Review article — Oorsigartikel

Possible application of a nematophagous fungus as a biological control agent of parasitic nematodes on commercial sheep farms in South Africa

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ABSTRACT

Biological control of parasitic nematodes of livestock is currently under development and represents another tool that may be integrated into helminth parasite control strategies. This paper presents a brief introduction to commercial sheep farming in South Africa and currently available nematode parasite control methods. These include the FAMACHA® clinical assay, strategies of pasture management, dilution of resistant worm species by introduction of susceptible worms, breed resistant sheep and nutritional supplementation. The purpose of this paper is to outline the principles of biological control using nematophagous fungi and how it may be applied on sheep farms in South Africa.

Key words: *Duddingtonia flagrans, Haemonchus contortus,* integrated worm control strategies, *Nematodirus spathiger, Oesophagostomum columbianum, Trichostrongylus falculatus.*

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INTRODUCTION

Sheep farming in South Africa is predominantly an extensive enterprise and comprises approximately 29.2 million sheep[1]. Sheep farming is concentrated mainly in the Northern and Eastern Cape, Western Cape, Free State and Mpumalunga provinces[1]. The Western Cape represents the winter rainfall area of South Africa and is divided into 5 subregions: North West, Swartland, Boland, South Coast and little Karoo[7]. The predominant sheep breed is fine-woolled Merinos (50 %), the remaining breeds being locally developed Afrina and Letelle (woolled mutton breeds adapted to arid conditions), Döhne Merino, Merino land sheep and Dorper[1]. Mutton breeds of sheep are found mainly in the more arid areas, while dual-purpose breeds and pure wool breeds occur in the relatively higher-potential areas[7]. Animal production on irrigated areas is not confined to specific regions, whereas quick rotation systems of grazing are normally applied to irrigated pastures.

A number of studies have been conducted to determine the seasonality of intestinal helminth parasites in sheep. In one study, nematodes of sheep on dry land pasture on the Transvaal highveld were assessed[6]. Tracer lambs were placed on pasture periodically, exposed for a period of approximately 6 weeks, and then slaughtered. The peak numbers of parasites, which were predominantly *Haemonchus contortus,* appeared between December and May[6] (Fig. 1). This result was subsequently confirmed in a study by Krecek *et al.*[10]

The epidemiology of sheep nematode parasites in the semi-arid region of South Africa has been investigated in 2 experiments conducted in the Karoo. The Karoo extends westwards from the Drakensberg and covers most of the escarpment as far inland as the northern borders of the Cape Province[19,20]. Despite the prevailing arid conditions, helminth parasites were found to be of great importance, and drenching in April, September and December was recommended[19]. The April drench was aimed at all nematode species, the September drench against *Oesophagostomum columbianum,* and the December drench against *H. contortus.* This was revised later when the dominant species in the same area were found to be *Nematodirus spathiger* and *Trichostrongylus falculatus*[20] (Fig. 2). These parasites reached peak burdens in the winter. The following revised strategic dosing programme was recommended: a March drench to reduce overwintering 4th-stage larvae of *H. contortus,* as well as *N. spathiger* and *T. falculatus,* and a July drench to prevent late winter burdens of *N. spathiger* and *T. falculatus.*

In a workshop aimed at developing recommendations for worm control it was suggested that, except in wet years, no drench was necessary for stock in the dry summer rainfall area (J van Wyk, Faculty of Veterinary Science, University of Pretoria, pers. comm., 2000). However, regular worm egg counts were recommended to monitor worm levels and take steps to reduce excessive accumulation of worms.

METHODS TO CONTROL PARASITIC NEMATODES OF SHEEP

It is important when considering the control of helminths in livestock that the concept of life-long eradication should be abandoned. Not only is such a concept impossible, but it has led to an over-reliance on antiparasitic drugs, resulting in drug resistance within the parasite population. A more sustainable approach is required. Such an approach may seem unattainable upon first consideration, and the benefits received are often realised in the long term in comparison with the use of an effective, broad-spectrum anti-parasitic drug. Some control methods, currently either validated or in the process of validation, that may be applied on commercial sheep farms in South Africa are: smart drenching techniques, including the FAMACHA® (ocular mucous membrane clinical assay), grazing management, the introduction of susceptible worms, breeding for resistance and nutrition. Another tool that may be available to farmers in the near future is the application of biological control using the nematophagous fungus *Duddingtonia flagrans.*

Notwithstanding the prospect of such alternative non-chemical control methods, anthelmintics remain an essential element of parasite control. It should be remembered that most currently available alternatives are not curative and cannot remove a high worm burden from an animal suffering from clinical parasit-

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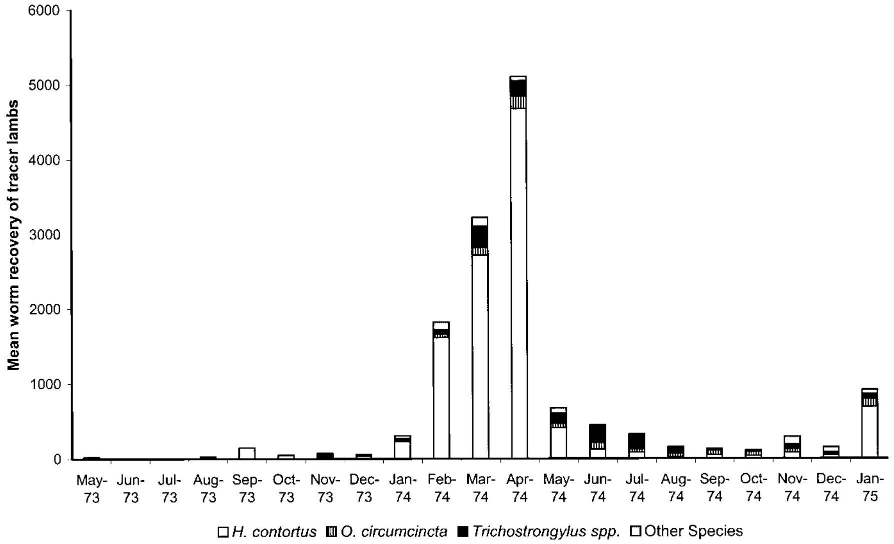

Fig. 1: **Seasonal incidence of helminth infection on a farm in the Transvaal Highveld as indicated by mean worm burdens of tracer lambs. Adaptation of Table 1 in Horak[6].**

ism. One might argue that preventive measures would reduce the need for antiparasitic drugs. However, when considering a sustainable approach to parasite control, all possible methods of treatment should be considered together. Although widespread resistance to anthelmintics exists in sheep in South Africa, so-called smart drenching techniques can be called upon to optimise anthelmintic activity[17]. A cost-effective approach is to reduce animal feeding before administering the drench. The reduced feed intake slows down digesta flow rate, increasing the amount of drench that is absorbed and hence the amount to which the parasites are exposed[5]. In South Africa, a tool aimed at reducing numbers of chemotherapeutic treatments in sheep infected with *H. contortus* has been validated. This is known as the FAMACHA® clinical assay and uses an ocular colour chart for assessing anaemia, the clinical picture associated with *H. contortus* infection in sheep and goats. Overdispersion of gastrointestinal helminths within a sheep flock is the typical picture. Since only a few sheep carry the majority of worms, it is not rational to drench the entire flock as is practised currently. Secondly, it is known that packed cell volume (PCV) is a good index of a sheep's ability to cope with a *H. contortus* infection. The colour of the ocular conjunctiva has proven useful to predict low PCV percentages[13]. An easy-to-use chart has been developed that will allow the farmer to identify the sheep requiring drenching. In some cases it may be advantageous to simply cull the animals with high *H. contortus* burdens as indicated by the level of anaemia with the application of the FAMACHA®. In the Malan and Van Wyk study 69 % of 269 ewes did not require anthelmintic treatment in the peak *Haemonchus* season[13].

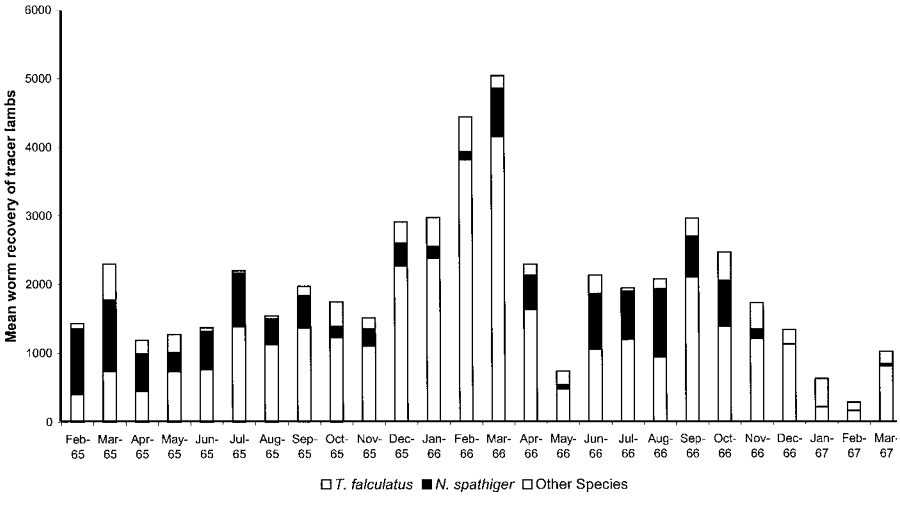

Fig. 2: **Seasonal incidence of helminth infection in the Karoo as indicated by mean worm burdens of tracer lambs. Adaptation of Tables 4, 5 and 6 in Viljoen[19].**

Previously, this flock had to be treated at 3-weekly intervals. Care should be taken when using the FAMACHA® chart, because this simply monitors anaemia without indicating its cause. The presence of *H. contortus* eggs in faeces should also be monitored, as indicated by a faecal culture.

A strategy that aims to increase the vigour of preferred grass species in South Africa to improve fodder flow and animal performance has been outlined[8]. The authors suggested 3 strategies of pasture management in sheep production (Fig. 3). The 2-block strategy is designed for sheep grazing alone, while the 3-block and 4-block strategies are designed for sheep grazing with cattle. Since livestock are concentrated on half of the pasture while the other half is rested, these strategies provide farmers with virtually worm-free pastures annually. This system of management is being promoted in South Africa and offers a simple method of improving pasture quality as well as aiding in the control of animal parasitic nematodes.

The focus on alternatives for parasite control has primarily resulted from the emergence of high level multiple resistance to anthelmintics by sheep parasitic nematodes. A trial was performed in which the resistant population of *H. contortus* was diluted by the addition of susceptible strains of *H. contortus*[18]. Six test paddocks were used in the trial. In 2 paddocks susceptible larvae were introduced in autumn, in another 2 the introduction was made in spring, in 1 paddock the introduction was made in summer, and the remaining paddock served as a control. A reversion to a susceptible population was recorded in the *H. contortus* larvae in 3 of the 5 paddocks, 2 introduced in spring and 1 in autumn. Although 1 autumn and the summer plot did not revert to susceptibility, further testing is warranted. Van Wyk *et al.*[17] suggested that this method should be tested on-farm in conjunction with the 2-block grazing management strategy developed by Kirkman and Moore[8].

One option under investigation for management of anthelmintic resistance is to breed resistant sheep. A clear distinction should be made here between the resistance and resilience of sheep to nematode infection. The different approaches necessary to breed for resistance compared to breeding for resilience have been outlined[14]. Breeding for resistance mainly involves selecting sheep with lower faecal egg counts on the premise that these animals suppress the establishment and/or development of parasite infection. Breeding for resilience normally involves assessing the number and timing of

	Year 1		Year 2		Year 3		Year 4	
	Growing season	Dormant season	Growing season	Dormant season	Growing season	Dormant season	Growing season	Dormant season
(A)								
Block 1	Burn Graze		Rest	Graze				
Block 2	Rest	Graze	Burn Graze					
(B)								
Block 1	Burn Sheep		Cattle		Rest	Graze		
Block 2	Cattle		Rest	Graze	Burn Sheep			
Block 3	Rest	Graze	Burn Sheep		Cattle			
(C)								
Block 1	Burn Sheep		Cattle		Cattle		Rest	Graze
Block 2	Cattle		Cattle		Rest	Graze	Burn Sheep	
Block 3	Cattle		Rest	Graze	Burn Sheep		Cattle	
Block 4	Rest	Graze	Burn Sheep		Cattle		Cattle	

Fig. 3: **Grazing strategies outlined by Kirkman and Moore[8]; A: two-block strategy; B: three-block strategy; C: four-block strategy. A is designed for sheep alone, whereas B and C are designed for sheep grazing with cattle.**

drench treatments required to maintain acceptable health and productivity. These selection criteria aim to breed sheep that are able to maintain an acceptable level of production in the face of helminth challenge. The heritability of resistance is higher than resilience in both Merino and Romney sheep[14]. However, the correlation of production traits with resistance appears to differ between Merino and Romney sheep. Before such schemes could be introduced in South Africa it should be decided which selection criteria would be beneficial for the breeds of sheep used. One option could be to use the FAMACHA® system developed by Malan and Van Wyk to select animals resilient to *H. contortus* infection[13].

The nutritional status of sheep affects infection with gastrointestinal nematodes. Logically, sheep that are well-nourished are better able to mount an immune response against helminth parasite infection and withstand the resulting effects of the infection. A key feature of infections with these parasites is loss of protein in the gastrointestinal tract[2]. Improving protein nutrition will, therefore, better equip livestock to withstand the effects of gastrointestinal infections. The effects of supplementing an animal's diet with protein have been described[2]. In general, protein supplementation increases the rate of acquisition of immunity and increases resistance to re-infection. Improving nutrition is a simple, effective way of improving resilience of livestock to nematode infection and this is immediately available to farmers.

BIOLOGICAL CONTROL OF PARASITIC NEMATODES USING NEMATOPHAGOUS FUNGI

For the past 10 years much attention has been given to the possible use of nematophagous fungi as biological control agents of animal parasitic nematodes. Nematophagous fungi are a group of fungi that use nematodes as a food source. These fungi are commonly grouped in 1 of 3 types: nematode-trapping fungi, endoparasitic fungi and egg-parasitic fungi. Much of the research activity has been focused on the use of nematode-trapping fungi and in particular the fungus *Duddingtonia flagrans* (Fig. 4). *D. flagrans* is not an obligate parasite but produces traps in the presence of nematodes. Nematophagous fungi such as *D. flagrans* do not affect the established populations of worms within the host and are thus not curative. *D. flagrans* targets the free-living larval stages outside the host. These stages exist as larvae within the faecal deposit, and as infective 3rd-stage larvae on pasture. The most obvious way to apply the fungus to the faecal environment is via passage through the gastrointestinal tract of the host. *D. flagrans* is unique in that it produces in vast numbers a survival structure known as a chlamydospore, which, when fed to livestock, can survive passage through the gastrointestinal tract to be deposited in faeces. Once voided in the faeces, *D. flagrans* grows within the faecal deposit and traps emerging larvae. This prevents 3rd-stage larvae from

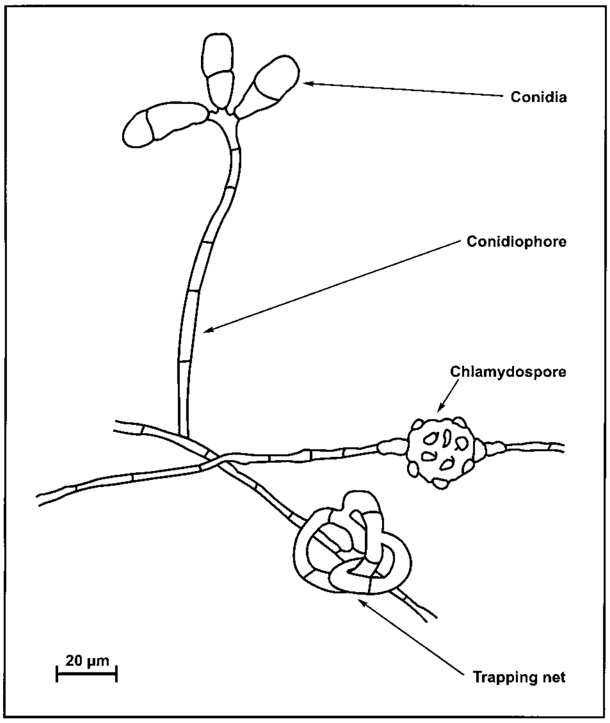

Fig. 4: **Line-diagram showing morphological features of the fungus *Duddingtonia flagrans*. Redrawn from Grønvold *et al.*[4].**

migrating onto pasture and infecting other hosts. Numerous field trials have been conducted that demonstrate successful reduction of parasitic nematode levels on pasture grazed by animals fed the fungus *D. flagrans.* The effectiveness of *D. flagrans* as a biological control agent of parasitic nematodes has been demonstrated in various animal species. Two Danish studies demonstrated that daily feeding of calves with *D. flagrans* during the first 2–3 months of the season led to lowered herbage infectivity and reduced acquisition of *Ostertagia* sp. and *Cooperia* sp. later in the season[12,15]. In another Danish study, feeding *D. flagrans* spores to foals resulted in a reduced herbage larval infectivity[11]. Tracer foals had significantly lower numbers of *Strongylus vulgaris, S. edentatus* and cyathostome larvae compared with controls. Only 1 field trial has been conducted involving pigs, which were fed *D. flagrans* spores over a 2-month period[16]. This resulted in lower herbage larval infectivity and lower worm recoveries (*Oesophagostomum dentatum* and *Hyostrongylus rubidus*) in tracer pigs. Only 2 field trials involving sheep have been conducted. In an Australian field trial, 6 groups of 10 sheep each were set up, with 3 groups offered barley grains on which *D. flagrans* had been cultured. Feeding of fungal spores resulted in reduced faecal egg counts and improved live weight gains[9]. This study demonstrated that *D. flagrans* is effective in warm climates in reducing the contamination of pastures with infective larvae. In the Danish field trial, dosing sheep with fungus led to a reduced number of parasites on pasture, but there was little impact on the parasite burden of the sheep[3]. Feeding *D. flagrans* did not prevent the sheep from picking up infective larvae already on pasture. It should be noted that the total worm burdens of introduced tracer lambs was significantly lower on plots grazed by lambs that had been fed fungi. Hence, if this experiment were carried out over several grazing seasons, the benefits from feeding *D. flagrans* spores might become more apparent. Biological control using *D. flagrans* does not eliminate all of the 3rd-stage infective larvae emerging from eggs deposited in the faeces. Its intended use is as a tool for lowering herbage infectivity at epidemiologically important times.

DISCUSSION

Duddingtonia flagrans potentially presents a farmer with a method of lowering pasture nematode parasite infectivity. The farmer will need to feed *D. flagrans* chlamydospores at times when many eggs are deposited on to pasture, which later lead to peaks in worm burdens in grazing livestock. Hence, a thorough understanding of the epidemiology of parasites is necessary when considering the application of *D. flagrans* as a biological control agent within a parasite control programme. An example of a suitable occasion to feed chlamydospores to livestock would be when ewes are pregnant and lactating. At such times ewes have higher worm egg counts and therefore heavily contaminate pastures.

Often, peaks in larval numbers on pastures can be predicted from previous studies and the prevailing climatic conditions in the summer rainfall region. According to previously published data[6], a beneficial time to feed chlamydospores to sheep would be between October and December (summer). This would theoretically reduce pasture infectivity sufficiently to lower the high *H. contortus* worm burdens seen between January and May (summer and autumn). According to another study, a longer period of feeding would be necessary to prevent the peak in *T. falculatus* and *N. spathiger* worm burdens[20]. Feeding chlamydospores daily from January through to May might not be sufficient to substantially lower pasture infectivity for these 2 nematode genera.

It is best to combine biological control with other tools when developing an integrated worm control programme. A possible combination would be *D. flagrans* and the FAMACHA® clinical assay. Sheep infected with *H. contortus* at levels that cause anaemia would be targeted for anthelmintic treatment using the FAMACHA® chart. The remaining sheep could be dosed with *D. flagrans,* not only lowering herbage infectivity with *H. contortus,* but other important parasite species, such as *O. columbianum* or *Trichostrongylus. Duddingtonia flagrans* chlamydospores could also be administered to sheep in the grazing management strategies outlined by Kirkman and Moore[8]. Thus, biological control application could further ensure that the infectivity on pastures is lowered on available grazing.

CONCLUSIONS

Biological control with *D. flagrans* is a potentially useful control strategy that may be available for use in South Africa in the near future. Before such a strategy can be applied on-farm, a thorough understanding of the epidemiology of the parasites is necessary. Biological control using *D. flagrans* is not designed to be used alone but in combination with other control techniques such as anthelmintic treatment and grazing management.

To date, experimental application of *D. flagrans* to livestock has involved daily feeding of chlamydospores incorporated in a supplement. Although this presents an excellent opportunity to combine good host nutrition strategies with biological control, the extensive farming practised in South Africa hinders application of such a technique. A delivery mechanism (*i.e.* bolus) that is suitable for the extensive farming situation will need to be developed before biological control can be applied to sheep farms in South Africa. It should be noted that all of the published field trials have been conducted in Denmark, a distinctly different climate to South Africa. Field trials need to be conducted in a tropical climate to ascertain whether such a control strategy will be useful in South Africa.

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REFERENCES

1. Anonymous 1998 *South Africa Year Book.* ABC Press, Epping, Cape Town
2. Coop R L, Holmes P H 1996 Nutrition and parasite interaction. *International Journal for Parasitology* 26: 951–962
3. Githigia S M, Thamsborg S M, Larsen M, Kyvsgaard N C and Nansen P 1997 The preventive effect of the fungus *Duddingtonia flagrans* on trichostrongyle infections of lambs on pasture. *International Journal for Parasitology* 27: 931–939
4. Grønvold J, Wolstrup J, Nansen P, Henriksen S A, Larsen M, Bresciani J 1993 Biological control of nematode parasites in cattle with nematode-trapping fungi: a survey of Danish studies. *Veterinary Parasitology* 48: 311–325
5. Hennessy D R 1997 Modifying the formulation or delivery mechanism to increase the activity of anthelmintic compounds. *Veterinary Parasitology* 72: 367–390
6. Horak I G 1978 Parasites of domestic and wild animals in South Africa. V. Helminths in sheep on dryland pasture on the Transvaal highveld. *Onderstepoort Journal of Veterinary Research* 45: 1–6
7. Joubert J G V 1980 Veld and pastures in animal production systems in the Western Cape and South West Africa/Namibia. *South African Journal of Animal Science* 10: 299–303
8. Kirkman K P, Moore A 1995 Veld management strategies for livestock farmers in the Sourveld regions of South Africa. *Bulletin of the Grassland Society of South Africa* 6: 25–28
9. Knox M R, Faedo M 2001 Biological control of field infections of nematode parasites of young sheep with *Duddingtonia flagrans* and effects of spore intake. *Veterinary Parasitology* 101: 155–160
10. Krecek R C, Groeneveld H T, Van Wyk J A 1991 Effects of time of day, season and stratum on *Haemonchus contortus* and *Haemonchus placei* third-stage larvae on irrigated pasture. *Veterinary Parasitology* 40: 87–98
11. Larsen M, Nansen P, Grøndahl C, Thamsborg S M, Grønvold J, Wolstrup J, Henriksen S A, Monrad J 1996 The capacity of the fungus *Duddingtonia flagrans* to prevent strongyle infections in foals on pasture. *Parasitology* 113: 1–6
12. Larsen M, Nansen P, Wolstrup J, Grønvold J, Henriksen S A, Zorn A 1995 Biological control of trichostrongyles in calves by the fungus *Duddingtonia flagrans* fed to animals under natural grazing conditions. *Veterinary Parasitology* 60: 321–330
13. Malan F S, Van Wyk J A 1992 The packed cell volume and colour of the conjunctiva as aids for monitoring *Haemonchus contortus* infestation in sheep. *Proceedings of the SAVA Biennial Veterinary Congress, Grahamstown, South Africa,* 1–10 September 1992: 139
14. Morris C A, Bisset S A 1997 Breeding sheep which require minimal anthelmintic treatment: a review of the genetics of resistance and resilience of sheep to nematode parasites. *Proceedings of Workshop held at the 16th International Conference of the World Association for the Advancement of Veterinary Parasitology, Sun City, South Africa*: 51–63
15. Nansen P, Larsen M, Grønvold J, Wolstrup J, Zorn A,, Henriksen S A 1995 Prevention of clinical trichostrongylidosis in calves by strategic feeding with the predacious fungus *Duddingtonia flagrans. Parasitological Research* 81: 371–374
16. Nansen P, Larsen M, Roepstorff A, Grønvold J, Wolstrup J, Henriksen S A 1996 Control of *Oesophagostomum dentatum* and *Hyostrongylus rubidus* in outdoor-reared pigs by daily feeding with the microfungus *Duddingtonia flagrans. Parasitological Research* 82: 580–584
17. Van Wyk J A, Malan F S, Randles J L 1997 How long before resistance makes it impossible to control some field strains of *Haemonchus contortus* in South Africa with any of the modern anthelmintics? *Veterinary Parasitology* 70: 111–122
18. Van Wyk J A, Van Schalkwyk P C 1990 A novel approach to the control of anthelmintic resistant *Haemonchus contortus* in sheep. *Veterinary Parasitology* 35: 61–69
19. Viljoen J H 1964 The epizootiology of nematode parasites of sheep in the Karoo. *Onderstepoort Journal of Veterinary Research* 31: 133–142
20. Viljoen J H 1969 Further studies on the epizootiology of nematode parasites of sheep in the Karoo. *Onderstepoort Journal of Veterinary Research* 36: 233–264