BRIEF REPORT

BOVINE PROTOTHECOSIS

A brief report of ten cases

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Prototheca zopfii was isolated repeatedly from milk samples from ten cows (of a herd of 192 dairy cows) with reduced milk yield and indurated mammary glands. The strain was moderately sensitive to streptomycin, polymyxin and gentamycin, but resistant or relatively resistant to other antibiotics and antmycotics commonly used in clinical practice.

An attempt to treat the infection with Ethidium bromide, which was found effective in vitro, did not succeed. The number of Prototheca excreted decreased, but a complete cure was not obtained.

In histological sections of the udder, Prototheca cells were demonstrated both intracellularly and interstitially.

Key words: Prototheca zopfii: protothecosis, bovine: mastitis, bovine.

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Accepted as submitted 26.1.78

During routine examination of 192 dairy cows from one farm, microorganisms were isolated from milk samples from ten cows. At first the organisms were assumed to be atypical yeast cells, but a closer study revealed that they actually belonged to genus Prototheca.

Prototheca – first described by Kriiger in 1894 – is a group of non-pigmented, unicellular microorganisms belonging to the colourless algae.

Whereas numerous microorganisms among bacteria, fungi, protozoa, spirochaetes and viruses are well-known pathogens, algae are usually not associated with disease. Only in the last 20-25 years have they been mentioned at all in the medical literature, and then only rarely.

Geographically, Prototheca has been demonstrated in localities as far apart as Argentine, Germany, Puerto Rico, Sierra Leone, USA and Denmark.

Prototheca has been isolated from various sources such as slime flux of trees, lake and sea water, sewage, sludge, from skin scrapings and tissues from humans and animals, and from human sputum and faeces.

Protothecosis, the infection which may be produced by certain Prototheca species, was first described in a cow by Lerche in 1952. Lerche showed experimentally that the Prototheca strain concerned could give rise to mastitis, but histopathological studies were not performed.

Available evidence strongly suggests that the skin is the primary site of infection in both man and animals. The exact mechanism of infection is not known.

The most common form of protothecosis in man and animals seems to be the cutaneous variety which occurs either as a demarcated local skin lesion or as a generalized disease. Cutaneous manifestations were, in fact, described by Davies et al., who in 1964 were the first to prove that Prototheca can be pathogenic for man. They isolated a Prototheca strain from skin affections in an African rice farmer and also demonstrated its presence in the tissues.

Present material

During examination of a herd of 192 dairy cows, Prototheca was isolated repeatedly at weekly intervals from milk samples from ten of the cows. Clinically the cows were in good condition, though the affected quarters were indurated and the milk yield reduced.

For epidemiological reasons, it was attempted to trace the source of infection by inoculations from the milker.
the fodder and the environment (including water holes and marlips on the farm), but without result.

The actual strain grew well aerobically at 30–32°C on conventional culture media. In young cultures the colonies resembled colonies of yeast, but in older cultures on blood agar they were duller and drier. On Sabouraud agar, they were finely shagreened, opalescent and transparent. The smell of the cultures on Sabouraud agar was unpleasant.

*Prototheca* cells are Gram-positive and catalase positive. In physiological studies of the isolated *Prototheca* strain by the method of Lodder 1970 no carbohydrates were fermented, but the strain assimilated glucose, glycerine, ethanol, n-propanol and lactate. Using Arnold and Ahearn’s identification key (Arnold & Ahearn 1972) the strain in question was identified as *Prototheca zopfii*. Comparisons with type strains from the American Type Culture Collection have verified the diagnosis.

The cases of protothecosis reported in the literature were difficult to treat because of the relative resistance of *Prototheca* to the drugs usually applied against infections.

This observation is in good agreement with the results of sensitivity tests performed on the *Prototheca zopfii* strain under consideration here. The strain was moderately sensitive to streptomycin, polymyxin and gentamycin, but resistant or relatively resistant to other antibiotics and antimycotics commonly used in clinical practice.

As the antibiotic therapy applied to the affected cows proved ineffective, one of us (S. M.) thought that Ethidium bromide might prove effective in the treatment of the algal infection. Ethidium bromide—a phenanthridine derivative which can form a complex compound with DNA in cell nuclei—is used under the proprietary name of Homidium® in the treatment of trypanosomiasis diseases in the tropics.

In serial dilutions on Sabouraud agar plates, the minimum inhibition concentration of Ethidium bromide was found to be 5 μg/ml.

However, mastitis was still present, even though treatment with Ethidium bromide of both the affected animals and an experimentally infected cow resulted in a fall in the excretion of *Prototheca* cells.

The experimentally infected and Ethidium bromide treated cow was killed and subjected to autopsy. *Prototheca* cells were demonstrated both intracellularly and interstitially in histological sections of the udder tissue.