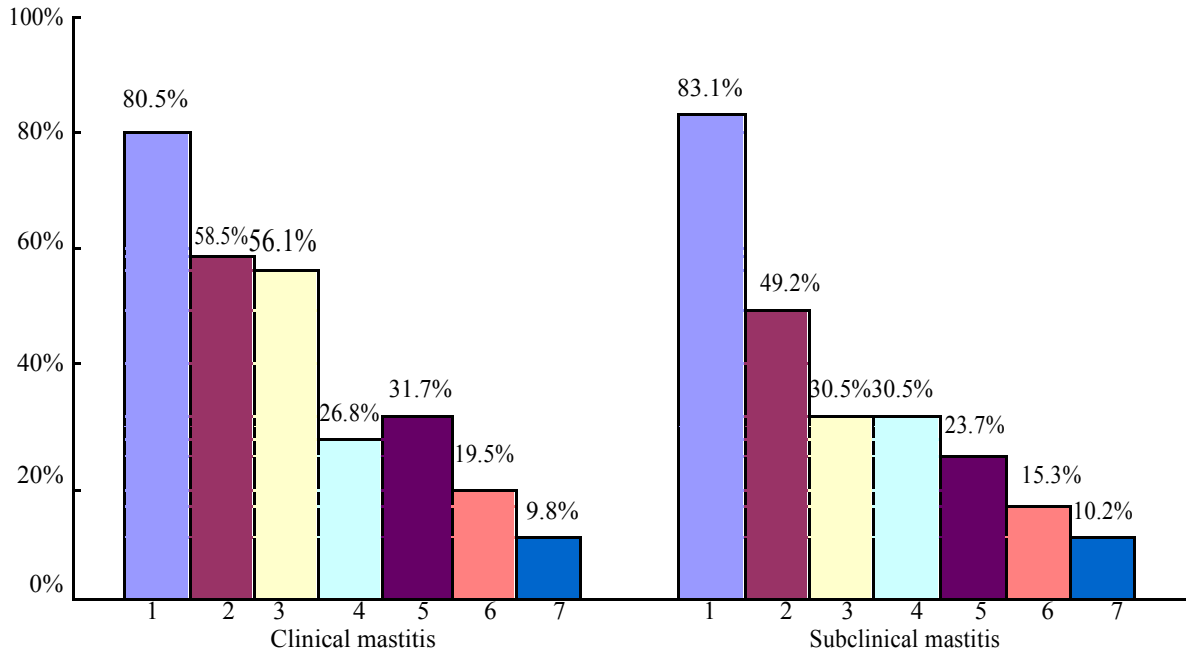

Figure 1. Different bacterial infection between clinical and sub-clinical mastitis.
1. *E. coli*, 2. *Str. Uberis*, 3. *S. aureus,* 4. *Str. Dysgalactiae*, 5. *Str. Agalactiae*, 6. *S. epidermids* and 7. *S. saprophyticus*.

(particularly *E. coli*) and *Str. uberis* (Aarestrup et al., 1999; Watts, 1988).

Probably the biggest challenge facing the modern dairy industry is the pressure to reduce the incidence of mastitis, and the extensive investigation and research of mastitis etiology may be capable of helping to provide an important and optimistic approach to control this disease. The statistics of this research not only confirmed the prevalence of the five vast major species of bacteria (*E. coli*, *Str. uberis*, *S. aureus*, *Str. dysgalactiae* and *Str. agalactia.*) in part region of China, but also found *S. epidermids* and *S. saprophyticus*, previously considered as naught pathogenic bacteria, were existed in the diseased mammary gland of the problem cows. It revealed that *E. coli* was the commonest organism in most mastitis cases, and *Str. uberis* was continues to be a prevalent pathogen closely followed by *S. aureus*, *Str. dysgalactiae* and *Str. agalactiae*. In addition, *Str. uberis* and *S. aureus* were more frequently associated with clinical mastitis than sub-clinical case, while the infection rates of *E. coli*, *Str. dysgalactiae*; *Str. agalactiae*, *S. epidermids* and *S. saprophyticus* were similar. But on the other hand, whether the bacteria previously considered as purely environmental or naught pathogenic could directly cause disease or engender host immune response, it was not study in this research and the mysteries remain continual and deep research.

Finally, we have to regret to say that we didn't develop the work to confirm whether the 100 diseased cows were infected with other organisms, which were also important in bovine mastitis.

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