

Aust. Soc. Parasitol. (1988), Sydney, 27-30 Sept.

Analysis of *Cryptosporidium* antigens by Western blot technique and ^{125}I radiolabelling.

R. Lumb¹, J.A. Lanser¹ and P.J. O'Donoghue²

Division of Clinical Microbiology¹, Institute of Medical and Veterinary Science, and Central Veterinary Laboratories², Department of Agriculture, Adelaide, South Australia.

Cryptosporidium spp. are coccidian parasites which infect epithelial cells lining the gastrointestinal and respiratory tracts of animals and man. Infections may cause acute, self-limiting gastroenteritis in immunocompetent individuals or severe, unremitting illness in immunocompromised patients.

Laboratory diagnosis is usually made by visualisation of the oocysts in clinical material by direct microscopy or following concentration. Although such techniques are rapid and easy to perform, recognition of *Cryptosporidium* oocysts may be difficult due to their small size and confusion with yeasts and faecal debris.

Antigen characterization studies were performed with immune sera from naturally infected humans and goats to determine whether common antigens exist between isolates which would allow the production of a specific immunoreagent for use in the detection of oocysts in clinical material.

Acute or convalescent sera from 10 humans and 5 goats were reacted with an intact *Cryptosporidium* oocyst preparation derived from a human patient using the Western blot technique. Nine of the 10 human sera and all of the goat sera reacted with a 23,000 MW antigen (previously described by Ungar and Nash, 1986) as well as a 32,000 MW antigen. A 15,500 MW antigen was also recognized by 4 of the 10 human sera and all of the goat sera, whereas various other antigens above 40,000 MW were recognized by individual sera.

Surface labelling of 3 human isolates of intact *Cryptosporidium* oocysts with ^{125}I was performed using the Bolton and Hunter reagent. The oocysts were solubilised, separated on 12 and 18% PAGE gels and then autoradiographed. Common bands were seen at 15,500, 32,000, 44,000, 47,500, 79,000 and 96,000 MW.

These studies demonstrate that the 32,000 MW antigen is located on the external surface of the oocyst wall and is common to many isolates. This antigen therefore appears to be a suitable candidate for the manufacture of polyclonal or monoclonal antibody probes.

Ungar B.L.P. and T.E. Nash. 1986. Quantification of specific antibody response to *Cryptosporidium* antigens by laser densitometry. *Infect. Immun.* 53: 124-128.