The effectiveness of hydrogen peroxide liquid or gas plasma on protozoan oocysts

To the Editor:

Gastroendoscopy is increasingly in demand to diagnose and treat illnesses of the gastrointestinal tract. Because endoscopes may become contaminated with infectious agents from the intestine or rectum, they have to be reprocessed. The infectious agents that are most difficult to destroy (second to prions) are the oocysts of coccidian parasites such as *Cryptosporidium* and *Cyclospora* (1,2). No liquid disinfectants, including the much-studied aldehydes, have been shown to be entirely effective. We used hydrogen peroxide, a newer oxygenated product to the market, in both liquid and gaseous form, in a bioassay to test its effectiveness in inactivating oocysts. Because human coccidian parasites, such as *Cryptosporidium*, are hazardous to handle in the laboratory, we used a close alternative, *Eimeria acervulina*, as a surrogate (3). This parasite causes coccidiosis in chickens. In this bioassay, if oocysts are inactivated, they will not produce evidence of infection, lesions or replicate themselves to produce oocysts in chickens.

Our studies confirmed that despite a 3 h soak in any of the hydrogen peroxide liquid disinfectants, some oocysts remained infectious (Table 1). In contrast to liquid disinfectants, a

hydrogen peroxide gas plasma system, operating at less than 50°C, inactivated all *E. acervulina* oocysts (Table 2) from the surface (aluminum foil) and from the inside of narrow tubing (inner diameter of 3 mm). None of the 40 chicks inoculated with gas plasma-treated oocysts produced lesions (n=15 chicks inoculated) or oocysts (n=25 chicks inoculated). Oocysts that were either air-dried on aluminum foil or inside narrow tubing, but not treated with gas plasma, remained infectious for all chicks (n=25). Heat-treated controls did not produce infection.

These results were similar to the study by Vassal et al (4), in which 10⁵ *Cryptosporidium parvum* oocysts treated in a 75 min cycle, hydrogen peroxide gas plasma system (STERRAD 100s [Advanced Sterilization Products, USA]), were found to be non-infectious to immunocompromised Sprague-Dawley rats. This similarity in behaviour between *E. acervulina* oocysts and *Cryptosporidium* oocysts in their response to hydrogen peroxide also supports the usefulness of *E. acervulina* as a surrogate for testing the sterilization of endoscopes. In addition, *E. acervulina* oocysts are safe and economical to handle and provide verifiable and clear evidence of inactivation.

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TABLE 1
Effect of hydrogen peroxide disinfectants on sporulated *Eimeria acervulina* oocysts after a 3 h soak. Inocula between 10,000 and 15,000 oocysts were given orally to each chick on the first or second day of hatch

Treatments	Trial 1		Trial 2		Total positive
	Lesions	Oocysts	Lesions	Oocysts	
No treatment	5/5	—	5/5	—	10/10
25% hydrogen peroxide, 5% peracetic acid	5/5	—	9/9	3/3*	14/14
2.5% hydrogen peroxide, 0.5% peracetic acid	5/5	—	5/5	—	10/10
7.5% hydrogen peroxide	5/5	—	5/5	—	10/10
2% hydrogen peroxide (product A)	5/5	—	5/5	—	10/10
2% hydrogen peroxide (product B)	5/5	—	5/5	—	10/10
Heat-treated controls	0/3	0/2	0/5	0/5	0/15

*Fecal collections of three chicks before necropsy showed an abundance of oocysts. Five of five means that five birds produced evidence of infection, either lesions or oocysts, out of five birds that were inoculated. Likewise, zero of five means that no birds produced evidence of infection, neither lesions nor oocysts, out of five birds that were inoculated

TABLE 2
Infectivity of sporulated *Eimeria acervulina* oocysts treated by a hydrogen peroxide gas plasma system*. Inocula between 2000 and 20,000 oocysts were given orally to each chick on the first or second day of hatch

Treatment	Preparation 1				Preparation 2		Total positive
	Sample 1		Sample 2		Sample 1	Sample 2	
	Lesions	Oocysts	Lesions	Oocysts	Oocysts	Oocysts	
Untreated controls (oocyst culture)	ND	ND	5/5	5/5	5/5	5/5	20/20
Untreated controls (oocysts on aluminum foil, air-dried only)	5/5	ND	5/5	5/5	ND	ND	15/15
Hydrogen peroxide gas plasma (oocysts on aluminum foil)	0/10	0/5	0/5	0/5	ND	ND	0/25
Untreated controls (oocysts in tubing, air-dried only)	ND	ND	ND	ND	5/5	5/5	10/10
Hydrogen peroxide gas plasma (oocysts in tubing)	ND	ND	ND	ND	0/5	0/10	0/15
Heated controls	0/5	ND	0/5	0/5	0/5	0/5	0/25

*STERRAD 100s (Advanced Sterilization Products, USA). ND Not determined