Does the use of silvopastoral systems increase or reduce the levels of parasitism in cattle in Colombia?

Abstract

Silvopastoral systems are being adopted in Colombia as an alternative to open pasture systems. Silvopastoral systems bring benefits such as increased local biodiversity, animal production and welfare; however, it is not known what effect these systems have on endoparasite and ectoparasites burdens. This study examined faecal samples and compared tick counts from 5 silvopastoral farms and 7 open pasture farms in the Eje Cafetero, Colombia. On this occasion, there was no statistical difference between faecal egg counts of cows grazing the two systems ($P = 0.23$), nor was there a statistical difference in tick counts ($P = 0.92$). Other studies with a greater scope or which more directly observe aspects of silvopastoral systems which may affect parasite burden are required to confirm these findings.

Introduction

In the last 30 years, livestock production has intensified in the Eje Cafetero, a traditionally coffee producing area on the western slope of the Central Cordillera of the Andes in Colombia. This has led to widespread deforestation and other environmental effects: soil compaction, erosion, organic and chemical pollution of water-systems and loss of biodiversity, among others. Recently, silvopasture is being adopted as an alternative system, thanks to the efforts of several NGOs, including Fundación CIPAV. Silvopasture is the practice of combining forestry and animal production. Silvopastoral systems (SPS) have various benefits over open pasture systems (OPS). They have been shown to increase local biodiversity, livestock production and benefit animal welfare (Murgueitio, 1999). SPSs have been identified by the UK Department of Energy and Climate Change as a viable method of climate change mitigation and poverty alleviation. A report published in 2012 concluded that converting 48,000 hectares of OPS to SPS in Colombia would:

1) Reduce green house gas emissions by about 2MtCO_2 over 8 years.
2) Improve the livelihoods of small cattle ranchers by:
    a) Increasing farm income. A 10% increase in milk and beef productivity is predicted by the end of the project.
    b) Natural resource optimisation on farms. Providing secondary sources of income such as timber.
3) Bring environmental benefits such as biodiversity conservation and reduced soil erosion and water pollution. (Department of Energy and Climate Change)

SPSs in Colombia typically use two different shrubs to provide forage for cattle: *Leucaena leucocephala* or *Tithonia diversifolia*.

L. leucocephala is a fast-growing legume which brings the added benefit of nitrogen fixation. This characteristic makes it suitable for improving previously depleted tropical soils. As well as providing forage for cattle, *L. leucocephala* also provides a source of firewood and green manure. An intensive SPS planted with *L. leucocephala* can increase stocking densities by 200 to 500% (Murgueitio, 1999). *Tithonia diversifolia* is also used as a forage plant is SPS and has been shown to increase milk yields and milk quality when included in cows' diets.

It has been suggested that the increased biodiversity of SPS could directly lower parasite burdens by increasing predation of parasite species, or indirectly by the action of other species on the environment. However, little research has been done to quantify this effect. It is also possible that SPS increases parasite burdens by providing a more favourable environment for parasites to reproduce and find a host

The Importance of Dung Beetles

Some work has been done into the impact on parasite burden of grazing cattle on an SPS instead of OPS. Giraldo et al (2011) found that *Haematobia irritans* (horn fly) adults and larvae numbers are lower in SPS than OPS at 3 different farms in Quindio, Colombia. The reduction in numbers of horn flies has been linked to the presence of dung beetles. Giraldo speculates that the beetles cause mechanical damage to fly eggs and remove the flies' food source, dung, from pasture. The presence of predatory species such as phoretic mites may also contribute to the lower populations found in SPS than OPS.

Studies of the effect that dung beetle activity has on the number of helminth larvae released from faeces at pasture have produced conflicting results. Bryan (1973), working in Australia, found that the dung beetle species *Onthophagus gazella*, reduced the number of infective larvae which hatched from cattle faeces left at pasture. Similarly, Fincher (1975) found that calves grazing pasture previously contaminated by parasitised steers were infected with lower levels of *Ostertagia* and *Cooperia* with increasing populations of dung beetles present on the pasture.

In contrast, Chirico et al (2003), colonised faecal pats of high (250-600epg) and low (<100epg) egg counts with dung beetles (*Aphodius rufipes* and *A. scybalarius* syn.*rufus*). After 12 days incubation at 21°C and 90%RH, greater numbers of infective L3 were retrieved from pats colonised by the dung beetles than control pats. After 12 further days of incubation, similar numbers of L3 were recovered from beetle colonised pats, and there was a significant increase in L3 from the control dung. These results suggest that *Aphodius* spp. of dung beetle can optimise conditions for L3 nematode development and so increase the parasite burden of pasture. This may be due to the fact that these species of dung beetle place brood chambers for their larvae within faeces, rather than removing and burying the dung.

Giraldo et al (2011), found increased numbers and diversity of dung beetles in SPS when compared with improved pasture (IP). The species recovered from SPS were: *Ontherus lunicollis*, *Aphodius brasiliensis*, *Aphodius* sp, *Onthophagus curvicornis* and *Dichotomius satanas*. It is unknown which of these species will act like *O. gazelle* and decrease levels of endoparasites, and which will act like *A. rufipes* and increase L3 emergence.

Ticks

It has been suggested that the increase in biodiversity of SPS can reduce levels of ticks on farms by the introduction and increased populations of natural predators. Indeed, Sáenz (2007) found that agricultural systems which use live fences or partially forested pasture increase the number and diversity of birds in the area. However, an increased predator population does not necessarily result in reduced prey numbers.

Although SPS increases the number of tick predators within the pasture ecosystem, this may not cause a reduction in the tick population. Whilst many predators and parasites of ticks have been identified, it has not been established whether or not their presence affects tick survival and reproduction to the extent that population density is reduced. There are studies reporting that the presence of certain species of ants and spiders can stabilise the population of ticks in an area under certain conditions. However, since spiders and ants are generalist predators, and as such do not depend on any particular prey, their populations should be independent of tick populations. The same should be true for other vertebrate predators of ticks such as cattle egrets, chickens and shrews (Wilson, 1994).

Another group of organisms that may impact the population of ticks within an ecosystem are parasitic wasps. Yet various studies have found no difference in the population of ticks in areas where parasitic wasps are present and those where they are not. Furthermore, controlled releases of these wasps have failed to control tick populations. The effect of tick pathogens, such as fungi and protozoa, is poorly understood. Infection with fungi has been reported in Europe, but is often non-lethal, although it may affect reproduction and survival of ticks in more subtle ways (Wilson, 1994).

This study aimed to test that hypothesis that cattle grazing SPS have lower nematode and tick burdens than those grazing OPS.

Methodology

Area of Study:

The study was conducted in the La Vieja River watershed, close to the towns of Alcala, Quimbaya, Filandia and Cartago. Initial visits to farms in the area were conducted to determine their suitability for inclusion.

Definition of farm types:

All farms visited were part of Fundación CIPAV’s programs. A farm was defined as SPS if cows exclusively grazed pastures sown with a mixture of grass and rows of either *Leucaena leucocephala* or *Tithonia diversifolia* (Photo 1). These systems also included live fences. A farm was defined as OPS if only pasture was available for the cattle to graze. All farms selected had established live fences and some additional tree cover at pasture (Photo 2).

Selection of farms:

Both SPS and OPS farms were visited to assess current parasite treatment regimes. The purpose of the study and benefits to the farmers were explained. Each farmer was offered a report of faecal egg counts and tick counts of their cattle to discuss with their veterinary


Photos 1 & 2 – Examples of SPS (left) and OPA with live fences and some tree cover (right).

surgeon as an incentive for participation. The following questions were asked to assess the farm's suitability for inclusion in the study:

1. When was the last treatment for endoparasites given to the cattle?
2. What product was used and how was it applied?
3. When was the last treatment for ectoparasites given to the cattle?
4. What product was used and how was it applied?
5. Have you used fumigation to control ticks at pasture?

With the above parameters in mind, those farms which had used ectoparasites treatments and anthelmintic treatments least recently were selected for participation in the study.

Farms were excluded if:
1. Slow or pulse-release anthelmintic products were used.
2. Fumigation at pasture was used to control ticks.

Quantifying Parasites
Once the selection process was finished, a second visit to the farm was scheduled for sampling.

Ticks
An attached tick sampling technique was used to sample ticks from 10 randomly selected cows on each farm. The BCS, age and sex of each individual was recorded as these factors have been identified as affecting tick burden (Tadesse et al, 2012). Each cow was examined for 5 minutes. Ticks were removed using forceps and preserved in 70% ethyl alcohol. The examination began at the base of the tail, and progressed distally (making sure to lift the tail and remove ticks from around the anus and vulva) to the perineum, base of the udder, udder and hind legs. If possible, and if time was available, the axillae and dewlap were also examined, although not all cattle handling systems provided ready and

safe access to this area. The head and ears were not examined due to the difficult handling conditions on some farms.

To control for operator skill, the same person checked for ticks each time. Where possible, visits to different types of farm were intercalated to control for increase in operator skill during the study period.

After collection, each tick was observed microscopically at the Veterinary Parasitology Laboratory at the *Universidad Nacional de Colombia*, and identified to the species level.

Endoparasites

After the examination for ticks, a faecal sample was taken from the rectum of each animal. An air tight container was filled with faeces. Samples were immediately transferred to a cool box with ice. At the end of each day, the samples were transferred to a larger cool box and stored on ice. Temperature was monitored twice a day to ensure that the temperature was below 4˚C.

The time period between collection and examination was 4 days. Each sample was examined using the modified McMaster Technique described by the RVC/FAO Guide to Veterinary Diagnostic Pathology (FAO):

1. Weigh out 8g of faeces.
2. Mix with 52ml of saturated salt solution.
3. Filter the mixture to remove large particles.
4. Take a subsample using a pipette and fill both chambers of a McMaster slide.
5. Leave the slide to settle for 5 minutes before examination.
6. Examine the slide using the 10x magnification lens.
7. Count Strongylida eggs found in both chambers (not just the area under the grid).
8. Multiply by 12.5 to give the number of eggs per gram (epg) of faeces.

Data Analysis

For faecal egg counts (FECs), a Mann-Whitney test was used to determine if there was a significant difference between the FECs of cattle grazing SPS and OPS. A non-parametric test was chosen as the median was considered to be the best representation of the central tendency of the data. Tick counts were analysed using Welch’s t-test to test for significant differences in the mean number of ticks recovered from cows in different pasture systems. An F-test was used to determine significant differences in the variances. Statistical analysis and graphs were generated using GraphPad Prism 7.01.

Results

Of 29 farms visited, 12 were selected for sampling. Faecal samples were taken from all farms, but only nine were sampled for ticks – two due to lack of handling facilities (Farm 6 and Farm 9), one because topical insecticide had been applied five days before sampling (Farm 5). The information about parasite treatments used on each farm is displayed in Table 1.

A total of 113 faecal samples were examined for Strongylida eggs. Of these, 47 were from cows grazing SPS, and 66 from cows grazing OPS. The majority of eggs observed were from the family Trichostrongyloidae (Photo 3), although *Oesophagostomum* eggs were also present in some samples (Photo 4). Most samples had an egg count of <12.5epg because eggs were not observed (64% of samples from cattle grazing SPS and 73% from cattle grazing OPS). The average FEC and tick count for each farm, as well as breed and average age of cattle sampled, is displayed in Table 2.

Photo 3 – *Trichostrongyloidae egg,* x100 magnification.

Photo 4 – Oesophagostomum egg, x100 magnification.

The mean FEC was greater for cattle grazing SPS than OPS (31.38epg and 15.34epg respectively, Fig. 1a), although the median for both data sets was 0. There was no significant difference between the faecal egg counts of cattle grazing SPS and those grazing OPS (P = 0.23). Subsequently, FECs were reorganised into 2 groups, one for farms that regularly use injectable ivermectin (n=4), and another for farms which use other products (n=8). Farms using ivermectin had a mean FEC of 43epg (Fig. 1b), and those using other products a mean of 14epg. A Mann-Whitney test returned a P value of 0.0025, showing a significant difference between the two groups.

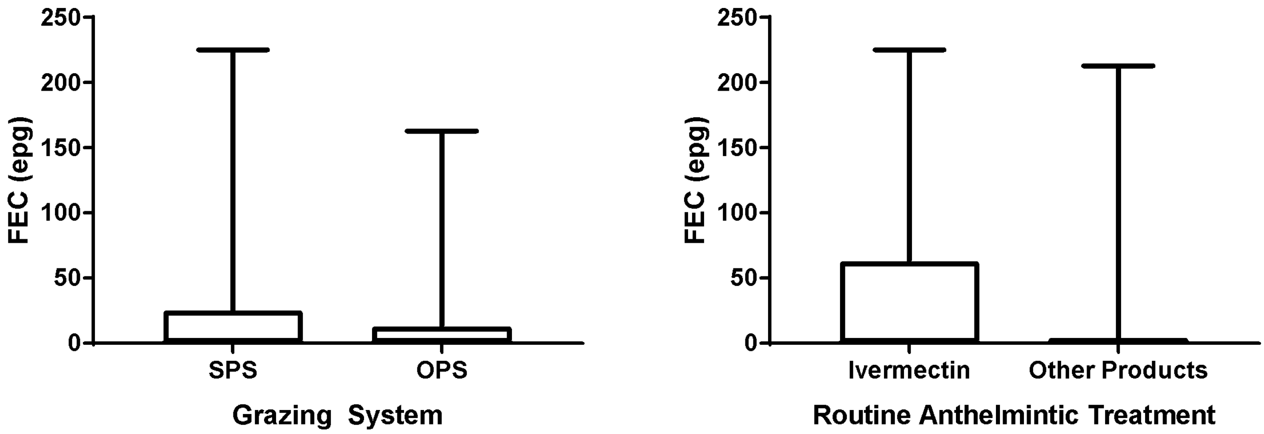

Figure 1a & b – Box and whisker plots of FECs from cattle grazing SPS and OPS (left) and FECs from cattle on farms which use ivermectin and other products as their principle anthelmintic treatment

Farm	Grazing System	Last Endoparasite Treatment	Product Used	Last Ectoparasite Treatment	Producto Used
Farm 1	Extensive SPS	44 days	Levamisole, Oral	44 days	Fungal spores, Topical
Farm 2	Intensive SPS, Boton de Oro	180 days	Fenbendazole, Oral	120 days	Amitraz, Topical
Farm 3	Open Pasture	At calving – 147 days (average)	Fenbendazole, Oral. Ivermectin 1.5%, Injectable	21 days	Organophosphate, Topical
Farm 4	Intensive SPS, Boton de Oro	180 days	Albedazole, Oral	27 days	Cypermethrin and Fluazuron, Topical
Farm 5	Open Pasture	90 days	Fenbedazole, Oral	5 days	Cypermethrin and Chlorpyrifos, Topical
Farm 6	Open Pasture	210 days	Ivermectin, Injectable	120 days	Seaweed extract, Topical
Farm 7	Open Pasture	90 days	Fenbedazole, Oral	60 days	Amitraz, Topical
Farm 8	Open Pasture	30 days	Fenbendazole, Oral	30 days	Amitraz, Topical
Farm 9	Intensive SPS, Leucaina	180 days	Ivermectin 3.15%, (Injectable)	100 days	Amitraz, Topical
Farm 10	Open Pasture	44 days	Fenbendazole, Oral	10 days	Organophosphate, Topical
Farm 11	Intensive SPS, Leucaina	At drying off - 130 days (average)	Fenbendazole, Oral	28 days	Amitraz, Topical
Farm 12	Open Pasture	150 days	Ivermectin, Injectable	120 days	Cypermethrin and Chlorpyrifos, Topical

Table 1 – Details of the selected farms, including grazing system, last parasite treatments and the product used.

Farm	Breed	Average Age (Years)	Average Tick Count	Average FEC (epg)
Farm 1	Brangus	3.6	39.1	3
Farm 2	Limousin	3.2	31.8	3.8
Farm 3	Jersey X Brahman	5.6	35.0	2.5
Farm 4	Brangus	3.7	10.2	35.4
Farm 5	Jersey X	6.6	N/A	17.5
Farm 6	Brahman	3.2	N/A	56.3
Farm 7	Zebu	6.0	24.2	28.8
Farm 8	Jersey X	4.8	41.9	1.3
Farm 9	Zebu	3.0	N/A	108.3
Farm 10	Jersey x Holstein	3.4	41.1	<12.5
Farm 11	Jersey x Holstein	5.5	32.8	16.7
Farm 12	Holstein x Brahman	3.0	12.4	17.5

Table 2 – Average tick counts and FECs for all farms.

A total of 86 cattle were examined for ticks. Of these, 36 cattle grazed SPS and 50 grazed OPS. The mean number of ticks removed was 30.5 for cows grazing SPS, and 30.9 for cows grazing OPS (Fig. 2). There was no significant difference between the means or the variances of the SPS or OPS groups (P = 0.92 and P = 0.1 respectively).

The ratio of female**:**nymph**:**male ticks removed from cattle grazing SPS was 9.5 **:** 13 **:** 10. The same ratio for OPS was 9.5 **:** 15 **:** 10.

A total of 2644 ticks were removed for identification. All specimens were identified as *Rhipicephalus microplus* (Photo 5).

Discussion

Although this study suggests that there is no difference in parasite burden in adult cows grazing SPS and those grazing OPS, constraints on time and inability to control for various important variables do not contribute to the reliability of the results. A wide variety of different chemical and biological treatments were used across all farms. The frequency, dose and timing of previous treatments as well as host factors such as age and breed, were not controlled for and ultimately will define the current parasite situation on farms regardless of which pasture system is used. While there was a difference in the mean FEC between SPS and OPS, this effect was produced by the results from Farm 9.

It should also be emphasised that all OPS farms that participated in the study were involved in CIPAV's program of planting live fences and increasing tree cover. Although these farms were not using intensive planting of shrubs, the pasture still had a relatively high tree cover. It is possible that there is no additional benefit in terms of parasite control between the SPS and OPS which include live fences and trees at pasture. The previous study which evaluated dung beetle populations (Giraldo et al, 2011) compared treeless pasture with SPS.

Photo 5 – From left to right: Adult female, adult male, and nymphs of *Rhipicephalus micoplus*. Photo courtesy of Oscar Cruz, Veterinary Parasitology Laboratory, Universidad Nacional de Colombia.

Increasing the tree cover and using live fences may be sufficient to increase dung beetle populations to a level where mechanical disruption and removal of faeces from pasture reduces parasite levels. Unfortunately, it was not possible to analyse dung beetle populations on this occasion, so no conclusions can be drawn in this respect.

A possible reason for the increased FECs in farms using ivermectin regularly may be resistance to ivermectin products in the area. Multiple anthelmintic resistance in nematodes in sheep has recently been confirmed in Colombia, with farms showing reduced efficacy of albendazole, fenbedazole and ivermectin (Garcia et al, 2016). However, it would be unusual to find resistance to only ivermectin and not to other commonly used drugs such as albendazole.

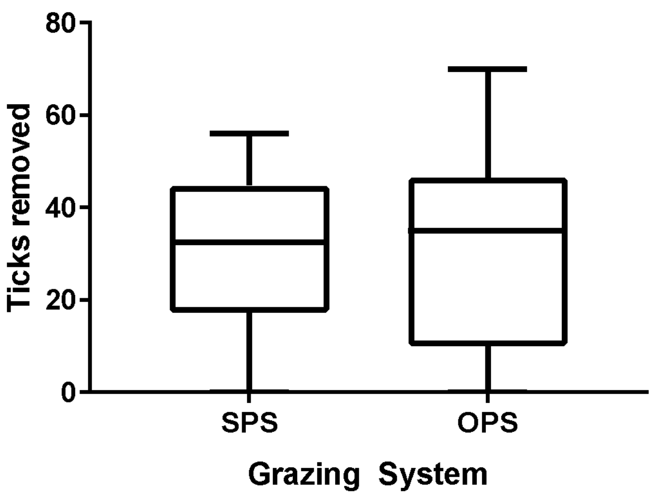

Figure 2 – Box and whisker plot of tick counts from cattle grazing SPS and OPS.

Another possible explanation is the negative impact that the use of ivermectin has on dung beetle populations and biodiversity. Recent studies have documented negative effects such as slowed larval development, decreased larval survival (O'Hea et al, 2010) and decreased olfactory and locomotor capacity of adult dung beetles which affect their ability to perform activities such as foraging (Verdu et al, 2015). Verdú reports that the adverse effects of ivermectin can be induced by concentrations as low as 1µg kg^{-1} of dung (fresh weight). For reference, ivermectin concentrations of 49µg kg^{-1} of dung can still be found 28 days after topical application of 500 µ g kg^{-1} bw. This explanation relies on the assumption that the presence of dung beetles in both SPS and OPS reduces parasites at pasture as discussed in the introduction. Further studies that directly observe the effects of dung beetles on parasite emergence from faecal pats in SPS and OPS are required to elucidate the role that dung beetles play.

The most likely explanation for the difference between both comparisons, ivermectin vs other products and SPS vs OPS, is the influence of the high mean FEC from Farm 9. This single result is responsible for the differences for both comparisons which are much reduced if Farm 9 is excluded from the analysis. As can be seen in Figure 3, there is a relationship between FEC and time since the last treatment (Pearson's Rank Coefficient = 0.57, P = 0.05, Spearman's Rank Coefficient = 0.71, P = 0.01). This relationship is to be expected. From this graph it can also be seen that 2 of the 4 farms which treated livestock with ivermectin, also rank as the farms which have treated least recently, giving the false impression that farms treating with ivermectin have higher average FECs. From this data set it is not possible to specify which variable (use of ivermectin or time since last treatment) caused farms 6 and 9 to have the highest FECs, however, it is most likely that the effect is due to time since the last treatment.

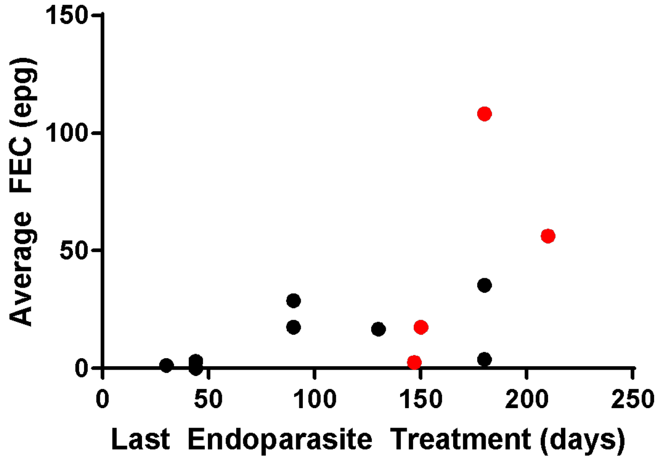

Figure 3 – Relationship between average FEC and time since last endoparasite treatment. Farms that use ivermectin are represented by red points.

There was no statistical difference between the number of ticks removed from cattle grazing SPS and OPS. Nor was there a difference in the species, or the ratio between adult females, nymphs and adult males. Tick control regimes on farms varied greatly, although the majority relied on topical application of insecticides. The frequency of application varied depending on the current tick burden observed by the farmer. Some farmers reported having to use insecticides every 8 days at times. There was also some concern about resistance to common products, especially amitraz, due to frequent use. Other variables which couldn't be controlled for include local altitude and climatic conditions, local vegetation and pasture management techniques. Farmers also reported that breed had an effect on tick burdens. Jerseys were considered to be the most susceptible, and Zebu or Brahman the least. More detailed and specific studies will be required to tease apart the complex variables involved in tick ecology.

Conclusion

The ecology of endoparasites and ectoparasites is a complex interaction of many variables. In this case, no significant difference in parasite burden between SPS and OPS was found. Other factors such as type of anthelmintics used, frequency of use and potential resistance to these products, geographical location and breed may be more important than grazing system in controlling parasites.

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