

Association of *Prototheca* with Bovine Mastitis

Mahendra Pal¹ and Chang-woo Lee*

Department of Veterinary Public Health, College of Veterinary Science,
Gujarat Agricultural University, Anand-388001, India

*Department of Veterinary Clinical Pathology, College of Veterinary Medicine,
Seoul National University, Seodundong 103, Republic of Korea

*Prototheca*에 의한 소 유방염 발생에

팔 마헨드라 · 이창우*

인도 구자라트농과대학교 수의과대학 공중보건학교실

*대한민국 서울대학교 수의과대학 임상병리학교실

요 약 : 7세의 홀스타인 암소에서 염록소가 없는 *Prototheca*에 의한 만성적인 진행성 유방염의 발생에 대해 보고한다. 유방염 우유로 만든 습윤표본에서 명확한 세포벽과 내생포자를 갖고 있는 구형 내지 난원형의 병원체가 직접 관찰되었고, Sabouraud배지에서의 순수배양물에 대해 Wright염색 및 PHOL염색을 했을 때 algae가 증명되었으며, nutrient배지에서 세균이 증식하지 않아서 *Prototheca*가 유방염의 원인으로 제시되었다. 이 병원체는 자연상태에서는 사물기생성이지만 감염원은 확실하지 않았다. algae와 진균의 형태학적인 연구를 하는데 있어서 PHOL염색액을 광범위하게 응용할 수 있음을 제시하였다. *Prototheca*의 ecology와 병원성을 규명하기 위해 더 많은 연구가 이루어져야 될 것으로 생각된다.

Key words : cattle, mastitic milk, PHOL stain, *Prototheca*

Introduction

Despite advancement in veterinary medicine, mastitis still remains an important health problem in milch animals of many dairy farms of the world. The disease is mainly caused by bacteria and less frequently by fungi^{1,2,9,11,12}. Very occasionally *Prototheca* species have been implicated as a cause of bovine mastitis⁴. There is paucity of literature on protothecosis in man and animals in India. The present paper records the occurrence and etiologic significance of *Prototheca*, a colourless algae, in chronic progressive mastitis of seven year-old Holstein Friesian cow. In addition, the efficacy of PHOL technique for morphological study of *Prototheca* is also reported.

Materials and Methods

The mastitic milk collected aseptically in a sterilized wide mouthed capped glass bottle from the hind quarters of a 7-year-old Holstein Friesian cow was submitted to the Laboratory of Veterinary Public Health, College of Veterinary Science, Anand. The cow had a history of chronic mastitis which was treated for bacterial mastitis by the veterinary assistant. Intramammary administration of numerous antibiotics and hydrocortisone inconsistently failed to show clinical cure. The milk from the affected hind quarters were inoculated on to the duplicated slants of Sabouraud dextrose agar without cycloheximide and plates of nutrient agar. The former medium was kept at 25°C and later at 37°C. The inoculated media were examined for microbial growth. The suspected colony was subcultured on Sabouraud

¹Corresponding author.

medium for morphology in PHOL stain¹⁰. This new stain contained 3 ml of glycerol, 5 ml of 4% aqueous solution of 35% formaldehyde and 0.3 ml of 3% aqueous solution of methylene blue. No chemotherapy was attempted, and environmental samples were also not screened for the presence of *Prototheca*.

Results

On palpation, the affected hind quarters of the cow appeared hard and slightly reduced. The milk from the diseased udder contained white floccules and showed whitish-cream colour. There was no evidence of blood in the milk. Direct microscopical examination of mastitic milk in unstained wet mount, Wright stain and PHOL stain revealed round to ovoid cells with endospores morphologically compatible with *Prototheca* (Fig. 1). No other organisms like *Nocardia*, *Cryptococcus neoformans*, yeast and molds could be detected in the milk. Many white to cream coloured yeast-like colonies were observed on Sabouraud medium at 25°C after 4 days. No bacterial growth was seen on nutrient agar at 37°C. The isolates of *Prototheca* grew on blood agar at 37°C. The cultures in PHOL stain showed the similar morphology as was noticed in mastitic milk. The cultural and morphological examination confirmed the identity of the isolates as *Prototheca*. No detailed study of the organisms could be made, as the isolates were lost during subsequent subculturing.

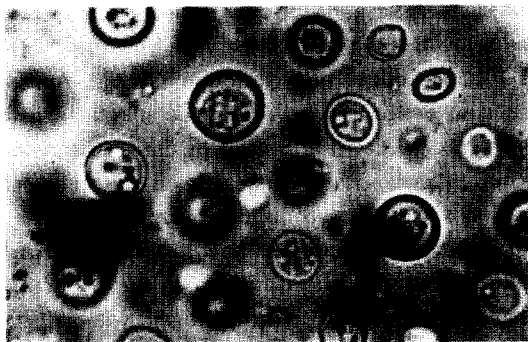


Fig 1. Microphotograph showing circular to oval unicellular cells with definite wall and endospores of *Prototheca* organisms in mastitic milk of a Holstein Friesian cow with chronic mastitis. PHOL stain $\times 600$.

Discussion

The genus *Prototheca* has two important species, *P. wickerhamii* and *P. zopfii* which are considered rare pathogen of man as well as animals. The algae can infect skin, subcutaneous tissues, eye, gastrointestinal tract, central nervous system, lymph nodes besides mammary gland^{3-6,13}. The infection of the udder in a cow due to *P. zopfii* was probably reported for the first time by Lerche⁷. The absence of bacteria on nutrient agar, unresponsiveness of conventional mastitis treatment, growth of isolate on blood agar at 37°C and direct demonstration of algae in mammary gland exudate suggest that *Prototheca* would probably be associated with chronic bovine mastitis. It is emphasized that etiologic role of *Prototheca* should be investigated in various clinical disorders of man and animals.

Though *Prototheca* are widely distributed in a variety of environmental material like sewage, soil, water, vegetation etc, the infrequent prevalence of protothecosis indicates the low virulence of the organisms in animals. In an experimental study Pal⁸ reported that none of 12 albino mice injected one million cells of *P. wickerhamii* exhibited any clinical evidence of protothecosis even at 60 days post inoculation. Further studies are imperative to understand the pathogenesis of the disease.

Authors did not conduct any epidemiological investigation to establish the source of infection in the present case. Moreover, authors had no facilities to perform immunological test to know whether the cow was immunocompromised. Since the organisms occur as saprobe the ecological studies on *Prototheca* in India should be conducted by covering different types of environmental materials. This may help to understand the epidemiology of protothecosis.

Conclusion

The occurrence and etiologic role of *Prototheca*, an achlorophyllous agent has been described in a 7-year-old Holstein Friesian cow which had a history of chronic progressive mastitis. The direct detection of spherical to oval bodies with distinct wall

and endospores in mastitic milk in wet mounts, PHOL stain and Wright stain, isolation of algae in pure growth on Sabouraud medium and absence of bacteria on nutrient agar indicated that *Prototheca* was implicated in bovine mastitis. Source of infection remains uncertain, though the organism grows as saprophyte in nature. The wider application of PHOL technique for studying morphology of algae as well as fungi is emphasized. In addition, further work on the ecology and pathogenesis of *Prototheca* organisms should be conducted.

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