

Experimental Infection of Bovine Mammary Gland with *Prototheca zopfii* Genotype 1

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ABSTRACT. *Prototheca zopfii* is divided into three genotypes, one of which, *P. zopfii* genotype 2, appears to be the main causative agent of bovine protothecal mastitis. However, the difference in pathogenicity between genotypes 1 and 2 has not been well investigated. In the present study, we experimentally infected normal bovine mammary gland with *P. zopfii* genotype 1 to investigate its pathogenicity. The mammary gland infected with *P. zopfii* genotype 1 showed no clinical signs. However, the histopathologic features of the infected mammary gland consisted of interstitial infiltrates of macrophages, plasma cells, lymphocytes, and fibroblasts with neutrophils in acinar lumens. Algae were present in macrophages and free in the alveolar lumens and the interstitium. Histopathology of the resultant tissue samples revealed that genotype 1 also induced a granulomatous lesion in the cow teat, similar to the mastitis lesion due to genotype 2. **KEY WORDS:** bovine *Prototheca* mastitis, experimentally infection, genotype, *Prototheca zopfii*.

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Prototheca zopfii causes bovine mastitis, which is associated with reduced milk production, and is characterized by a thin watery secretion containing white flakes. *P. zopfii* can be differentiated biochemically and serologically into at least two biotypes [1, 6, 7], *P. zopfii* genotype 1 and *P. zopfii* genotype 2 [7]. Isolates from bovine mastitis in Germany and Japan have all been identified as *P. zopfii* genotype 2, suggesting that it is the main causative agent of bovine protothecal mastitis [4, 5]. However, the difference in pathogenicity between genotypes 1 and 2 has not been well investigated. In the present study, we experimentally infected bovine normal mammary gland with *P. zopfii* genotype 1 to investigate its pathogenicity.

The animal experimentally infected was a 5-year-old Holstein cow with chronic protothecal mastitis in the back right quarter of the udder and a front left teat blinded from the mastitis. Culture of a milk sample containing congelations from the back right quarter of the udder resulted in the isolation of *Prototheca* in pure culture; the isolate was identified as *P. zopfii* genotype 2, based on morphological and molecular features [2, 5]. However, culture of milk samples from the front right and back left quarters of the udder yielded no growth of *Prototheca*. On physical examination, the cow was normal without slight swelling of the back right quarter of the udder. The owner of this cow agreed to the experimental infection since mastitis due to *Prototheca* is a chronic infection with no effective treatments. The experimental infection was performed in isolation from the other cows at the dairy.

The *P. zopfii* genotype 1 strain, NUBS 68, from bovine

feces was used in the present study [2]. The strain was cultured in physiologic saline supplemented with 1% glucose as a carbon source at 27°C for 2 days. The final concentration of the inoculums was 1×10^6 CFU/50 ml, as advised by Dr. U. Roesler (Institute of Animal Hygiene and Veterinary Public Health, Faculty of Veterinary Medicine, University of Leipzig) who carried out experimental infection of cows with *P. zopfii* (personal communication). The 1×10^6 CFU of organisms/50 ml was inoculated into the front right quarter of the normal udder through the right fore teat. Starting on the day after inoculation, the cow was then milked every day for 10 days. Ten days after inoculation, no clinical signs of mastitis were evident in the front right quarter of the udder. The algae-free left back quarter received sham treatment as the control in this experimental infection.

The cow was euthanized according to the laws of the Japanese Meat Inspection Center and a postmortem examination was conducted at the Meat Inspection Center in Toyohashi City.

Prototheca mastitis is most frequently recognized as a chronic and symptom-less disease with a very high somatic cell count (SCC) and low milk yield [8]. In the present study, the only clinical sign of the cow was slight swelling of the back right quarter that was not found in the front right quarter of the udder. The histopathologic features of the mammary glands from both quarters (of which genotype 1 was isolated from the front right and genotype 2 was isolated from the back right), consisted of interstitial infiltrates of macrophages, plasma cells, lymphocytes, and fibroblasts with neutrophils in acinar lumens (Fig. 1). Algae were present in macrophages and free in the alveolar lumens and the interstitium (Fig. 1). Histopathology of tissue samples from both quarters revealed the typical granulomatous features of mastitis by *P. zopfii* and indicated mild clinical or

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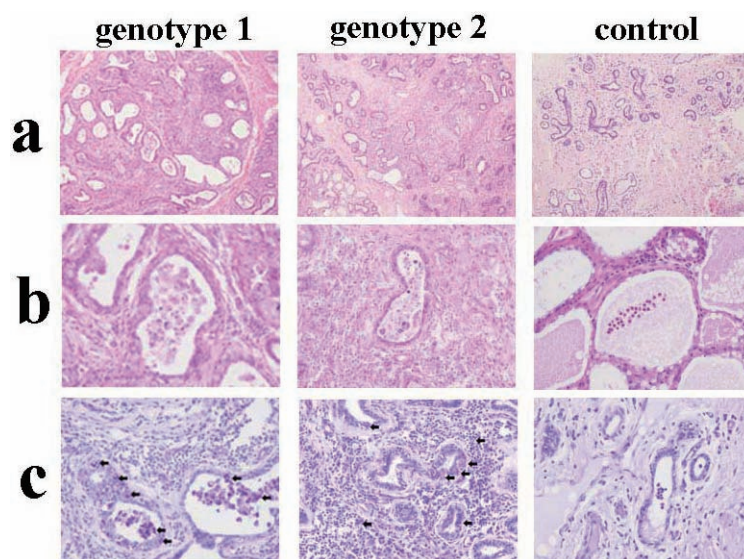


Fig. 1. The histopathologic features of *P. zopfii* genotype 1-infected, genotype 2-infected and non-infected (control) mammary glands. The genotype 1-infected and genotype 2-infected mammary glands include interstitial infiltrates of macrophages, plasma cells, lymphocytes, and fibroblasts with neutrophils in acinar lumens. Algae were present in macrophages and free in the alveolar lumen and interstitium (arrows). (a), HE stain $\times 100$; (b), HE stain $\times 400$; (c), periodic acid-Schiff (PAS) stain $\times 400$.

chronic subclinical mastitis [3].

In one of our previous studies, the genotype 1 isolates all came from cow-barn surroundings and not from *Prototheca* mastitis [5]. Therefore, this genotype was thought to be nonpathogenic for bovine mastitis, and a difference in the virulence between the two genotypes was not examined. Molecular epidemiologic studies have shown that the vast majority of clinical isolates from *Protothecal* mastitis are genotype 2. The source of *P. zopfii* genotype 2 infection is thought to be environmental, since *P. zopfii* is often found in flowing water, standing water and water tanks. However, our previous study confirmed that almost all isolates of *P. zopfii* from the environment, including cow feces, are genotype 1 [5]. To investigate this idea more thoroughly, in the present study we experimentally infected a teat canal with genotype 1. Histopathology of the resultant tissue samples revealed that genotype 1 induced a granulomatous lesion in the cow teat, similar to the mastitis lesion due to genotype 2. However, we still have no explanation for why genotype 1 is not isolated from mastitis, despite being more predominant in the environment than genotype 2. We expect that the genotype 2 isolates from mastitis would vary depending on individual predisposition. This distribution pattern might be related to an interaction between *P. zopfii* and microenvironment factors in the teat canal (e.g. bacterial flora, pH, salts, immune response, biochemistry and physiology). Further investigation of the mechanisms behind the difference in virulence between the two genotypes of *P. zopfii* is required.

All procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council [9].

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