Evaluation of the disinfective effect of Neopredisan™ 135-1 on eggs of *Heterakis* sp. *in vitro* and *in vivo*

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1. Introduction

The trial was performed following the guidelines of the German Veterinary Association (Deutsche Veterinärmedizinische Gesellschaft, DVG) in its present form (as of May 1996).

Deviations from the DVG guidelines are set forth in part 2, Methods.

2. Methods

2.1 Test Preparation

- Customary 10 litre cask of Neopredisan™ 135-1, lot No. 02010, to be used before 08/07.

2.2 Test Organism

- Field isolate of *Heterakis* sp. from domestic hens
- Extraction of female worms from the appendices of naturally infected animals and isolation of the eggs with subsequent incubation at ambient temperature until the development of infective larvae (storage: < 6 months)
- Extraction of eggs from the faeces of experimentally infected hens for immediate use *in vitro* (2.3 development inhibition test)

2.3 Evaluation of the inhibitive effect on the development of larvae *in vitro*

Freshly extracted eggs (from experimental infection) were cleaned by flotation, washed, sown into 24-hole-plates in tap water (density: approx. 20 eggs/double test) and disinfected according to the following scheme:

Active time: 1 – 2 – 4 hours (at ambient temperature); concentration 0 % (reference control), 0.25 – 1 – 2 % Neopredisan™ 135-1.

To this, 100 µl ice suspension with 900 µl disinfectant solution (1.1fold concentration of the final concentration) were added. After the respective incubation times, the eggs were rinsed with tap water.

For the development of the eggs, the plates were incubated at ambient temperature and aired daily. In order to control the development, the plates were examined daily for the development of larvae by microscope for a period of 4 weeks.

2.4 Evaluation of the inactivation of embryonated eggs *in vivo*

Previously incubated (see above), infective eggs (content: moving larvae) were disinfected with 0 % (control) or 2 % Neopredisan™ 135-1 for 2 h as described under 2.3, washed and counted. 2 groups (A: not disinfected control; B: disinfected group) of 4 chicken each (age: 1 week, female fattening hybrides) were orally infected with ice suspensions (50±5 eggs/animal). The faeces of the animals was collected beginning with the 21st day after the infection and qualitatively analyzed for egg excretion by coproscopy. Six days after the beginning of the excretion, all animals were killed and their appendices were analyzed for the presence of *Heterakis*.

3. Results and conclusions

3.1 Inactivation *in vitro*

In order to examine the effect of Neopredisan™ 135-1 on freshly excreted, not yet infective eggs of *Heterakis*, eggs were isolated from faeces, cleaned and disinfected and subsequently incubated for the evaluation of the development of larvae.

In an average of 91% of the untreated eggs a development of larvae took place that was completed 22 days after sowing. At a concentration of 0.25 % or 1 % Neopredisan™ 135-1 already (depending on the time) a formation of segmentation globules but no development of larvae took still place; at even higher concentrations, the eggs were deformed and a further development of the egg cell was repressed completely (table 1).

Table 1: Development of larvae (in % of the eggs) after disinfection of fresh eggs of *Heterakis*

concentration Neopredisan™ 135-1	**1 h incubation**	**2 h incubation**	**4 h incubation**
0 % (control)	87.5	90	95
0.25 %	0 (segmented: 94.7)	0 (segmented: 84.2)	0 (segmented: 80.0)
1 %	0 (segmented: 55.5)	0 (segmented: 33.3)	0 (segmented: 28.6)
2 %	0	0	0

A concentration of 0.25 % and an active time of 1 h were sufficient *in vitro* for the inhibition of the development of infective larvae in a field isolate of *Heterakis* sp..

3.2 Inactivation *in vivo*

In order to evaluate the effect of Neopredisan™ 135-1 on infective eggs of *Heterakis*, previously incubated, embryonated eggs were disinfected and used for the infection of hens. The excretion of eggs was examined in groups and all were killed after the beginning of the patency and analyzed for infestation with worms.

The first excretion of the control group was recorded on the 36th day after the infection. Eggs were present in the faeces of the control group until the killing 40 days after the infection, whereas the disinfected group remained negative altogether.

In the control group (A), adult worms were found in the appendices of 2 of the 4 animals, whereas the disinfected group B (4 animals) was free from infestation with worms.

At the recommended concentration of 2 % for worm eggs and an active time of 2 h with Neopredisan™ 135-1, even already developed eggs of *Heterakis* are killed; an infection *in vivo* took not place under the selected conditions.

Wien, 2004-09-21 *signature*

(Prof. Dr. Anja Joachim)