

# This Week's Citation Classic®

**Henriksen S A & Pohlenz J F L.** Staining of cryptosporidia by a modified Ziehl-Neelsen technique. *Acta Vet. Scand.* 22:594-6, 1981.  
[National Veterinary Laboratory, Frederiksberg, Copenhagen, Denmark]

A modified Ziehl-Neelsen staining technique was found to be a reliable method for diagnosing cryptosporidia in smears from fecal samples or scrapings of intestinal mucosa. It provides a magnificent differential staining with a high degree of sensitivity. [The SCI® indicates that this paper has been cited in more than 135 publications, making it the most-cited article published in this journal.]

## A Parasite Diagnosed by a Bacteriological Staining Method

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In the 1970s, several publications indicated that protozoa of the genus *Cryptosporidium* might be of etiological importance in the diarrhoea complex in calves.<sup>1</sup> Earlier, only little attention was paid to these protozoa. Up to 1980, demonstration of infections with *Cryptosporidium* was, on the whole, based on the observation of the parasite in histological sections or in smears stained by the Giemsa technique.<sup>2</sup>

In 1980, at the National Veterinary Laboratory, Copenhagen, Sv. Aa. Henriksen analyzed the extent to which infections with *Cryptosporidium* were occurring among calves in Denmark. However, he had no experience in diagnosing such organisms—in fact, nobody in Denmark seemed to have observed the organism at all.

Smears, prepared from the mucosal membrane of the small intestine of calves that died from diarrhoea, were stained by Giemsa, and a search was made for round organisms 4.5-5 µm in diameter with a faint blue-stained cytoplasm and a few red granules. From one of the first calves, a great number of such organisms were encountered. However, Henriksen found it quite difficult to distinguish the organisms in the smears, due to lack of differential staining. This prompted him to try staining with other techniques.

For this, a series of smears was prepared from the intestinal tract of a similarly infected calf. The smears were stained by Gram, Ziehl-Neelsen, Köster, or other staining techniques routinely used in the bacteriological laboratory next door. It was soon obvious that techniques that made use of the so-called acid-fast character of specific groups of bacteria (e.g., mycobacteria), namely, the Ziehl-Neelsen technique and the Köster technique, also seemed to be applicable for the demonstration of oocysts of *Cryptosporidium*. However, the techniques employed by the bacteriologists quite obviously had to be modified or improved to provide a distinct differential staining of the parasite. The attention was focused on the Ziehl-Neelsen technique,<sup>3</sup> and within five to six hours, a recipe for a staining technique was established, resulting in a magnificent differential staining of the *Cryptosporidium* oocysts. They appeared as densely stained red bodies, clearly distinguishable against a green background.

To confirm the identity of the organisms, Henriksen contacted J. Pohlenz, Iowa State University, Ames, whom he had met earlier, and who already had described infections with *Cryptosporidium* in calves. Pohlenz confirmed that the organisms found in the smears were actually *Cryptosporidium*. With a few additional adjustments, suggested by Pohlenz, the modified Ziehl-Neelsen technique was ready for publication.

Henriksen has received numerous international inquiries regarding the modified Ziehl-Neelsen technique. Clearly, this was coincident with the growing perception that cryptosporidiosis was a widespread disease within the diarrhoea complex in neonatal calves<sup>4,5</sup> and a rather frequent complication in human patients suffering from AIDS.<sup>6,7</sup>

In his characteristically modest way, Henriksen stated that the technique was developed on a completely empirical basis, taking advantage of techniques already applied in the bacteriological laboratory.

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4. Angus K W. Cryptosporidiosis in man, domestic animals, and birds: a review. *J. Roy. Soc. Med.* 76:62-70, 1983.
5. Henriksen S A & Krogh H V. Bovine cryptosporidiosis in Denmark. 1. Prevalence, age distribution, and seasonal variation. *Nord. Vet.-Med.* 37:34-41, 1985.
6. Current W L, Reese N C, Ernst J V, Bailey W S, Heyman M B & Weinstein W M. Human cryptosporidiosis in immunocompetent and immunodeficient persons. *N. Engl. J. Med.* 308:1252-7, 1983. (Cited 320 times.)
7. Soave R. Clinical cryptosporidiosis in man. (Angus K W & Blewett D A, eds.) *Cryptosporidiosis*. Proc. 1. Int. Workshop. Edinburgh, 7-8 September 1988. p. 19-26.

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