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# The effect of Stalosan F on selected poultry parasites

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**Summary**

Production losses are significantly higher in free-range table egg production systems compared to conventional intensive indoor production systems. No methodical analysis of the cause of the higher morbidity and mortality is available at present. However, investigations have shown that infe-tions with intestinal roundworms such *as Ascaridia galli*, *Heterakis gallinarum and Capillaria obsignata* are more prevalent in free-range production systems. These infections might cause production losses in the range of 10-20% due to impaired feed conversion, reduced growth and egg production, and increased mortality. The potentially high level of disease in the organic production of broilers and eggs for consumption, together with anthelmintic regulations in force, show a marked need for alternative methods of parasite control in organic poultry production. The aim of this study was to examine whether Stalosan F could be an alternative or supplement in the control of selected endoparasites in poultry.

Three experiments were conducted to examine the effect of Stalosan F on *A. galli, H. gallinarum* and *C. obsignata* eggs under *in vitro* and *in vivo* conditions. In short, the results suggest that Stalosan F does have a sublethal effect on these parasite eggs. Under laboratory conditions eggs treated with Stalosan F showed less ability to develop into infective stages and establish in subsequently experimentally infected chickens. Under field conditions, an area heavily contaminated with *A. galli, H. gallinarum and C. obsignata* eggs, was divided into two pens. One pen was treated with Stalosan F after which tracer animals were inserted into both pens. Tracer animals from the Stalosan F treated pen, were found to harbour significantly fewer adult worms than the chickens from the untreated pen. However, no differences were found between the two groups in the total number of worms (larvae and adults) recovered. It therefore seems that the development from larvae to adult worm was arrested by the use of Stalosan F. This could be of importance to the epidemiology of the worms, since it would decrease the reproduction rate of the worms hereby reducing the number of parasite eggs in the pen. Based on these results it seems that regular use of Stalosan F in free-range poultry production systems may reduce the infectivity of *A. galli, H. gallinarum and C. obsignata* eggs.

Key words: Stalosan F; *Ascaridia galli; Heterakis gallinarum; Capillaria obsignata; in vivo; in vitro;* infectivity

## Introduction

Poultry production in Denmark and other industrialised countries has gone through major changes since the 1950'es due to the development of new technology, abolition of tariff barriers and international competition. From mainly being a “backyard business”, poultry is now being produced intensively at relatively few, but large units consisting of up to 275,000 birds. Today, Denmark has a production of approximately 80 million kilogram eggs and 130 million broilers per year (Anon., 2002). In Denmark, recent changes in consumer demands have resulted in an increasing number of table eggs being produced in free-range systems. The production of broilers in free-range systems are also increasing, although not yet fully established due to bacterial as well as parasitic problems.

Production losses are significantly higher in free-range production systems compared to conventional intensive indoor production systems. No systematic analysis of the cause of the overall mortality and morbidity in free-range production systems is available at present. However, a crosssectional study has shown that infections with intestinal roundworms such as *A. galli, H. gallinarum and C. obsignata* are highly prevalent in free-range systems (Permin *et al.*, 1999). Infections with such endoparasites have been estimated to cause production losses in the range of 10-20 % due to impaired feed conversion, reduced growth and egg production, and increased mortality (Ikeme, 1971; Soulsby, 1982). *H. gallinarum* is of additional importance due to its ability to transfer the protozoa *Histomonas meleagridis*, which causes Blackhead in poultry (Soulsby, 1982). Furthermore, a study have shown that *Salmonella enterica* can be transferred by eggs from *A. galli* to chickens (Chadfield *et al.*, 2001). Also, ongoing investigations suggest that *A. galli* have a negative impact on concurrent infections with *Pasteurella multocida* and *E. coli* (Dahl *et al*., 2002; Permin *et al*., 2002).

The high prevalence of parasites in free-range systems is probably a result of several factors. The animals have access to outdoor areas where a range of parasites have optimal conditions, and where the increased contact to wildlife constitute an additional risk of contamination of the farm. Also, the typical large number of animals in the flocks, together with regulations in force that have prohibited the use of prophylactic anthelmintics, greatly enhances the risk of parasitic diseases. Therefore, there is a marked need for alternative methods of parasite control in free-range poultry production systems.

The dry powder Stalosan F is used in pig, cattle and poultry houses as a disinfectant. Several studies have demonstrated the capability of Stalosan F to reduce numbers of bacteria, virus and fungi (Anon., 2000). Furthermore, it has been shown that the disinfectant has an effect on sporulation rates of coccidian oocysts (Anon., 2000). These findings have raised expectations that Stalosan F might have an effect on eggs from other parasites, and that the disinfectant consequently can function as an alternative or a supplement to conventional control of parasites in free-range poultry production systems.

The main purpose of the present study was therefore to evaluate the effect of Stalosan F on the commonly occurring poultry parasites *A. galli, H. gallinarum*, and *Capillaria* spp. in three experiments. First, the effect of *in vitro* application of Stalosan F to different developmental stages of the parasite eggs was evaluated. Secondly, it was examined whether this treatment had any effect on the infectivity of the eggs. The third experiment assessed the impact of applying Stalosan F to a parasite egg contaminated environment on infection of chickens.

## Materials and Methods

*Parasite material and isolation of mixed parasite eggs from faeces*
Twenty hens, excreting *A. galli, H. gallinarum* and *Capillaria* spp. eggs, were purchased from an organic egg production system and caged. Approximately 5 kilogram fresh faeces was collected from the hens during a 48 hour period. The pooled faeces was mixed with tap water before being washed through a series of sieves with mesh apertures of 200, 90, 20, respectively. The material (including parasite eggs) retained in the last sieve was collected and kept in a small volume of water. After counting the eggs, the suspension was diluted to contain 2500 eggs/ ml and then divided into three portions: One portion was stored at 5°C to prevent embryonation of the eggs (two days later, this portion was used to evaluate the effect of Stalosan F on newly excreted parasite eggs). The second portion was stored in 0.1 N sulphuric acid ($H_2SO_4$) according to the method described by Permin *et al*. ($1997_a$) at 18°C for 5 weeks to embryonate in order to study the effect of the disinfectant on the infective $L_3$-stage of the parasites. The third portion was also stored at 5°C to prevent embryonation of the eggs. These eggs were later used in an experimental infection to evaluate the effect of Stalosan F on the infectivity of parasite eggs.

*Isolation of A. galli eggs from adult female worms*
A batch of *A. galli* eggs was obtained according to a method described by Permin et al. (1997$_a$). In short, the hens were slaughtered and adult female *A. galli* collected from the small intestine. The worms were dissected and their uteri transferred to a Petri dish where the eggs were squeezed out using a wooden spatula. Tissue debris were removed and the eggs mixed with a small amount of tap water. The eggs were counted and the suspension diluted to contain 2500 eggs/ml and then divided into two portions. One portion was stored at 5°C to prevent embryonation of the eggs. Two days later, this portion was used to evaluate the effect of Stalosan F on nonembryonated *A. galli* eggs. The second portion was stored in 0.1 N sulphuric acid at 18°C for 5 weeks until the eggs were embryonated in order to study the effect of the disinfectant on the infective $L_3$-stage of *A. galli*.

*Experiment 1: in vitro treatment and examination*
The effect of *in vitro* treatment with Stalosan F was examined and compared with commercial bird sand as control for all four batches of parasite eggs: 1) newly excreted non-embryonated mixed eggs; 2) embryonated mixed eggs; 3) freshly extracted nonembryonated *A. galli* eggs; and 4) embryonated *A. galli* eggs. A 300 µl egg suspension (~750 eggs) was spread over a layer of neutral agar in labelled Petri dishes. Stalosan F or bird sand was then sprinkled evenly over the egg suspension in the Petri dishes at a concentration of 100 g/m$^2$ and incubated at 18°C for 1, 2, 3, 7 or 21 days (see Table 1). After the required exposure time the eggs were washed off the Petri dishes in tap water. The disinfectant or sand was subsequently removed by a light spin in the centrifuge leaving the eggs in the supernatant. The eggs were examined under microscope and judged either as “normal appearing” or “abnormal”. The experiment was done in triplicate.

*Experiment 2: Experimental infections*
A batch of parasite eggs was made to evaluate the effect of Stalosan F on the infectivity of newly excreted mixed parasite eggs. Half of the batch was, together with the disinfectant (100 g/m$^2$), applied over a layer of neutral agar in large Petri dishes and incubated at 18°C for one week, after which the parasite eggs were washed in tap water and then incubated in 0.1N sulphuric acid at 18°C. The other half of the batch was treated similarly, except that commercial bird sand was used instead of the disinfectant. After 4 weeks of further incubation (i.e. a total of 5 weeks), the eggs were counted and diluted to contain 1000 eggs/ml in both batches and two groups of 25 parasitenaive chickens, 4 weeks of age, were subsequently inoculated with 500 eggs. Group 1 received the Stalosan F -treated eggs and group 2 received the untreated eggs. All animals were slaughtered 8 weeks after inoculation, and their intestines examined for the presence of parasites (Permin and Hansen, 1998).

**Table 1. Trial design of the *in vitro* study to evaluate the effect of Stalosan F on four different batches of parasite eggs**

| Group | Powder | Parasite eggs | Time |
|---|---|---|---|
| | | Newly excreted (non-embryonated) mixed eggs[a] | |
| 1 | Stalosan | | 24h |
| 2 | Sand | | 24h |
| 3 | Stalosan | | 48h |
| 4 | Sand | | 48h |
| 5 | Stalosan | | 72h |
| 6 | Sand | | 72h |
| 7 | Stalosan | | 1 week |
| 8 | Sand | | 1 week |
| 9 | Stalosan | | 3 weeks |
| 10 | Sand | | 3 weeks |
| | | Embryonated ($L_3$ stage) mixed eggs[a] | |
| 11 | Stalosan | | 24h |
| 12 | Sand | | 24h |
| 13 | Stalosan | | 48h |
| 14 | Sand | | 48h |
| 15 | Stalosan | | 72h |
| 16 | Sand | | 72h |
| 17 | Stalosan | | 1 week |
| 18 | Sand | | 1 week |
| 19 | Stalosan | | 3 weeks |
| 20 | Sand | | 3 weeks |
| | | Freshly extracted (non-embryonated) *A. galli* eggs[b] | |
| 21 | Stalosan | | 24h |
| 22 | Sand | | 24h |
| 23 | Stalosan | | 48h |
| 24 | Sand | | 48h |
| 25 | Stalosan | | 72h |
| 26 | Sand | | 72h |
| 27 | Stalosan | | 1 week |
| 28 | Sand | | 1 week |
| 29 | Stalosan | | 3 weeks |
| 30 | Sand | | 3 weeks |
| | | Embryonated ($L_3$ stage) *B. galli* eggs[b] | |
| 31 | Stalosan | | 24h |
| 32 | Sand | | 24h |
| 33 | Stalosan | | 48h |
| 34 | Sand | | 48h |
| 35 | Stalosan | | 72h |
| 36 | Sand | | 72h |
| 37 | Stalosan | | 1 week |
| 38 | Sand | | 1 week |
| 39 | Stalosan | | 3 weeks |
| 40 | Sand | | 3 weeks |

**a** *consisted mainly of A. galli, H. gallinarum and Capillaria spp. eggs*
**b** *extracted from the uteri of female worms*

*Experiment 3: Natural infection study*
A pen, naturally contaminated with *A. galli* eggs, was divided into two smaller but equally sized pens. In one pen Stalosan F was applied as recommended by the producer, i.e. 50 g/$m^2$ for three consecutive days and then every week. The other pen was left as an untreated control. Twenty-five parasite-free chickens, two weeks of age, were introduced into each pen. The chickens had free access to water and feed, and their weight was recorded at week 1, 3, 5 and 6 after the introduction to the pens. All animals were slaughtered at week 7, and the intestines were examined for the presence of adult, as well as larval stages of *A. galli*.

*Statistical analysis*
Differences in means of egg excretion between the different groups of chickens were analysed for each observation time using the unpaired t-test with a 95 % confidence limit in the data processing software GraphPad Prism®. Worm burdens were similarly compared between the different groups.

## Results

*Experiment 1: in vitro experiments*

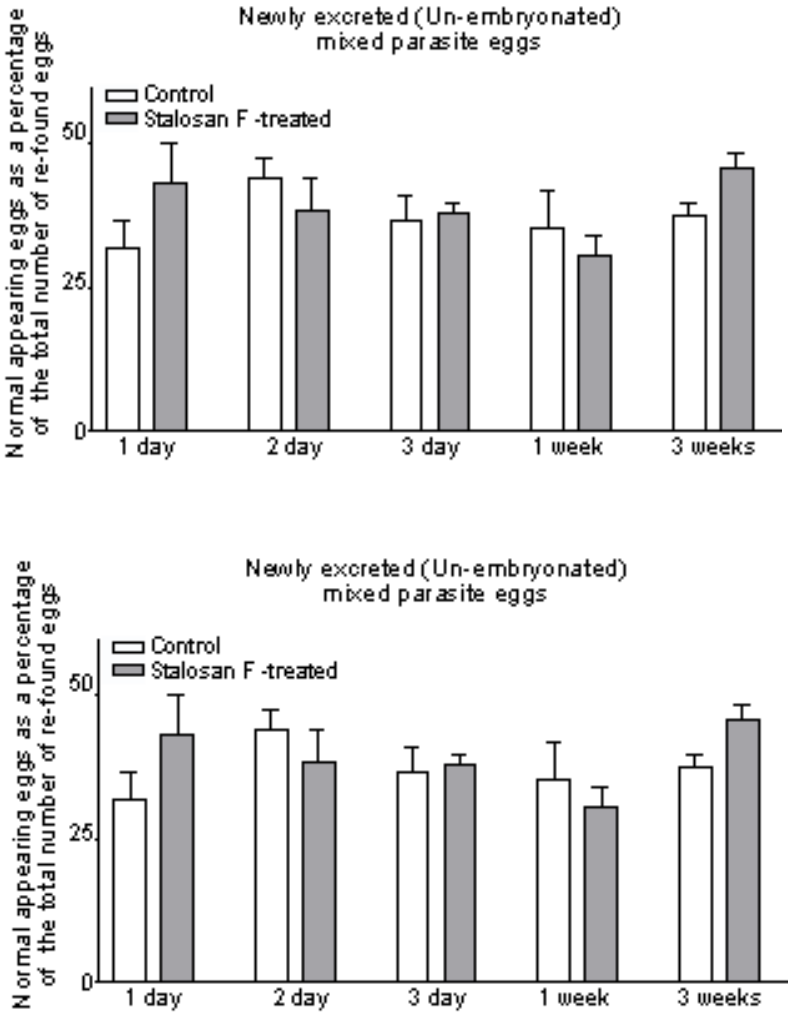


**Fig. 1. Normal appearing eggs as a percentage of the total number of re-found eggs after 1, 2, 3, 7 or 21 days of *in vitro* exposure to Stalosan F or commercial bird sand (control). A batch of newly excreted non-embryonated mixed eggs of *A. galli*, *H. gallinarum* and *Capillaria* spp. was used.**

After the required exposure time the eggs were examined and counted. It was only possible to recover between 126 and 315 of the initially incubated 750 parasite eggs. Thus, between 58 % and 83 % of the eggs were lost during the incubation time or during the procedure where the eggs were washed free of disinfectant or sand. On average, 179 eggs, i.e. ~24 % of the total number of incubated eggs were recovered in the Stalosan F - treated groups. In the control groups, an average of 231 eggs, i.e. ~31 % of the incubated eggs were recovered. However, the difference in recovery of eggs was not insignificant (P > 0.05) in all four parasite egg batches. In the following, the results are presented as normal appearing eggs as a percentage of the total number of recovered eggs.

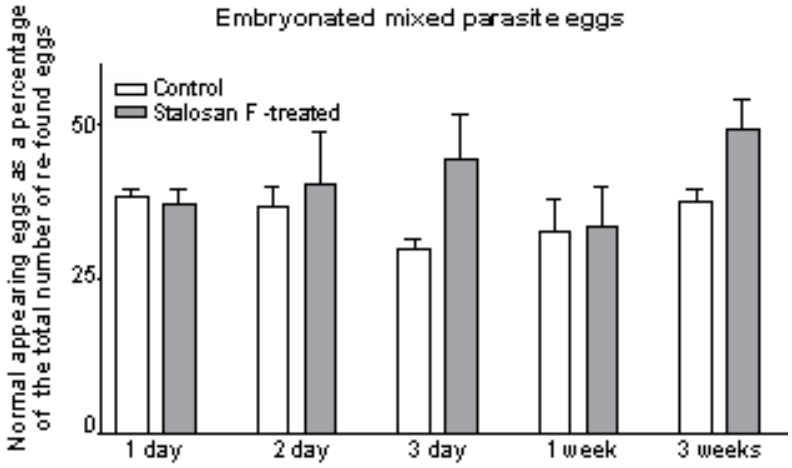


**Fig. 2 Normal appearing eggs as a percentage of the total number of re-found eggs after 1, 2, 3, 7 or 21 days of *in vitro* exposure to Stalosan F or commercial bird sand (control). A batch of embryonated ($L_3$ stage) mixed eggs of *A. galli*, *H. gallinarum* and *Capillaria* spp. was used**

As shown in Fig. 1-4, the proportion of normal appearing eggs was between 25 % and 50 % of the total number of recovered eggs, i.e. between 50 % and 75 % of the total number of recovered eggs were either dead or abnormal. The proportion of normal appearing eggs was very similar in both the Stalosan F -treated and in the control group at most examination times in all four parasite egg batches. Statistically significant difference (P < 0.05) between the Stalosan F - treated and the control group was only found at one occasion: In the batch of embryonated *A. galli* eggs originally extracted from the uteri of female worms, significant more normal appearing eggs were found in the Stalosan F - treated than in the sandtreated control group (Fig. 4).

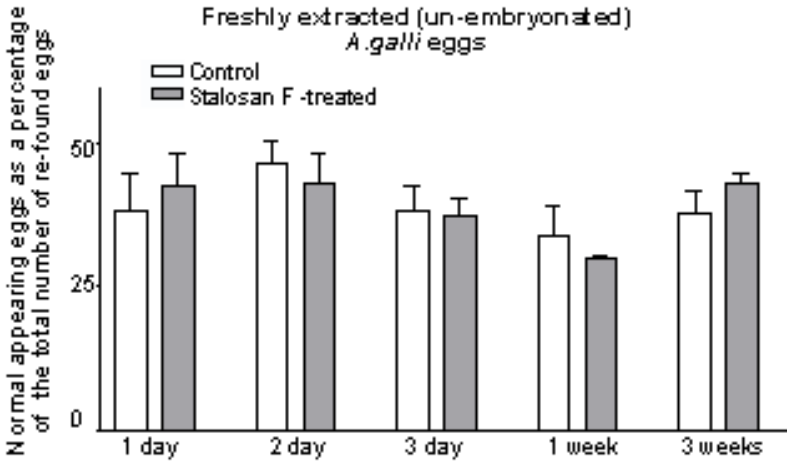


**Fig. 3. Normal appearing eggs as a percentage of the total number of recovered eggs after 1, 2, 3, 7 or 21 days of *in vitro* exposure to Stalosan F or commercial bird sand (control). The egg batch consisted non-embryonated *A. galli* eggs freshly extracted from the uteri of female worms**.

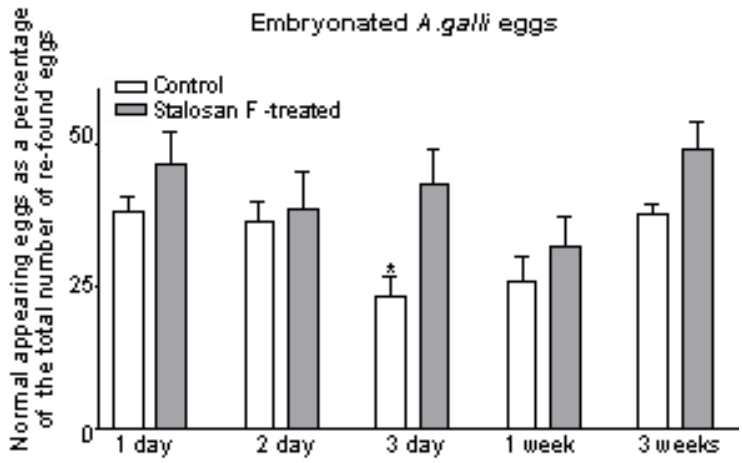


**Fig. 4. Normal appearing eggs as a percentage of the total number of recovered eggs after 1, 2, 3, 7 or 21 days of *in vitro* exposure to Stalosan F or commercial bird sand (control). The egg batch consisted embryonated ($L_3$ stage) *A. galli* eggs originally extracted from the uteri of female worms. The * signifies a statistically significant difference between the Stalosan F -treated and the control group.**

The time of exposure to Stalosan F did not seem to have an effect since the proportion of normal appearing eggs did not change significantly during the 3 weeks of incubation in any of the four parasite egg batches

*Experiment 2: Experimental infection study*

Only two of the 25 chickens in the group that was infected with Stalosan F -treated mixed parasite eggs were found to harbour worms when slaughtered, and each harboured only one adult *A. galli*. In contrast, 12 of the 25 chickens of the control group were found to harbour a number of worms, ranging from 1 to 25. The average worm burden, which was 5.3 (+/- 8.89), was found to be significantly higher ($P < 0.01$) compared to the average worm burden of 0.08 (+/- 0.28) in the group infected with Stalosan F - treated eggs.

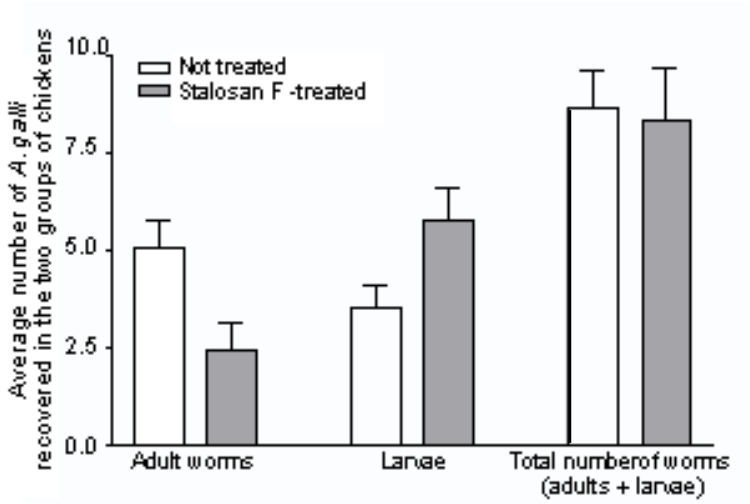


**Fig. 5. The average number of *A. galli* found at the time of slaughter in two groups of 25 chickens. Both groups had been placed in a pen contaminated with *A. galli* eggs for 6 weeks prior to slaughter. One group was confined to half of the pen which was treated with Stalosan F on a weekly basis. The other group was confined in the other half of the pen which was left untreated as control.**

*Experiment 3: Natural infection study*
Fig. 5. illustrates the average number of *A. galli* (adults, larvae and total number) found at the time of slaughter in the group of chickens from the Stalosan F - treated pen compared to the group from the non-treated pen (control). On average, statistically significant ($P < 0.05$) more adult worms were found in the control group. In contrast however, the average number of larvae were found to be significantly higher ($P < 0.05$) in the Stalosan F - treated group. The number of all recovered worms (adults + larvae) were almost the same in both groups (the two groups to the right), with an average of 8.6 ± 1.0 in the control group and an average of 8.3 ± 1.3 in the chickens from the Stalosan F - treated eggs.
No difference in weight gain was found between the two groups of chickens ($P > 0.05$).

## Discussion

The observed loss of between 58 % and 83 % of the incubated parasite eggs during the *in vitro* experiments probably occurred during the procedure of washing the eggs free of the disinfectant or the sand, rather than during the incubation time as the washing procedure involved several steps where eggs could be lost. Some might have been impossible to wash out of the agar in the Petri dish and others might have been lost during centrifugation. Although between 50 % and 75 % of the total number of recovered eggs were dead or appeared abnormal after exposure to Stalosan F a similar proportion of the eggs in the control groups also died or became abnormal during exposure to commercial bird sand. Furthermore, the only time significant difference was found, the proportion of normal appearing eggs was higher in the Stalosan F -treated group. Additionally, time of exposure did not have an effect as the proportion of normal appearing eggs did not change during the 3 weeks of incubation. Thus, *in vitro* application of Stalosan F apparently did not have an effect on parasite eggs either originating from the uteri of female *A. galli* worms or from faeces with a mixture of *A. galli*, *H. gallinarum* and *Capillaria* spp. However, when the infectivity of the batch of Stalosan F - treated mixed parasite eggs was evaluated by inoculating chickens, we found that infection was only established in two chickens out of twenty-five, each harbouring only one *A. galli*. In contrast, significant more worms established in the control group, which received non-treated eggs. This suggests that, although the *in vitro* examination could not detect any difference between treated and non-treated eggs, the exposure to Stalosan F may have had a sub-lethal effect on the parasite eggs which was reflected in a lower infectivity. However, the average worm burden was also lower at slaughter in the control group than normally seen with similar infection doses (Permin *et al.*, 1997b).

In the natural infection study we introduced tracer animals into two pens contaminated with *A. galli* eggs. The chickens from the Stalosan F - treated pen were found to harbour significantly fewer adult worms than the chickens from the untreated pen. However, we also found that the Stalosan F - group harboured significantly more larvae and when comparing the total number of worms (larvae and adults) recovered, no difference between the two groups was found. It therefore seems that the development from larvae to adult worm was arrested in the chickens from the Stalosan F - treated pen. Whether or not these larvae would develop into adults at a later stage remains uncertain, but even a short period of arrested development could be of importance in the epidemiology of the worms, since this would decrease the reproduction rate of the worms hereby reducing the number of parasite eggs in the pen. The average number of *A. galli* recovered at the time of slaughter in the natural infection study was relatively low in both groups, indicating that the pens might have been less contaminated than expected. It is therefore not surprising that no differences in weight gains were found between the two groups of chickens. However, it is generally agreed that severe infections with parasites such as *A. galli* causes impaired feed conversion resulting in reduced growth and egg production. It is thus possible that a stronger contamination of the pens with *A. galli* eggs would have resulted in a difference in weight gain between the two groups of chickens.

Management practices largely determine the extent of helminthosis in chickens (Morgenstern and Lobsiger, 1993; te Winkel, 1997). Total enclosure, improvement of cleaning, disinfection procedures and production according to the “all in - all out” principle have decreased the significance of helminth infections in the modern industrialised poultry production (te Winkel, 1997). With the ban on battery cages, new free-range systems have developed in which the prevention of helminth infections has proved to be difficult (Permin et al., 1999). The use of outdoor areas, where parasite eggs may persist in the environment for years have increased the risk of helminth infections (Ackert, 1931). Management practices including alternate use of the pen and strict disinfection of the house might subsequently reduce problems with helminth infections (Permin and Han-sen, 1998).

In addition to management practices prevention and control can also be obtained through a range of available drugs. Traditionally *A. galli* infections have been treated with piperazine compounds (Horton-Smith and Long, 1956; Nilsson and Alderin, 1988). Piperazine dihydrochloride is highly effective and may be 100 % effective against immature and mature stages of *A. galli*. Comparative investigations of the efficacy of piperazine adipate, levamisole hydrochloride and pyrantel pamoate against *A. galli*, revealed that piperazine adipate was 89.4 % effective against adult worms and 82.2 % effective against immature worms. Levamisole hydrochloride was 91.8 % and 95.8 % effective against immature and adult *A. galli*, respectively. The drug pyrantel pamoate was not as effective as the previous compounds being only 53.8 % and 70.7 % effective against immature and adult stages of *A. galli*, respectively (Verma et *al.*, 1991). Likewise, Pyrantel tartrate was 100 % effective against adult stages of *A. galli*, but had only limited effect against immature worms (Okon, 1975). Also Hygromycin B is highly effective against *A. galli* and *H. gallinarum* when given in the feed. However, the drug was more effective against *H. gallinarum* infections than *A. galli* infections (Shumard et *al*., 1958). Coumaphos was reported to be effective against concurrent infections with the three poultry parasites: C. obsignata, *H. gallinarum*, and *A. galli*. When Coumaphos was given in the feed, it was 99 %, 93 % and 100 % effective against *Capillaria* obsignata, *H. gallinarum*, and *A. galli*, respectively (Eleazer, 1969). Ivermectin was introduced as an antiparasitic drug in 1981. The drug was 90 % and 95 % effective against immature and mature stages of *A. galli*, respectively. The drug was considered of limited use against parasites in poultry until it was available for use in feed or water (Sharma et *al.*, 1990). The efficacy of flubendazole against Amidostomum anseris, *Capillaria* anseris, Trichostrongylus tenuis, and Syngamus trachea in geese was determined. The flubendazole medication resulted in a 100 % worm elimination, no consistent change in egglay performance, and a highly significant increase in hatchability (Vanparijs, 1984). Although proving to be very effective many of these drugs are not registered for use against poultry helminths in poultry in a range of countries. No drugs are currently registered for the treatment of blackhead infections in poultry. Furthermore, the long withdrawal period possibly creates a situation where poultry owners are reluctant to treat their animals due to economic reasons. These aspects further increases the demand of alternative drugs for use in specially table egg production systems.

In conclusion, the results of the present study suggests that alternative ways to control poultry parasites, such as the application of Stalosan F, might be possible. It seems possible that regular use of Stalosan F may contribute to the control of parasites in free-range poultry production systems, although this needs to be confirmed by larger onfarm trials. Furthermore, additional studies are needed to explore other alternatives, i.e. vaccination against parasites (as it is seen done against coccidiosis), alternative drugs (i.e. herb extracts) or the use of genetic resistant animals (Permin and Ranvig, 2001).

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