



Formulation of microorganisms for rodent control in bait gel

The present invention relates to compositions containing different stages of pathogenic protozoans in a gel and to their use for controlling rodents.

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It is known that protozoa from the group of the Sarcosporidia (phylum *Protozoa*, class *Sporozoa*, subclass *Coccidia*, order *Eucoccidiida*, suborder *Eimeriina*) are pathogenic to rodents (Genera *Rattus*, *Bandicota*, *Arvicanthis*, *Nosokia*, *Mus*) and that a sufficient dose leads to the animals' death (Rzepczyk 1974, Intern. J. Parasitol. 4, 447-449; Brehm, Frank 1980, Z. Parasitenkd. 62, 15-30). A parasite from among this group which is especially pathogenic to several rat species is *Sarcocystis singaporensis* (Zamann et al. 1975, Z. Parasitenkd. 47, 169-185).

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The definite hosts of the parasites are predators or omnivores; for *S. singaporensis*, the definite hosts are snakes, for example the reticulated python. The parasite forms sporocysts in the gut of the definite host. These persistent stages are excreted together with the faeces. If an intermediate host, in the case of *S. singaporensis* for example a rat, is infected orally with these sporocysts, sporozoites hatch in the rat's intestines and penetrate the intestinal wall. Via the blood circulation, these stages reach other organs, where they can multiply by division (Schizogony).

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Two schizogonies have been detected, the first one around day 6 post-infection and the second around day 14-16 post-infection. The schizonts (merozoites) are found in particularly high numbers in the lungs, where they multiply in the endothelial cells.

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If severe infection results in mass multiplication of the merozoites in the lung, the rat falls ill during the second schizogony of the parasite around day 16 post-infection and, in most cases, dies within a few hours.



It is possible to administer the sporocysts of these pathogens to rodents so that the latter die from the infection. This principle can be exploited for controlling rodents (Wood 1985, J. Plant Protect. Trop. 2, 67-69). Rats have already been controlled successfully with *Sarcocystis singaporensis* (Jäkel et al. 1996, J. Parasitol. 82, 280-287).

As a rule, infections of *Rattus rattus* and *norvegicus* with over 20.000 Sporocysts (depending on the strain) are lethal. Up to 14 days post-infection the animals show no symptoms. Then, they weaken rapidly and die of the infection around day 16 post-infection.

Sublethally infected rats show virtually no symptoms of disease, even after 14 days post-infection, and survive the infection without loss of vitality. Only a few days after the infection, these animals have established immunity to the pathogen. These animals are now insensitive to any further control with a *Sarcocystis singaporensis* product.

The sporozoites in aqueous suspension have a shelf life of up to two years without substantial loss of pathogenicity. This suspension can be administered to rats by gavage and, with appropriate dosing, has a lethal effect. However, the sporocysts do not survive in the dry state.

To control rats, baits must be used which are eaten by the animals. Such baits with *S. singaporensis* sporocysts remain pathogenic as long as they are moist. Once dried out, the sporocysts very rapidly lose their pathogenicity. This is why conventional cereal-based baits cannot be used for the effective administration of these parasites. In the applicant's own studies it was impossible to retain the infectivity of *S. singaporensis* sporocysts in cereal-based baits. One day after mixing, these baits had become ineffective.

Baits with sporocysts must be film-packaged in the moist state. The disadvantage of these moist products is the low shelf life since cereal would start to go mouldy, or would germinate, within a few days. One alternative is to dose the liquid suspension in cavities in the bait. For example, lumps of pasty bait whose interior contained a lethal dose of the suspension in a cavity were used successfully. However, these baits too must be used within a few days after preparation since water escapes or since the pasty matrix absorbs water and swells or ferments, rendering these baits useless.

The only possibility as yet for the practical use of protozoa such as *S. singaporensis* for rodent control is to prepare the baits immediately prior to use from a sporocyst-containing aqueous suspension and a bait matrix, for example cereals. The problem here is to withdraw a calculated amount of sporocysts since the latter settle very rapidly in the suspension and concentrate at the bottom of the vessel. This is why baits prepared in this manner contain an indeterminate amount of sporocysts which is not distributed homogeneously. These baits dry rapidly, frequently even during mixing. As a consequence, the sporocysts die and the bait stops being effective after as little as a few hours.

It has now been found, surprisingly, that *Sarcocystis* sporocysts retain their infectivity for a long time in specific gels, even though it is known from earlier studies that they are no longer infectious on cereals, in pastes and gels such as bentonites after as little as a few days. The fact that they survive over months in the gels described herein was very surprising and unexpected. It is known from the literature that even highly robust sarcosporidia from temperate climates, such as *S. gigantea*, are very sensitive to changes in the pH and chemicals (McKenna et al., Veterinary Parasitology 1992, 45 1-16). It is therefore particularly surprising that even the pH can be varied and in addition preservatives can be added without substantially reducing the parasite's chances of survival.

The present invention therefore makes it possible to preserve sporocysts in gels for the later use in the control of rodents. They remain distributed homogeneously in this

matrix. This makes possible the withdrawal of defined quantities of sporocysts. A defined quantity of this gel can be mixed with the bait matrix as long as a homogeneous state has been achieved since the gel is only adsorbed very slowly by the matrix. Since the loss of water, by evaporation, from the gels used is only very slow, the bait remains moist, and thus infectious, even when used in a dry atmosphere.

In this manner, it is possible to store sporocysts over months without suffering gel separation or a considerably reduced infectivity of the pathogens. The appended example, in which a Carbopol gel is used, confirms that such mixtures can be used over several months.

The present invention claims inter alia the principle of preserving stages of single-celled parasites in gels. These gels are used for controlling rodents, either alone or admixed to baits.

Suitable pathogenic organisms are, in principle, all protozoa from the class Sporozoa. Especially suitable are species from the subclass Coccidia. Very specially suitable are representatives of the suborder Eimeriina. Examples which may be mentioned, but not by limitation, are the species of the genus *Sarcocystis*, such as *S. singaporensis*.

The composition according to the invention can be incorporated into all formulas conventionally used for the production of feed baits for rodents, for example into cereal-based baits, into pastes, gels and wax blocks and into extruded mixtures of cereals and formulation auxiliaries. It is furthermore possible to offer the pathogens in water-based drink baits. The baits for the purposes of the invention are used like conventional baits for controlling harmful rodents. They can be placed in enclosed spaces and in the open, with 5 g to 500 g per bait station, depending on the target species and the bait station density. They can also be placed in the burrow entrances of the rodents to be controlled.

Dosing of the pathogens in the gel can be set as desired. Dosages of from 1000 to 10,000,000 sporocysts/ml are to be preferred. 500,000 to 2,000,000 sporocysts/ml are especially preferred.

5 The dosage of the pathogens in the bait should amount to 1000 to 200,000 sporocysts per gram of bait. Concentrations of from 2000 to 100,000 sporocysts per gram are preferred, concentrations of 10,000 – 30,000 are especially preferred and 20,000 sporocysts per gram are particularly preferred. Higher dosages are also possible, but, as a rule, not required. It is possible to employ sporocysts of only one pathogen
10 species or else mixtures of sporocysts of different pathogens.

Harmful and other rodents to be controlled are, for the purposes of the invention, rodents from the order Rodentia. The genera *Rattus*, *Mus*, *Bandicota*, *Nosokia* and *Microtus* are particularly emphasized. The representatives of the genera *Rattus* and
15 *Bandicota*, e.g. *R. rattus*, *R. norvegicus*, *B. bengalensis*, *B. indica*, are especially to be emphasized.

Gels for the purposes of the invention are water-based and treated with suitable thickeners. Suitable thickeners are macromolecules such as cellulose derivatives, for
20 example hydroxypropylcellulose, hydroxyethylcellulose, methylcellulose, carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxyethylmethylcellulose, hydroxyethylpropylcellulose or xanthans, alginates, polyvinyl alcohols, polyvinylpyrrolidone, polyacrylic acids and their derivatives, or inorganic gellants such as highly-dispersed silica (see, for example, Rudolf Voigt, Pharmazeutische
25 Technologie [Pharmaceutical Technology], pages 362-385, Ulstein Mosby). Particularly suitable are cellulose derivatives and polyacrylic acids.

The water content of the gels can be varied within wide ranges, for example between 30 and 98% by weight, in particular between 50 and 90% by weight and very
30 especially between 70 and 80% by weight.

The pH of the gels can also be varied within wide ranges, for example between pH 2 and 10, in particular between pH 4 and 9, especially preferably between pH 6 and 8.

5 The gels can be treated with preservatives such as parabens, benzoates, sorbic acid, citrates or parabens, humectants such as glycerol or propylene glycol, antioxidants such as butylhydroxytoluene or butylhydroxyanisole, tocopherol, ascorbic acid, flavourings or other formulation auxiliaries.

10 In the compositions according to the invention, the sporocysts of the pathogenic protozoa can also be combined with further, including, for example, chemical, active compounds for rodent control. The coumarins may be mentioned here by way of example, but not by limitation.

15 The compositions according to the invention are prepared by mixing the components in the relevant proportions. Mixing is performed mechanically, for example in stirred vessels or other apparatuses which are suitable for this purpose.

20 The examples which follow are intended to illustrate the present invention, but without imposing any limitation.

Example:

Method

5 The used sporocysts of strain S5 (available at Hohenheim University, Parasitology
Department) originate from a Passage in Thailand dated 17th August 1998. A
Neubauer haematocytometer was used for counting. The gels were prepared with 1%
Carbopol 974 P (Carbopols: weakly crosslinked polyacrylic acids) in water
10 (342 ppm). 2N NaOH was added by titration to adjust the pH. The preservatives used
were 0.02% of Solbrol P and 0.14% of Solbrol M (Solbrols: para-hydroxybenzoic
acid esters (methyl, ethyl, butyl)). One sample contained 20% of glycerol. 50,000
sporocysts per ml were added to all gels and the gels were mixed thoroughly in order
to achieve homogeneous distribution. The gels were kept in the refrigerator at
approx. 6°C and administered at the respective experimental days in accordance with
15 the experimental schedule.

All the experimental animals used were wild *R. norvegicus*. 14 hours prior to being
offered the sporocyst bait, the animals were starved and had ad libitum access to
water. The bait was based on crushed wheat. Each rat was given 5 g of crushed wheat
20 treated with 1 ml of gel. The rats were again offered standard feed ad libitum only
after they had eaten the *Sarcocystis* bait. Two rats which were observed up to 21 days
after dosing were used for each dosage. The parameters determined were the
mortality as a function of the gel formulation and the storage period. Rats which
showed severe symptoms within the period during which an effect of the pathogen
25 was expected (days 13-17) which would make survival under natural conditions
unlikely were destroyed.

Results

Table 1:

5 Mortality after the uptake of bait with 50,000 sporocysts (* with 100,000) in various gels on crushed wheat as a function of gel storage time.

Gel			Mortality in (%) after a week's storage time									
pH	Solbrol	Glycerol	0	2	4	6	8	10	12	14	19	24
6.17	+	-	100			100		100		50	0	
7.04	+	+	100			50	0			0	0	
7.05	-	-	100		100		100		0			50*
8.19	+	-	100			100		100		0	0	

Patent Claims

1. Composition containing sporocysts of pathogenic protozoa in an aqueous gel.
- 5 2. Composition according to Claim 1, characterized in that the aqueous gel is based on water and a thickener from the group consisting of cellulose derivatives, xanthans, alginates, polyvinyl alcohols, polyvinylpyrrolidone, polyacrylic acids and inorganic gellants.
- 10 3. Compositions according to Claims 1 and 2, characterized in that they contain sporocysts of pathogenic protozoa at a concentration of 1000 to 10,000,000 per ml.
- 15 4. Compositions according to Claims 1 and 2, characterized in that they contain between 30% and 98% of water.
- 20 5. Composition for controlling rodents, characterized in that it contains the compositions according to Claims 1 and 2 in addition to the customary bait materials.
6. Use of compositions according to Claims 1 and 2 for the preparation of baits for controlling rodents, characterized in that the compositions are mixed with customary bait materials.
- 25 7. Use of compositions according to Claims 1, 2 and 5 for controlling rodents, characterized in that the compositions are placed in the rodents' environment.