Institute for Parasitology
Tierärztliche Hochschule Hannover
(University forVeterinary Medicine)

MENNO CHEMIE GmbH
Langer Kamp 104

D-22850 Norderstedt
attention Mr. Nevermann

Dr.med.vet.habil.Arwid Daugschies
Bünteweg 17 - Building 217
D-30559 Hannover
phone: (0511) 953-8794/18
fax: (0511) 953-8870
e-mail:ardaug@parasit.tiho-hannover.de

Hannover, 03.061999

EXPERT OPINION

Test of the NEOPREDISAN 135-1 disinfecting action on pig coccidia oocysts

(*isospora suis*)

Orderer:

MENNO CHEMIE-Vertrieb GmbH
Langer Kamp 104
D-22850 NORDERSTEDT

comprises: 4 pages

1. Introduction

The test was carried out according to the current version (state of May 1996) of the "Richtlinien der Deutschen Veterinärmedizinischen Gesellschaft" (DVG) (Guidelines of the German Veterinary Association).

2. Method

2.1 Test preparation

The disinfectant to be tested is the preparation NEOPREDISAN E-135-1 of MENNO CHEMIE-Vertrieb GmbH, Norderstedt. The test preparation batch bears the number 9801 and has been delivered on 27.5.1998. The preparation properties are listed in table no.1.

Table no. 1: Properties of the test preparation

Preparation	Color	Precipitations	Composition (Producer's declaration)	Water solubility	pH value (Application concentration)
NEOPREDISAN E-135-1	colorless turbid	without	p-chloro-m-cresol 25% 1-propanol 25% 2-propanol 15%	good	pH 2-2,5

2.2 Test organism

The freshly passed *isospora suis* oocysts (HannIS) were used for testing the disinfecting action. The oocysts had been kept in a 2% potassium dichromate solution and stored in a refrigerator for a period of less than 6 months. For passing them, they were washed out three times by diluting the solution with water, centrifuging (200 x g, 5 minutes) and sucking off the supernatant fluid. A total of 9 sucking pigs were inoculated with a dosis of 5000 oocysts/pig in the first life week. The pig excrements were gathered between the 5^{th} and 7^{th}, and between the 10^{th} and 12^{th} day after infection and stirred with tap water to get a liquid suspension. After a 5-minutes centrifugation, the supernatant fluid was decanted, and the sediment resuspended in a 25% Percoll solution. After another centrifugation, the sediment was washed in water by centrifuging it. The oocysts were, then, kept for sporulation in a 2% potassium bichromate solution at room temperature in shallow Petri culture

dishes. After sporulation, the oocysts were enriched with a saturated solution of sodium chloride/sugar (specific weight 1,27), washed with water and kept in the refrigerator at 4-6° C for 1-2 days.

2.3 Lysis test

The passed oocysts were counted in the Fuchs-Rosenthal chamber and adjusted to a density of about 120.000 oocysts/ml. The portion of sporulated oocysts in the oocyst suspension was of 65%. For the special properties of the test preparation, its starting solution was not applied in a double concentration, but a 1,11-fold concentration, in exception to the guidelines. The desired final concentration was obtained by adding 0,1 ml of the oocyst suspension to 0,9 ml of the 4,44 or 2,22 % starting solution. The exposure times were of 30, 60, 90, 120, 180 and 240 minutes. The finishing of disinfection, the oocyst washing out, counting and assessment processes were performed as explained in the DVG guidelines for *eimeria tenella*, by using, however, sporulated oocysts, exclusively. The morphologically deformed oocysts were classified as to be lysed.

The disinfecting effect was assessed by applying the following the relative identification rate (rel. IR) formula and the lysis rate:

$$\text{rel IR (\%)} = \frac{\text{number of oocysts in the disinfected batches x 100}}{\text{number of oocysts in the control batches}}$$

Lysis rate (%) = 100 - rel. IR

3. Results and conclusions

The test was carried out by strictly observing the currently valid DVG guidelines for testing disinfectants, as far as deviations were not especially mentioned. The only deviations consisted in the way of preparing the disinfection batches. The test preparation was set in a 1,11-fold instead of the doublefold application concentration. This modification of the guidelines resulted necessary, as, according to the producer, we had to take into account precipitations when using a higher concentration, which would negatively influence the disinfection result. The identification rate of the

non-disinfected oocysts was of 73,4 % and, thus, relatively low. This deviation from the guidelines, however, is not relevant for the informative value of the test.

The tested disinfectant NEOPREDISAN E-135-1 showed a good effectiveness in the 2 % and the 4 % application concentration and an incubation time of 30 minutes. 60 minutes after applying the higher concentration and 90 minutes after applying the 2 % batch, there could not be detected any intact oocysts.

Table 2: Relative effectiveness and lysis rate after disinfection of *isospora suis* oocysts with NEOPREDISAN E-135-1

Application concentration	Exposure time (minutes)	relative identification (%)	Lysis rate (%)
2 %	30	4,35	95,65
2 %	60	3,04	96,96
2 %	90	0	100
2 %	120	0	100
2 %	180	0	100
2 %	240	0	100
4 %	30	1,43	98,57
4 %	60	0	100
4 %	90	0	100
4 %	120	0	100
4 %	180	0	100
4 %	240	0	100

The current DVG guidelines for *eimeria tenella* require an effectiveness of at least 95 % in the lysis test. NEOPREDISAN proved to have an excellent effect on sporulated *isospora suis* oocysts in the 2 and 4 % application concentrations after short exposure times already, satisfying, thus, the DVG requirements for disinfecting coccidia, even after the shortest time choosen of 30 minutes.

Hannover, on 03.06.1999

signed: signature

(PD Dr. Arwid Daugschies)