CENTRAL VETERINARY LABORATORY, MAFF

Trial to evaluate the efficacy of Stalosan F disinfectant against coccidial oocysts

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AUTHENTICATION:

I declare that this work was done under my supervision according to the procedures described herein and that this report represents a true and accurate record of the results obtained.

SIGNED:	Janet Catchpole BSc Contract Manager
DATED:	----------
Report Authorised by:	
	Miss S Bellworthy Parasitology Department Central Veterinary Laboratory Weybridge, Surrey
DATED:	----------

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SCHEDULE 1

OUTLINE PROTOCOL FOR TRIAL
to evaluate the efficacy of test disinfectant against coccidial oocysts.

Objective

To evaluate the efficacy of the test disinfectant in the prevention of sporulation of coccidial oocysts (Stage 1), and in the killing of sporulated oocysts (Stage 2).

Test material

"STALOSAN F" dry disinfectant powder.

Parasites

A fresh culture of oocysts will be prepared by infecting coccidia-free chicks with the Raglington strain of E.acervulina. Faeces will be collected six days later into ice water and oocysts extracted from the faeces using standard CVL methods, in the cold to prevent the start of sporulation. Half of the oocysts will be put to sporulate by the normal CVL methods for stage 2 of the trial.

Trial Design
Oocysts will be spread as a thin layer in a shallow, flat dish, and disinfectant powder will be spread evenly over the layer at the required concentration, as in Tables 1 and 2. Talc powder or quartz or fine sand will be used as a control, at similar concentrations.

The dish will be placed in a closed box and maintained between 16 - 22ºC.

Unsporulated oocysts will be used for Stage 1, and sporulated oocysts for Stage 2. Oocysts will be left in contact with the powder for 24 or 48 hours. An outline of the trial design in shown in Tables 1 and 2.

Table 1. Unsporulated oocysts for Stage 1

Group	Powder	Concentration	Time
1	Stalosan	30g/sq. m	24 hours
2	Stalosan	50g/sq. m	"
3	Stalosan	80g/sq. m	"
4	Talc	30g/sq. m	"
5	Talc	50g/sq. m	"
6	Talc	80g/sq. m	"
7	Stalosan	30g/sq. m	48 hours
8	Stalosan	50g/sq. m	"
9	Stalosan	80g/sq. m	"
10	Talc	30g/sq. m	"
11	Talc	50g/sq. m	"
12	Talc	80g/sq. m	"

Table 2. Sporulated oocysts for Stage 2.

Group	Powder	Concentration	Time
13	Stalosan	30g/sq. m	24 hours
14	Stalosan	50g/sq. m	"
15	Stalosan	80g/sq. m	"
16	Talc	30g/sq. m	"
17	Talc	50g/sq. m	"
18	Talc	80g/sq. m	"
19	Stalosan	30g/sq. m	48 hours
20	Stalosan	50g/sq. m	"
21	Stalosan	80g/sq. m	"
22	Talc	30g/sq. m	"
23	Talc	50g/sq. m	"
24	Talc	80g/sq. m	"

After the required exposure time the oocysts will be washed free of disinfectant by addition of water and repeated centrifugation.

The unsporulated oocysts will then be put to sporulate at 27 ºC for seven days, using the standard CVL sporulation method.

The sporulated oocysts from each trial dish will be dosed to six 12-day-old , coccidia free chicks

Parameters recorded

Stage 1

After seven days an assessment of sporulation will be made by examining oocysts under the microscope and the percentage of sporulated oocysts recorded.

This assessment will be done blind and in triplicate.

Stage 2

The number of parasites produced seven days later by each group of chicks will be counted using standard CVL methods.

Results and report

Stage 1

Comparison of sporulation rates between untreated and the various concentration of disinfectant will give a measure of the efficacy of the disinfectant in preventing sporulation of the oocysts.

Sporulation does not necessarily measure the infectivity of these oocysts although it is usually regarded as such a measure.

Stage 2

Comparison of parasite burdens of chicks dosed with treated and untreated oocysts will give a measure of the efficacy of the disinfectant in the killing of sporulated oocysts.

A short report on the results will be submitted within three weeks of completion of the work.

It is anticipated that this trial could be carried out in July/August 1996. Exact dates to be forwarded to the company when the contract is signed.

This method of determining efficacy of a dry powder disinfectant is untried. It is proposed to set up a small scale pilot using talc or quartz or fine sand and stock oocysts to find the optimum volume of coccidial suspension needed to test the system.

Materials and Methods

Test material

Following consultation with E. Jones silver sand was substituted for talc as the control carrier in the protocol.

The trays over which the oocysts were spread measured 11.2cm x 21.8cm giving an area of 244.2 cm^2 , or 0.0244m^2.

The amount of Stalosan F or silver sand control material required for each tray is shown in table 1.

Table 1. Amount of test material needed for each concentration

Test concentration	Amount / tray
30g/sq. m	0.73g
50g/sq. m	1.22g
80g/sq. m	1.95g

The required amounts of Stalosan F or silver sand were weighed into separate small bottles with perforated lids.

Preparation of parasites

A group of 20 chickens was infected with E.acervulina oocysts and the faeces containing fresh unsporulated oocysts collected from beneath their cages six days later.

The oocysts were extracted from the faeces using standard CVL methods and the final suspension of oocysts in water was divided into two portions. One portion, used for stage 1, was initially placed at 4 ºC to prevent sporulation. The second portion was put to sporulate at 27 ºC for seven days before exposure to the disinfectant in stage 2 of the trial. Antibiotics were added to control any bacterial contamination.

Stage 1. Exposure of unsporulated oocysts

The number of oocysts present in the suspension was determined using standard CVL methods at 4,356,000 oocysts/ml.

This was adjusted to give 500,000/ml in water.

10ml of suspension containing 5,000,000 oocysts was added to labelled trays and disinfectant powder or silver sand sprinkled evenly over the liquid and left to stand for 24 or 48 hours as in table 2.

Table 2. Designation of test for unsporulated oocysts.

Group	Powder	Concentration	Time
1	Stalosan	30g/sq. m	24 hours
2	Stalosan	50g/sq. m	"
3	Stalosan	80g/sq. m	"
4	Silver sand	30g/sq. m	"
5	Silver sand	50g/sq. m	"
6	Silver sand	80g/sq. m	"
7	Stalosan	30g/sq. m	48 hours
8	Stalosan	50g/sq. m	"
9	Stalosan	80g/sq. m	"
10	Silver sand	30g/sq. m	"
11	Silver sand	50g/sq. m	"
12	Silver sand	80g/sq. m	"

After the required time the residual material on the trays was washed off thoroughly, mixed with water and passed through muslin to remove any coarse particles. The remaining powder was removed by a light spin in the centrifuge leaving the oocysts in the supernatant. The oocysts were recovered by repeated washing in water and were put to sporulate at 27 ºC for seven days. Antibiotics were added to control any bacterial contamination.

After seven days a sporulation assessment was made on each batch of treated material.

Stage 2. Exposure of sporulated oocysts.

The oocysts put to sporulate after the initial extraction from the faeces were counted, the sporulation rate determined.

The suspension contained 4,023,000 oocysts per ml with 80% sporulated, equivalent to 3,218,400 sporulated oocysts/ml.

80% was taken as the expected sporulation rate for this batch of coccidial oocysts.

The suspension of oocysts was adjusted to give 500,000/ml in water.

10ml of the suspension, containing 5,000,000 oocysts, was added to labelled trays and disinfectant powder or silver sand sprinkled evenly over the liquid and left to stand for 24 or 48 hours as in table 3.

Table 3. Designation of test for sporulated oocysts.

Group	Powder	Concentration	Time
13	Stalosan	30g/sq. m	24 hours
14	Stalosan	50g/sq. m	"
15	Stalosan	80g/sq. m	"
16	Silver sand	30g/sq. m	"
17	Silver sand	50g/sq. m	"
18	Silver sand	80g/sq. m	"
19	Stalosan	30g/sq. m	48 hours
20	Stalosan	50g/sq. m	"
21	Stalosan	80g/sq. m	"
22	Silver sand	30g/sq. m	"
23	Silver sand	50g/sq. m	"
24	Silver sand	80g/sq. m	"

After the required time the residual material on the trays were washed off thoroughly, mixed with water and passed through muslin to remove any coarse particles. The remaining powder was removed by a light spin in a centrifuge leaving the oocysts in the supernatant. The oocysts were recovered by repeated washing with water until all traces of the powder was removed.

The number of oocysts present was determined using standard CVL methods.

The sporulated oocysts were then dosed to groups of eight coccidia-free chickens, such that each chicken received 100,000 infective oocysts based on a sporulation rate of 80%.

The total bodyweight of each group of birds was recorded on the day of infection (day 0), and six days later (day 6).

Faeces were collected from each group of birds from trays below the cages.

RESULTS

The trays containing the test material dried up with both Stalosan and silver sand. After 24 hours the material was slightly damp but by 48 hours was completely dry.

Stage 1. Exposure of unsporulated oocysts

Effect of Stalosan F on the sporulation rate of coccidial oocysts

The data sheets for the sporulation assessments made on each batch of treated material are in appendix 1 and the mean values for each group and a calculation of the rate compared to the equivalent control level are summarised in table 4.

Table 4. Sporulation percentages of oocysts treated before sporulation

Group	Powder	Concentration	Time	% sporulated	% of control
1	Stalosan	30g/sq. m	24 hours	69	87
2	Stalosan	50g/sq. m	"	40	52
3	Stalosan	80g/sq. m	"	35	47
4	Silver sand	30g/sq. m	"	79	100
5	Silver sand	50g/sq. m	"	77	100
6	Silver sand	80g/sq. m	"	75	100
7	Stalosan	30g/sq. m	48 hours	53	88
8	Stalosan	50g/sq. m	"	46	67
9	Stalosan	80g/sq. m	"	36	40
10	Silver sand	30g/sq. m	"	60	100
11	Silver sand	50g/sq. m	"	69	100
12	Silver sand	80g/sq. m	"	90	100

Oocysts treated with silver sand for 24 hours showed only slight reduction in sporulation rate when compared to the 80% seen with oocysts sporulated by usual methods, after 48 hours the reduction was greater in two of the silver sand groups. The sporulation rates obtained for group 12 cannot be easily explained.

The sporulation rates of oocysts treated with Stalosan F are reduced in comparison to the oocysts treated with silver sand and also markedly reduced when compared to the 80% seen with untreated oocysts shown in table 5.

Table 5. Reduction in sporulation rate of treated oocysts

Group	Powder	Concentration	Time	% reduction cf control	%reduction cf untreated

1	Stalosan	30g/sq. m	24 hours	13	14
2	Stalosan	50g/sq. m	"	48	50
3	Stalosan	80g/sq. m	"	53	56
4	Silver sand	30g/sq. m	"	0	1
5	Silver sand	50g/sq. m	"	0	4
6	Silver sand	80g/sq. m	"	0	6
7	Stalosan	30g/sq. m	48 hours	12	43
8	Stalosan	50g/sq. m	"	33	42
9	Stalosan	80g/sq. m	"	60	55
10	Silver sand	30g/sq. m	"	0	25
11	Silver sand	50g/sq. m	"	0	14
12	Silver sand	80g/sq. m	"	0	-

The reduction in sporulation rate appeared to increase at the higher concentrations of the Stalosan disinfectant powder.

Stalosan at 50g/sq.m for 24 hours gave a 50% reduction and similar results were seen at all concentrations after 48 hours exposure.

Stage 2. Exposure of sporulated oocysts.

Effects on growth rate of chicks

The infected chicks were batch weighed by group on day of infection and six days later. The actual weight sheets are in appendix 2 and group mean growth rates from day 0 to 6 summarised in table 6.

Table 6. Growth of birds infected with oocysts that were exposed to disinfectant after sporulation

Group	Powder	Concentration	Time	weight gain, day 0 to 6	% of control
13	Stalosan	30g/sq. m	24 hours	418	97
14	Stalosan	50g/sq. m	"	440	88
15	Stalosan	80g/sq. m	"	378	78
16	Silver sand	30g/sq. m	"	432	100
17	Silver sand	50g/sq. m	"	500	100
18	Silver sand	80g/sq. m	"	488	100
19	Stalosan	30g/sq. m	48 hours	430	98
20	Stalosan	50g/sq. m	"	418	90
21	Stalosan	80g/sq. m	"	428	100
22	Silver sand	30g/sq. m	"	440	100
23	Silver sand	50g/sq. m	"	465	100
24	Silver sand	80g/sq. m	"	430	100

The growth rates achieved by all infected groups except group 15 were very similar, birds infected with oocysts treated with silver sand appeared to perform slightly better than those receiving oocysts treated with disinfectant.

Faeces collected from each group of birds were counted using MacMaster counting chambers to determine the total number of oocysts produced by each group of birds and the average number produced per bird.

The data counting sheets are in appendix 2 and results are summarised in table 7.

Table 7. Oocyst output from chicks infected with oocysts that were exposed to disinfectant after sporulation.

Group	Powder	Concentration	Time	Total x 10^6	Oocysts per bird x 10^6
13	Stalosan	30g/sq. m	24 hours	848	106

14	Stalosan	50g/sq. m	"	544	68
15	Stalosan	80g/sq. m	"	608	76
16	Silver sand	30g/sq. m	"	1408	176
17	Silver sand	50g/sq. m	"	1312	164
18	Silver sand	80g/sq. m	"	1504	188
19	Stalosan	30g/sq. m	48 hours	1216	152
20	Stalosan	50g/sq. m	"	1232	154
21	Stalosan	80g/sq. m	"	1264	158
22	Silver sand	30g/sq. m	"	960	120
23	Silver sand	50g/sq. m	"	1552	194
24	Silver sand	80g/sq. m	"	1648	206

Very high numbers of oocysts were produced by every group of infected birds. There is a very slight reduction in the number of oocysts produced by the birds infected with Stalosan F treated oocysts.

In coccidial infections high oocyst output can be produced by birds dosed with low numbers of infective oocysts.

However the birds infected with oocysts treated with silver sand, which was assumed to be inert, grew slightly better than those infected with the treated oocysts. So it is unlikely that the high oocyst output is due to a reduction in viable oocysts from Stalosan F treated oocysts compared to silver sand treated oocysts as the birds would have shown improved growth rates.

CONCLUSIONS

Effects of Stalosan F disinfectant on unsporulated oocysts.

The unsporulated oocysts exposed to Stalosan F showed reduced sporulation rates compared both to the silver sand control material and to untreated oocysts. The reduction in sporulation rate increased with exposure time.

Effects of Stalosan F disinfectant on sporulated oocysts.

The oocysts exposed to Stalosan F disinfectant after sporulation caused a very slight weight reduction in infected birds compared to control oocysts treated with silver sand, suggesting no loss of activity of the oocysts.

There was no significant difference in oocyst output between oocysts treated with Stalosan F disinfectant and those treated silver sand.

Stalosan F would appear to have some activity against unsporulated oocysts and as this is the stage excreted by the host animal there made a role for this product in reducing the initial environmental challenge.

The trial design is untested and the oocysts used were of Eimeria genera rather than Isospora genera, the common coccidia causing problems with young pigs. It was assumed that both genera would behave similarly but this may not be the case as the sporulation time for Isospora sp. may be shorter.

Appendix 1 -

Treatment of unsporulated oocysts

Sporulation counts of oocysts treated before sporulation

Appendix 2 -

Treatment of sporulated oocysts

Counts of sporulated oocyst cultures

Raw data bodyweights for infected chicks
Faecal oocyst counts from chicks