Acta vet. scand. 2004, *45,* 149-160.

Evaluation of Copper Supplementation to Control *Haemonchus contortus* Infections of Sheep in Sweden

By *P. J. Waller*[1], *G. Bernes*[2], *L. Rudby-Martin*[3], *B.-L. Ljungström*[4] and *A. Rydzik*[1]

[1]Department of Parasitology (SWEPAR), National Veterinary Institute and Swedish University of Agricultural Sciences, Uppsala, [2]Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, Umeå, [3]Svenska Djurhälsovården AB (SvDHV), Kävlinge, and [4]Vidilab, Enköping, Sweden.

Waller PJ, Bernes G, Rudby-Martin L, Ljungström BL and Rydzik A: Evaluation of copper supplementation to control *Haemonchus contortus* infections of sheep in Sweden. Acta vet. scand. 2004, 45, 149-160. – A pen study was conducted to assess the effect of providing daily copper mineral supplement, or copper wire particle (COWP) capsules, on established or incoming mixed nematode infections in young sheep. For lambs with established (6 week old) infections, COWP resulted in 97% and 56% reduction of the adult and early L4 stages of *H. contortus,* respectively, compared with controls ($p<0.001$). Additionally there was a 74% reduction in *Teladorsagia circumcincta* infections in the COWP lambs compared with controls ($p<0.01$). However, no effect was observed when COWP were given at the commencement of a larval dosing period of 6 weeks. There was no significant effect of copper mineral supplement (given at the recommended rate to prevent Cu deficiency) on either established, or developing parasite infections. In addition, a field trial was conducted on a commercial farm to assess the effects of COWP in the management of recurrent *H. contortus* infections, but lack of parasites during the grazing season prevented an adequate assessment from being made. These results indicate that there is little, if any, benefit from a parasite control standpoint in recommending copper therapy, specifically to control parasites in Swedish sheep flocks.

helminth; sheep; copper; organic; production; Sweden

Introduction

Recently, a large-scale survey was completed that was aimed at determining the prevalence and intensity of nematode infections in organically reared sheep flocks throughout Sweden (*Lindqvist et al.* 2001). This study, conducted over 3 consecutive years, showed that internal nematode parasites were a major disease constraint and *Haemonchus contortus* was a particular problem. Further epidemiological studies have shown that this parasite has evolved to survive the long, cold winters in Sweden almost entirely within the host as the arrested larval stage, relying almost entirely on the lambing ewe to complete its life cycle (*Waller et al.* 2004). The Swedish Animal Health Service sheep veterinarians (*L. Rudby-Martin* pers. comm.) and the National Veterinary Institute (*D. Christensson* pers. comm.) report more clinical cases and more post-mortem causes of death due to *H. contortus* in recent years, respectively. This may be attributed to several factors that could include the general trend of warmer and wetter grazing seasons, the greater time animals spend on pasture, ineffective deworming practices, or the further development of anthelmintic resistance in this parasite. Resistance to benzimidazole anthelmintics in *H. contortus* infections of Swedish sheep flocks was detected more than a decade ago (*Nilsson et al.* 1993), but there have been no further investigations since this time.

Certainly the Swedish organic small ruminant (sheep and goat) producers have justifiable causes for concern. This is because the organisations which impose regulations that conform with the statutes developed for organic farming at both the EU level (IFOAM – International Federation of Organic Farming Movements) and nationally (KRAV), stipulate that prophylactic use of any drugs, including anthelmintics, is prohibited. In addition, organically reared animals have to spend more time on pasture, thus potentially exposing them to infective larval pick-up for longer periods. Thus, the problem of *H. contortus* control will inevitably get worse in organic flocks of Sweden.

However, there were findings that came from the survey of *Lindqvist et al.* (2001), which could assist in the management and control of *H. contortus* infections in organically farmed sheep. This was in relation to the effect of mineral supplementation, used particularly to safeguard against hypocuprosis, or copper deficiency, in Swedish pelt (Gotland Breed) sheep flocks (*Schwan et al.* 1987). In flocks provided with mineral supplementation, mean egg counts of lambs in the latter part of the grazing season were substantially less than the egg counts of lambs from flocks not receiving minerals (*Lindqvist et al.* 2001). Furthermore, research in New Zealand has shown that copper wire particle (COWP) boluses, or capsules, administered orally to sheep resulted in a high level anthelmintic effect against *H. contortus,* as well as extended protection against incoming infection of this parasite (*Bang et al.* 1990a, 1990b, *Reid* 1995). Other studies on examining the effects of COWP against *H. contortus* have been conducted in Brazil (*Nyman* 2000), France (*Chartier et al.* 2000), Australia (*Knox* 2002), USA (*Watkins et al.* 2003) and Mexico (*Canto-Dorantes et al.* 2004).

This study was designed to investigate the specific anti-*H. contortus* effects of mineral supplementation and COWP in young sheep in Sweden. The project was conducted in 2 phases. Firstly, in pen studies in young lambs, where parasite infections and mineral dose rates were controlled and other variables minimised. Secondly, a field trial was conducted on a farm in southern Sweden with a history of problems with *H. contortus,* with ewes and their lambs over an entire grazing season.

Materials and Methods

Pen trial

Experimental lambs and treatments. Thirty six female crossbred lambs (50% White Swedish Landrace; 50% Texel) were used in this study. Their pre-experimental history was that they were turned out, together with their dams, onto pasture in late May 2002, when they were 2-4 weeks of age. On 10 July they were dosed with ivermectin (Ivomec®vet, Veter AB, Södertälje, Sweden) and moved to pastures that had not been grazed by sheep since late September 2001, 10 months previously. In late August 2002, the lambs were treated again with anthelmintic (fenbendazole [Axilur®vet, Intervet, Stockholm, Sweden]) and moved onto a newly established pasture. After 3 weeks on this pasture they were housed in pens with straw bedding and fed a ration consisting of hay (*ad libitum*), supplemented with barley and soybean meal. Two weeks after housing the lambs, which were approximately 4 months of age, were allocated at random to the following:

<u>Established parasite groups</u>

Half the lambs (18) were each dosed with infective larvae L3 (1,200 L3 three times / week for 2 weeks: total 7,200 L3) to achieve adult

worm populations. During the first 6 weeks after the commencement of larval dosing, lambs received normal rations plus a daily mineral supplement that did not contain copper. Beginning week 7, the lambs were then divided into the following groups of 6 animals, each receiving their respective treatment:
Group 1 – Control: (daily mineral supplementation, without copper)
Group 2 – Cu Supplement: (daily mineral supplementation containing copper)
Group 3 – COWP: (copper wire particle bolus, with no additional mineral supplement.)
These 3 groups were slaughtered after a further 4 weeks on the above treatments, for worm recovery.

Developing parasite groups
The other 18 lambs each received L3 dosing over an extended period of time (400 L3 three times / week for 6 weeks: total 7,200 L3). At the start and for the entire duration of dosing, the lambs were apportioned into the following 3 groups of 6 animals, each receiving their respective treatment:
Group 4 – Control: (daily mineral supplementation, without copper)
Group 5 – Cu Supplement: (daily mineral supplementation containing copper)
Group 6 – COWP: (copper wire particle bolus at the start of daily dosing with L3, with no additional mineral supplement.)
These latter 3 groups were slaughtered 4 weeks after last larval dose was administered.

Source of infective larvae. Infective larvae (L3) were obtained from bulked cultures of faeces derived from several organic sheep farms in Sweden during the summer of 2002. These larvae were stored in small volumes of water in tissue culture flasks, laid on their side at 5 °C, the water was replaced each fortnight. When required for the experiment, >95% L3 motility was observed and the estimated species composition, based on the morphology of ensheathed and exsheathed L3, was approximately 60% *Haemonchus contortus,* 20% *Trichostrongylus spp,* 10% *Teladorsagia circumcincta,* with a very small percentage of *Cooperia* spp., *Nematodirus* spp. and *Chabertia ovina.* Larval numbers were accurately estimated in the bulked aqueous suspension and administered to each restrained lamb with the use of a repeating dose syringe fitted with an oral dosing attachment designed for lambs. Between dosing of each lamb, the larvae contained within the syringe were kept in a uniform suspension by continuous inversion of this apparatus.

Mineral supplement and copper wire particle (COWP) boluses. The mineral supplementation used was a commercial mix (Effekt Fårmineral® Lactamin, Kimstad, Sweden), which is available in formulations without and with copper (250 mg Cu/ kg). It was given at a rate of 10g / animal / day together with the concentrates to the groups, according to the schedule above. Lambs in Groups 3 and 6 (COWP treatments) each received a 4g copper oxide needle capsule (Copinox®, Bayer Animal Health, Suffolk, UK) at the times stated above.

Technical procedures. Daily concentrate rations (and minerals) were weighed prior to feeding, hay rations and residuals were weighed once / week. The lambs were weighed every second week. At the last weighing, a group faecal collection was made for analysis of copper content. At slaughter the viscera was collected for nematode parasite recovery and enumeration from the gut contents and abomasal mucosa by the methods described by *Donald et al.* (1978) and *Dobson et al.* (1990). Copper analyses were made, according to accredited analytical procedures, on muscle and liver samples taken at autopsy of one lamb from Group 1 and 3 lambs from each of the Groups 4, 5 and 6. Statistical analysis of parasitological data was conducted using the statistic programme NCSS 2000 (*Hintze* 1998) and variances were analysed with GLM-ANOVA.

Field trial

This study was conducted on a commercial sheep farm located in the southern province of Skåne in Southern Sweden. A recurrent problem with parasitism had been reported on this farm in recent years, particularly with *H. contortus.* Because of the potential threat of this parasite, the farmer was advised by veterinarians of the Swedish Animal Health Service to treat all pregnant ewes with ivermectin (Ivomec®vet, Veter AB, Södertälje, Sweden) during the time of housing. This was carried out in March 2003, approximately 6 weeks prior to lambing indoors.

In May 2003, a trial was initiated which involved the allocation of 2 groups of sheep, each of 20 ewes with their lambs (8 ewes with twins; 12 ewes with single lambs), to 2 adjacent paddocks (approx. 2ha) of newly established improved pasture, which had not been previously grazed. The ewes in Group A were untreated control animals, whereas ewes in Group B received a 4 g COWP capsule (Copinox®, Bayer). Ewes and lambs remained on these pastures until 1 July, when they were removed to graze in a common mob onto aftermath pasture, which had been reserved for silage cuts early in the season. On 19 August the lambs were weaned and re-introduced to their original pasture plots and those in Group B each received a 2g COWP capsule (Copinox®, Bayer). They remained on these pastures until housing on 29 October 2003.

The following measurements were undertaken:

Faecal egg counts and lamb performance. All ewes were sampled prior to turnout on 12 May 2003 to determine the presence of positive nematode egg counts, by the methods described by *Lindqvist et al.* (2001), and with the minimum level of detection being 50 epg. Subsequently, 10 ewes per paddock were faecal sampled for nematode egg counts and infective larval differentials on 2 June, 16 June and 1 July. Lamb egg counts and larval differentials were conducted on 20 lambs per paddock on 1 July, 18 August, 17 September and 29 October. Lambs were weighed each month.

Tracer tests. A tracer test was performed at the time of allocation of ewes and lambs to pasture using 2 worm-free lambs per plot, derived from the previous year lamb crop. Four sequential tracer tests were conducted in autumn starting when the lambs were re-assigned to their respective experimental plots, using lambs that were born in 2003. As for the initial tracer test, these tests used 2 lambs per plot. The tracers had been previously rendered worm-free by several anthelmintic treatments with ivermectin and managed as a separate group on pasture that had been previously un-grazed by sheep. For each successive group of 4 tracers, the last anthelmintic treatment was given no later than 4 weeks prior to allocation to the paddocks.

All tracer tests were approximately 3 weeks in duration and the tracer lambs were then housed for 2 weeks prior to slaughter to assess the true level of larval inhibition in parasite infections. Lambs were consigned to the local slaughterhouse and viscera of all lambs were collected and processed for worm recovery, speciation and enumeration by the methods described by *Donald et al.* (1978) and *Dobson et al.* (1990). Livers and kidneys from 2 control lambs and 2 COWP treated lambs were analysed for copper content.

Results

Pen trial

Effect of treatments on feed intake and growth. There was no differences between the groups in daily feed intake, with group means for the whole experiment period ranging between 1.33-1.42 kg DM / lamb / day (mean intake of ME 14,5 MJ / lamb / day). Also there were no differences in live weight gain between the groups. Daily gain during the experiment varied between 143-160 g / day gain in the different groups. Mean final weight for all lambs was 45.5 kg.

Effects of Cu supplement and COWP on established parasite infections [Gps. 1-3]. Total establishment rate of parasites in the Control group (Group 1) was approximately 42% of the total infective larval dose (45%, 67% and 13% for *H. contortus, T. circumcincta* and *Trichostrongylus* spp., respectively). Despite the fact that the parasite

Table 1. Mean worm burdens in the abomasum and small intestine of lambs with 6-week old nematode infections either untreated, receiving daily mineral supplement with copper, or receiving COWP.

	Group 1 Control	Group 2 Cu Supplement	p value 1 v's 2	Group 3 COWP	p value 1 v's 3
Abomasum					
H. contortus					
Adults	560	510	0.56	15	<0.001
Early L4	1525	1515	0.96	655	<0.001
Total	2085 (73%)*	2025 (75%)	0.74	670 (98%)	<0.001
T. circumcincta					
Adults	220	85	0.14	60	0.06
Early L4	265	125	0.06	65	0.01
Total	485 (55%)	210 (60%)	0.06	125 (53%)	0.01
T. axei					
Adults	100	135	0.41	60	0.30
Early L4	0	0		0	
Total	100	135	0.41	60	0.30
Small Intestine					
Trichostrongylus spp.					
Adults	200	185	0.79	125	0.25
L4	0	0		0	
Total	200	185	0.79	125	0.25
Nematodirus spp.					
Adults	135	15	0.10	85	0.50
L4	0	0		10	
Total	135	15	0.10	95	0.50
Cooperia spp.					
Adults	10	15		0	
L4	40	10		0	
Total	50	25		0	

* number in parenthesis represents the percentage of infection as early L4 larvae

Table 2. Mean worm burdens in the abomasum and small intestine of lambs with incoming nematode infections either untreated, receiving daily mineral supplement with copper, or receiving COWP.

	Group 4 Control	Group 5 Cu Supplement	p value 4 v's 5	Group 6 COWP	p value 4 v's 6
Abomasum					
H. contortus					
Adults	285	570	0.04	250	0.79
Early L4	1465	1830	0.22	1280	0.52
Total	1750 (84%)	2400 (76%)	0.04	1530 (84%)	0.44
T. circumcincta					
Adults	60	15	0.38	65	0.85
Early L4	50	70	0.66	40	0.82
Total	110 (45%)	85 (82%)	0.74	105 (40%)	0.84
T. axei					
Adults	110	30	0.06	50	0.13
Early L4	0	0		5	
Total	110	30	0.06	55 (10%)	0.13
Small Intestine					
Trichostrongylus spp.					
Adults	85	85	-	160	0.07
L4	65	0		0	
Total	150	85		160	0.07
Nematodirus spp.					
Adults	50	65	0.91	215	0.25
L4	0	0		35	
Total	50	65	0.91	250	0.25
Cooperia spp.					
Adults	10	30		0	
L4	10	30		0	
Total	20	60 (50%)		0	

* number in parenthesis represents the percentage of infection as early L4 larvae

populations were at least 10 weeks of age, 73% of the *H. contortus* and 55% of the *T. circumcincta* burdens in the Control lambs were in the early fourth larval stage (early L4) of development.

There was no significant difference in the worm burdens in any of the stages of development for all parasite species found between the Control lambs and those that received the Cu Supplement. In contrast, the comparison between the Control and the COWP treated lambs showed highly significantly less adults ($p<0.001$) and early L4 stages ($p<0.001$) of *H. contortus,* representing 97% and 56% reduction in these worm burdens, respectively. In addition the total number of *T. circumcincta* in the COWP group, was significantly less ($p<0.01$), than in the Control animals (74% reduction). The numbers of *T. axei* were low in all groups, with no significant difference between the Control and the COWP lambs, although the latter lambs had significantly lower ($p<0.05$) burdens than in the

Cu Supplement lambs. There was no significant difference between the groups in the parasite species found in the small intestine, although the worm burdens were low in all groups (see Table 1).

Effects of Cu supplement and COWP on incoming parasite infections [Gps. 4-6]. Similar levels of establishment were observed in the Control lambs in this larval dosing regime (Group 4) and the Control lambs above (Group 1). Apart from significantly more ($p<0.04$) *H. contortus* in the Cu Supplement group compared to the Controls, there was no significant difference between any of the stages of development for all parasite species in the Control, Cu Supplement, and the COWP groups (see Table 2).

Estimates of tissue and faecal copper levels. Estimates of muscle and liver copper levels from samples taken at slaughter from the pen trial are shown in Table 3. The mean baseline copper levels in liver and muscle for the Control lambs (Group 4) was 62 mg/kg and 0.62 mg/kg, respectively. The levels in the Cu Supplement group (Group 5) were within the same range as the Controls. For the lambs that received COWP 10 weeks before slaughter (Group 6), liver copper levels were substantially higher than the controls, particularly for one of the 3 animals tested, which had an estimate of 366 mg/kg. However, it should be noted that the muscle copper levels were within the range found in the Controls and the Cu Supplement groups. The liver and muscle copper levels of the one lamb from Group 3, which received COWP 4 weeks before slaughter, were in the same range as Group 6 lambs (COWP 10 weeks before slaughter). No tissue copper levels were taken from lambs in Groups 1 and 2.

Estimates of copper levels in the faeces showed levels in Group 3 to be 5-fold greater than for the Controls (Groups 1 and 4). The 2 Cu Supplement groups (Groups 2 and 5) showed no difference to the Control Groups (Groups 1 and 4). Neither was there a higher level in the group given COWP capsule 10 weeks before slaughter (Group 6), compared to the Control groups.

Table 3. Copper levels in liver, muscle and faecal samples taken at slaughter from lambs in a pen trial where they received no copper supplementation (Groups 1 and 4), mineral supplement containing copper (Groups 2 and 5), or COWP bolus (Groups 3 and 6).

Lamb	Group	Cu in liver (mg/kg)	Cu in muscle (mg/kg)	Cu in faeces (mg/kg) dwt.
Mean	1			22.9
Mean	2			19.7
2106	3	188	0,85	
Mean				147
2013	4	66	0,55	
2015	4	50	0,79	
2038	4	69	0,52	
Mean		62	0.62	27.9
2045	5	77	0,79	
2050	5	58	1,1	
2078	5	35	1,0	
Mean		57	0.96	22.7
2001	6	366	0,80	
2052	6	186	0,64	
2091	6	113	0,76	
Mean		222	0.73	21.9

Field trial

Apart from the occasional positive egg count in 1 or 2 ewes, faecal egg counts of ewes were zero on all sampling occasions. Faecal egg counts of lambs were very low for the entire study, with mean counts zero when first sampled (1 July) and increased slowly during the autumn, with COWP and Control groups showing mean egg

Table 4. Faecal egg counts of ewes and lambs for Control and COWP treatments in the field trial conducted in southern Sweden, for the grazing season 2003.

Date	Ewe Faecal Egg Counts		Lamb Faecal Egg Counts	
	Control	COWP	Control	COWP
11 May	0	0		
2 June	5 (1/10)*	0		
16 June	5 (2/10)	0		
1 July	0	0	0	0
18 Aug			13 (3/20)*	3 (1/20)
17 Sept			140 (19/20)	70 (16/20)
29 Oct			230 (19/20)	76 (13/20)

*(x/y) proportion of animals with positive egg counts.

counts of 76 and 230 epg. respectively, at the final sampling on 29 October (see Table 4). Tracer worm counts were zero for the first tracer test at turnout and the autumn tests showed only low numbers of *T. circumcincta,* the occasional *H. contortus* and *Nematodirus* spp., with no difference between the 2 treatments (see Table 5). Lambs grew equally well in both treatments, with final live weights at end October being 34.7 and 35.9 kg for the Control and the Copper groups, respectively.

Estimates of copper in liver and kidneys were conducted on 2 lambs from each group at slaughter in late October. The mean levels for the COWP and Control groups were 123 and 52 and 3.1 and 2.6 mg/kg for liver and kidney estimates, respectively.

Discussion

Although no faecal egg counts were conducted on the lambs used in the pen study, we are confident that they were effectively worm-free during their time on pasture. This is because they received 2 anthelmintic treatments of different drug classes (ivermectin and fenbendazole) with extremely high levels of efficacy, and they

Table 5. Mean worm burdens of *Haemonchus contortus, Telodorsagia circumcincta* and *Nematodirus* spp. in tracer lambs used in the field trial conducted in southern Sweden, for the grazing season 2003.

Tracer Test	Control Group			COWP Treatment		
	H.cont.	T. circ.	Nem. spp	H.cont.	T. circ.	Nem. spp
Turnout Test						
(12/5 - 3/6)#	0	0	0	0	0	0
Autumn Tests						
1(19/8 - 1/9)	0	100 (0%)*	50 (0%)	0	450 (0%)	50
2(1/9 - 15/9)	50 (100%)	650 (0%)	50 (0%)	100 (100%)	400 (0%)	0
3(15/9 - 6/10)	75 (100%)	900 (8%)	50 (0%)	50 (100%)	700 (5%)	0
4(6/10 - 27/10)	0	675 (100%)	50 (0%)	0	475 (100%)	0

Tracer test interval
*Percentage arrested development

grazed on helminthologically clean pastures from early July until the time of housing. The lambs were allowed 2 weeks to adjust to hand feeding in pens, before the parasite infection schedules were implemented. Although 2 different infection schedules were chosen, the total number of infective larvae given to all lambs was identical. The aim was to test COWP and Cu supplement on established (6-weeks-old) populations (Groups 1-3) and on incoming (developing) populations of parasites (Groups 4-6). The low-level, trickle dosing used for both infection schedules, has been shown to be the optimal method of achieving parasite establishment (*Barger et al.* 1985, *Dobson et al.* 1990).

The pen study showed that for parasites that were allowed 6 weeks to establish prior to the administration of COWP capsules, there was a 97% and 56% reduction in adult and early L4 stages of *H. contortus* respectively, compared with the Controls ($p<0.001$). There was also a significant ($p<0.01$) reduction in *T. circumcincta.* However, this finding did not occur in lambs given COWP at the same time as larval dosing commenced (Group 6). This result is difficult to explain, as the total abomasal worm burdens in the 2 Control groups (Groups 1 and 4) were similar, which vindicated our aim of achieving the same total numbers of parasitic stages of nematodes in all lambs.

Although the evidence of anthelmintic effect of COWP is clear, the results are not consistent. For example, *Bang et al.* (1990a) used pasture-reared lambs, which received 5 g COWP 5 days before being artificially infected with nematode larvae given over a nine-day period. These lambs were slaughtered 3 weeks after the last dose of infective larvae and there was a 96% and 56% reduction in *H. contortus* and *T. circumcincta* compared with controls. However, a study by *Knox* (2002), who used a similar design to *Bang et al.* (1990a), resulted in only a 30-50% reduction in *H. contortus* in groups of lambs receiving COWP (2.5g or 5g) treatments. In a trial where naturally infected, but housed, lambs received either 2 g or 4 g COWP capsules, approximately 75% reduction in both *H. contortus* and *T. circumcincta* worm burdens was observed after 5 weeks (*Nyman* 2000). Further experimentation in New Zealand with penned sheep showed that 2.5g COWP had an anthelmintic efficacy of 97% against established infections and 99% against incoming *H. contortus* larvae (*Familton AS, McAnulty RW, Harrison TR, Reid PR,* unpublished results – as cited by Knox 2002).

Early studies on the disposition of COWP in the gastrointestinal tract of sheep show that the copper particles move from the rumen with the ingesta flow to lodge in the folds of the abomasum, reaching maximum concentrations 5-6 days after dosing (*Stewart* 1950). The low pH in the abomasum induces the release of high concentrations of soluble copper, which remain elevated in treated sheep for up to 44 days (*Langlands et al.* 1989). However, the rate of passage of COWP is dependent on the temporal relationship between food intake and gut fill of treated animals. It has been observed in young sheep that were housed for some hours without feed before given COWP, that the particles rapidly passed through the proximal part of the gut, with only a very low percentage lodging in the abomasum (*MR Knox* – pers. comm.).

The negative result of Group 6 lambs that received the COWP at the same time that larval dosing commenced may be due to several factors. Firstly, for the first week following COWP administration (when maximum copper particle concentrations in the abomasum are likely), the lambs had only received 1,200 L3. Any anthelmintic effect at this time, may have been masked by a compensatory higher establishment rate later on in the 6-week larval dosing period. Secondly, at the start of dosing, the lambs had only been housed for 2 weeks after being raised on pasture, which may have resulted in variations in feed intake – although this was not observed on a group feed intake basis. Thirdly, and most likely, was the fact that the substantial proportion of worm populations in these artificial infections remained arrested in development (73%-84% in Control groups). It is well known that arrested, or hypobiotic, L4 stages are relatively more resistant to anthelmintics than the adult stages (*Eysker* 1997) and this is also clearly indicated in the composition of the *H. contortus* population in Group 3 of this study, where 98% of the survivors of COWP treatment against established infections were early L4.

The reasons why larvae of *H. contortus* and *T. circumcincta* used in this study showed such a high propensity to undergo arrested development, is a mystery. To our knowledge, this is the first time that such high levels of arrested development have occurred in these 2 different genera of sheep nematodes, following administration to lambs over a relatively short timeframe. The length of time (3-4 months), the temperature (~ 5 ºC) and the method of storage of infective larvae are routine in many veterinary parasitology laboratories.

It was disappointing to record that the mineral supplement containing copper had no effect on either established or developing parasite infections. The Swedish Animal Health Service recommends to all Gotland Breed sheep farmers that they should provide to their sheep 10g / day of commercial mineral mix consisting of 250-400 mg Cu per kg. This is particularly so in flocks raised in south-eastern Sweden, where soils are marginally copper deficient (*Pettersson* 1994). The results from a survey of parasite status amongst organic sheep farms in Sweden, showed that those that received copper supplement had lower faecal egg counts than those not receiving the supplement (*Lindqvist et al.* 2001). The implication being that this was due to reduced *H. contortus* infections and /or faecal egg counts. Although no faecal sampling was conducted in the pen trials of this study, the *H. contortus* worm burdens were unaffected, irrespective of what may have occured on nematode egg production.

The field trial also failed to produce any worthwhile information, largely because that the level of parasite infection was exceedingly low. The farmer whose sheep were used in this trial had previous problems with haemonchosis. Thus she was advised by the Swedish Animal Health Service to treat her ewes at the time of housing, as they were likely to have substantial burdens of arrested *H. contortus* (*Waller et al.* 2004). The combination of very effective anthelmintic treatment (ivermectin) and turning out lambed ewes onto helminthologically clean pasture, effectively eradicated *H. contortus* from this experimental treatment flock – at least in this year of study.

Notwithstanding the importance of maintaining adequate copper levels in sheep at risk of hypocuprosis, it would seem that there is little to recommend the use of copper, either as a supplement or as COWP, specifically as a prophylactic means of *H. contortus* control in sheep in Sweden. In addition, analysis of copper levels in tissue and faecal samples indicated that potentially toxic levels of copper could occur in the liver of COWP treated lambs and that high concentrations of copper are excreted in the dung of these animals for at least up to 4 weeks after treatment. Although there were no overt signs of copper toxicity in sheep used in either the pen or field trials, any further studies must also take into consideration the presence of copper accumulating plants in the grazing environment.

Acknowledgements
The authors would like to thank the technical staff of the Department of Agricultural Research for North-

ern Sweden, SLU, Umeå, for their assistance in managing the penned sheep trial. We wish to thank Drs. Johan Höglund and Lars Peterson for conducting the statistical and chemical analysis, respectively. Financial support for this project was provided by the Swedish Board of Agriculture (Jordbruksverket).

References

Bang KS, Familton AS, Sykes AR: Effect of copper oxide wire particle treatment on establishment of major gastro-intestinal nematodes in lambs. Res. Vet. Sci.1990a., *49,* 132-137.

Bang KS, Familton AS, Sykes AR: Effect of ostertagiasis on copper status in sheep: a study involving the use of copper oxide wire particles. Res. Vet. Sci.1990b., *49,* 306-314.

Barger IA, LeJambre LF, Georgi JR, Davies HI: Regulation of *Haemonchus contortus* populations in sheep exposed to continuous infection. Int. J. Parasitol. 1985, *15,* 529-533.

Canto-Dorantes JH, Torres-Acosta JFL, Calderon-Quintal JA, Perez-Garcia M, Aguilar- Caballero AJ, Vargas-Magana JJ, Hoste H: Improving resilience and resistance of naturally infected browsing kids through supplementary feeding and copper oxide particles in tropical Mexico. Proc. 8th Int. Goat Conf. Pretoria, South Africa. 2004 (in press).

Chartier C, Etter E, Hoste H, Pors I, Koch C, Dellac B: Efficacy of copper oxide needles for the control of nematode parasites in dairy goats. Vet. Res. Comm. 2000, *24,* 389-399.

Dobson RJ, Waller PJ, Donald AD: Population dynamics of *Trichostrongylus colubriformis* in sheep; the effect of infection rate on the establishment of infective larvae and parasite fecundity. Int. J. Parasitol. 1990, *20,* 347-352.

Donald AD, Morley FHW, Waller PJ, Axelsen A, Donnelly JR: Availability to grazing sheep of gastrointestinal nematode infection arising from summer contamination of pastures. Aust. J. Agric. Res.1978, *29,* 189-204.

Eysker M: Some aspects of inhibited development of trichostrongylids in ruminants. Vet. Parasitol. 1997, *72,* 265-283.

Hintze J. NCSS 2000. Manual. 1998. Kaysville, Utah.

Knox MR: Effectiveness of copper oxide wire particles for *Haemonchus contortus* control in sheep. Aust. Vet. J. 2002, *80,* 224-227.

Langlands JP, Donald GE, Bowles JE, Smith AJ: Trace element nutrition of grazing ruminants: copper oxide powder as a copper supplement. Aust. J. Agric. Res.1989, *40,* 187-193.

Lindqvist Å, Ljungström BL, Nilsson O, Waller PJ: The dynamics, prevalence and impact of nematode parasite infections in organically raised sheep in Sweden. Acta Vet. Scand. 2001, *42,* 377-389.

Nilsson O, Rudby-Martin L, Schwan O: Bensimidazol-resistenta *Haemonchus contortus* påvisade hos får i Sverige (Benzimidazole resistance demonstrated in *Haemonchus contortus* infections of sheep in Sweden). Sv. Vet. Tid. 1993, *45,* 303-307.

Nyman H: Alternative methods of treating gastrointestinal nematodes in sheep, using *Duddingtonia flagrans* and copper wire particles. Minor Field Studies No. 99. Swedish University of Agricultural Sciences, International Office, Uppsala, Sweden. 2000. pp, 54.

Pettersson O: Tungmetaller i odling och miljö (Heavy metals in agriculture and the environment). SLU Info. Aktuellt från lantbruksuniversitetet 422. 1994.

Reid PJ: The effect of timing of copper oxide wire particle treatment on the establishment of *Haemonchus contortus* in lambs. B.Ag.Sc.Dissertation. Lincoln University, Christchurch, New Zealand. 1995, pp: 125.

Schwan O, Jacobsson SO, Frank A, Rudby-Martin L, Petersson LR: Cobalt and copper deficiency in Swedish landrace pelt sheep. Application of diagnostics in flock-related deficiency diseases (case report). J. Vet. Med. Series A. Animal Physiology, Pathology and Clinical Veterinary Medicine. 1987. *34,* 709-718.

Stewart DF: Studies on resistance of sheep to infestation with *Haemonchus contortus* and *Trichostrongylus* spp and on the immunological reactions of sheep exposed to infestation. IV. The antibody response to natural infestation in grazing sheep and the "self cure" phenomenon. Aust. J. Agric. Res. 1950, *1,* 427-439.

Waller PJ, Rudby-Martin L, Ljungström BL, Rydzik A: The epidemiology of abomasal nematodes of sheep in Sweden, with particular reference to overwinter survival strategies. Vet. Parasitol. 2004, *122,* 207-220.

Watkins AD, Miller JE, Terrill TH, Larsen M, Kaplan RM: Effectiveness of copper-oxide wire particles on the control of *Haemonchus contortus* in sheep. Proc. 19thWAAVP Conference. New Orleans, August 10-14, 2003, p.198.

Sammanfattning

Utvärdering av koppartillskott för att kontrollera Haemonchus contortus-infektioner hos får i Sverige.

Effekten av koppartillskott, antingen i form av en daglig giva med mineralfoder eller som kapslar med kopparoxid (COWP) på parasitinfektion hos lamm har studerats. COWP gavs till lamm med en etablerad (6 veckor gammal) infektion. Behandlingen resulterade i en minskning av antalet vuxna *H. contortus* med 97%, jämfört med den obehandlade kontrollen (p<0.001). Motsvarande minskning av *H. contortus* i larvstadium (L4) var 56% (p<0.001). Dessutom reducerades den totala infektionen av *T. circumcincta* hos de COWP-behandlade lammen med 74%, jämfört med kontrollgruppen (p<0.01). Andra lamm fick först COWP och infekterades därefter med parasiter i 6 veckors tid. I denna grupp gav behandlingen inga signifikanta effekter. Inte heller hos de lamm som fick kopparberikat mineralfoder (i rekommenderad mängd) sågs någon effekt på parasitinfektionen. En uppföljande fältstudie gjordes på en gård där man tidigare haft stora problem med *H. contortus.* Några effekter kunde dock inte ses av de COWP-behandlingar som gjordes, då parasitsmittan detta år var mycket låg hos alla undersökta djur. Slutsatsen av dessa studier är att det inte är relevant att rekommendera koppartillskott för att behandla parasitinfektioner hos svenska får.

(Received March 5, 2004; accepted June 21, 2004).

Reprints may be obtained from: P. J. Waller, Department of Parasitology (SWEPAR), National Veterinary Institute and Swedish University of Agricultural Sciences, SE-751 89 Uppsala, Sweden.
E-mail: Peter.Waller@sva.se, tel: +46 18 67 41 27, fax: +46 18 30 91 62.