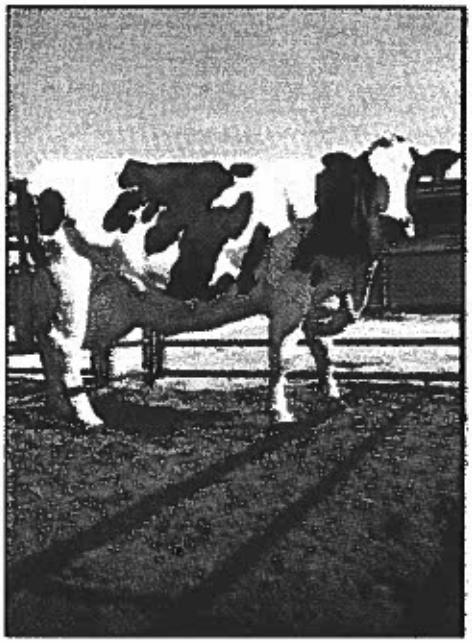
Lindsay Stenhouse
Independent project funded by the Weir and Barnson Bequest committees + JAMES KENNIE BEQUEST
September 2001

A comparative study of the parasite burden of dairy cattle between two regions surrounding Toluca, Mexico: Ejido de San Cristobal and Benito Juarez.



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Background

Parasites of the gastro-intestinal tract are of interest to a broad spectrum of scientists and researchers due to their diverse interactions with the host. There are many interactions to consider: immunological; nutritional; epidemiological and pathological to name the most obvious. The interest of this study aims to take a *basic* view of all of these factors in an investigation of the possible variation in parasite burden between two populations of dairy cattle. The two groups are managed with differing grazing strategies in a rural region surrounding Toluca, Mexico. The possibilities to be investigated are:

a) that the nutritional status of one group may be superior to the other, inferring greater resistance against (or tolerance to) parasitic infection;
b) that the parasite burden varies significantly between the populations depending upon the extent of exposure to parasites on the pasture, as determined by the proportion of natural grazing time allowed by the farmer;
c) that there may be environmental variations in habitat (topography, vegetation and drainage) that influence the number and species of parasite present on the pasture.

As the feeding and grazing strategies differ in each of the two regions, it is plausible that one may be more efficient in reducing parasitism of cattle, in terms of better nutrition, improved pasture management or environmental variations affecting the propagation of free-living stages of a parasite. A study investigating the gastro-intestinal and hepatic parasite burden of dairy cattle can yield important information about the efficiency of such a unit. Gastro-intestinal parasites can compete with the host for nutrients and/or cause damage to the intestinal villi. Villous atrophy is a common effect of helminth infections. The damage is caused by the direct feeding on the mucosa or by attachment of worms to maintain station in the gut. The absorptive area for nutrient uptake can be reduced and protein losses can result from excessive mucous secretions, increased epithelial cell turnover and blood loss. Both effects are correlated with the magnitude of infection. Nutrition and endogenous protein losses adversely affect milk production, which in these Fresian cattle, are already far lower than their European counterparts. Liver flukes are also important in terms of their pathological effects on the host. Large numbers can impair liver function and cause anaemia and oedema.

The farmers included in this study own very few animals, which are very expensive to replace. Hence, it is important to determine species and burden of parasites in the study group.

Aim

To conduct a comparative gastro-intestinal parasite survey of dairy cattle in two regions: Ejido de San Cristobal and Benito Juarez. The feeding and grazing strategies differs in each area and this project aims to identify the more favourable regime, in terms of reduced parasitaemia of cattle.

Methods

Faecal samples were obtained directly from the rectum of twenty cows from each region. Samples were taken twice from the same animals and collected one week apart for the purpose of repeating the experiments. Risks of cross-contamination

between samples were minimised as faeces were collected and sealed inside the inverted sampling gloves.

McMaster Technique/ Floatation

The modified McMaster Technique was used to liberate eggs from the centrifuged faecal pellet in a saturated sodium chloride solution. 0.2 ml of the thoroughly mixed sample was pipetted into each chamber to allow identification and counting of eggs to be conducted. As the weight of the faecal sample was known, the number of eggs per gram of faeces was calculated.

Sedimentation

A sedimentation technique was also conducted to estimate the number of *Fasciola hepatica* (liver fluke, see image 1) eggs in each sample. The latter are too large to float in a saturated salt solution. The number of eggs per gram of faeces was similarly achieved after screening with the McMaster apparatus. To determine whether *Fasciola* was present but not in large enough numbers to be detected under the McMaster slide, 10 ml of faecal sediment was stained with Crystal Violet and viewed under a dissection microscope.

Coproculture

Larval culture of the pooled faeces from each region was performed. The purpose of this experiment was simply to confirm the identification of the species of eggs found in the McMaster floatation. An equal amount of each sample was placed in a covered shallow tray with airflow permitted and incubated at a temperature of 22°C for 7 days. Following this treatment, the faeces were flooded with tepid water and left to steep for 2 hours. The liquid was poured off and sieved (1mm aperture) to remove most of the faecal debris. It was not necessary to Baermannise the filtrate as the samples were clear enough to screen. Larvae were stained for microscopy with the addition of a small volume of iodine. Exsheathment was unnecessary for identification.

Ziehl-Nielsen Stain for *Cryptosporidium* oocysts

Slides were coated with a thin layer of 1% bovine albumin solution in a phosphate buffer (pH 7.5) and allowed to dry for 2 hours. A small droplet of each faecal sample (prepared by sedimentation and stored in potassium dichromate solution at 4°C) was deposited onto the slides. A tiny droplet of a positive *Cryptosporidium* sample (provided by CIESA) was dotted onto the corner of each slide to allow comparisons to be made for correct identification of oocysts. Slides were stained in Carbofuschin and rinsed in acid alcohol before counter-staining with methylene blue. This experiment was not quantitative, but aimed simply to detect the presence of this protozoan (stains bright red) in the samples (see image 3).

Husbandry practices

Cattle kept in the area of San Cristobal are allowed to graze for around 5 hours per day on pasture largely comprised of ryegrass (*Lolium perenne*) and clover (*Trifolium repens*). They also receive a concentrate supplement ('Beefbuilder'), of which 5kg are fed to each animal per day. Stubble is available *ad libitum*.

Those in Benito Juarez have access to similar pastures, but their grazing times are extended to around 9 hours per day and they are fed with hay for the remainder of the

time. The drainage on Benito Juarez farms was much less efficient, and much of the grazing area was boggy.

Unfortunately, data detailing the nutritional value of the grasses and of the concentrated feed could not be obtained from the host university. However, the following assumptions can be made on the information available:

- Those cattle fed with the protein supplement should have a higher resistance to parasitism;
- The group which were spending more time grazing on the pasture should have a higher level of exposure to infective eggs and larvae;
- The bog nature of Benito Juarez can be assumed to support a greater population of aquatic snails, the intermediate host for *Fasciola hepatica*.

Statistical analysis

The unrelated two-sample t-test was used to analyse the difference between the means of the combined faecal egg counts of the two populations of cattle. The t values for each parasite were compared to statistical table H in Greene and D'Oliveira (1982) at 38 degrees of freedom.

Results

Table 1 Mean faecal egg counts (per gram faeces) of samples obtained

Sample	*F. hepatica*		*Dicroco-elium sp*		*T. colub*		*Ostertagia sp*		*Chabertia sp*		*Cooperia sp*		*A. bovis*		*Trichuris sp*	
	BJ	SC	BJ	SC	BJ	SC	BJ	SC	BJ	SC	BJ	SC	BJ	SC	BJ	SC
1	-	+	50	150	-	-	25	-	25	50	-	-	-	-	-	-
2	+	-	100	-	25	-	-	-	-	25	-	-	-	-	-	-
3	-	-	100	50	25	-	-	-	50	25	-	-	-	-	-	-
* 4	125	50	100	25	50	800	25	-	-	25	-	-	-	-	-	-
5	50	-	-	-	-	150	-	-	-	-	-	-	-	25	-	-
6	25	-	-	50	50	-	50	-	50	-	-	-	-	-	-	-
7	25	-	25	25	25	-	-	-	50	-	-	-	-	-	-	-
8	+	-	25	50	-	-	-	-	75	-	-	-	-	-	-	-
9	-	-	325	-	-	-	-	-	25	-	25	-	-	-	-	25
10	+	-	75	25	-	-	-	-	250	100	-	-	-	-	-	-
11	+	-	-	25	-	-	-	-	25	50	-	-	-	25	-	-
12	-	-	-	25	25	-	-	-	-	-	-	-	-	-	-	-
13	25	-	25	350	-	-	-	-	-	75	-	-	-	-	-	-
14	-	+	75	150	-	-	-	-	50	25	-	25	-	-	-	-
15	. 150	-	25	50	-	-	-	25	-	25	-	-	-	-	-	-
16	-	-	-	-	100	-	-	-	-	25	-	-	-	-	-	-
17	'+	-	-	25	-	175	-	-	-	25	25	-	-	-	-	-
18	-	+	-	75	-	-	-	-	-	-	-	-	-	-	-	-
19	100	-	-	75	-	75	-	-	25	50	-	-	-	-	-	-
20	25	-	-	25	25	-	-	-	-	25	-	-	-	-	-	-

Key to table 1

All counts represent the mean faecal egg counts (FEC) per gram faeces, obtained from the repeated analysis of samples used in the McMaster technique.
+ detected the presence of parasite in petri dish under low power (only *Fasciola hepatica* eggs were large enough to be seen with a dissection microscope).

- no parasite detected

* this individual from San Cristobal had a particularly high *Trichostrongylus colubriformis* egg count.

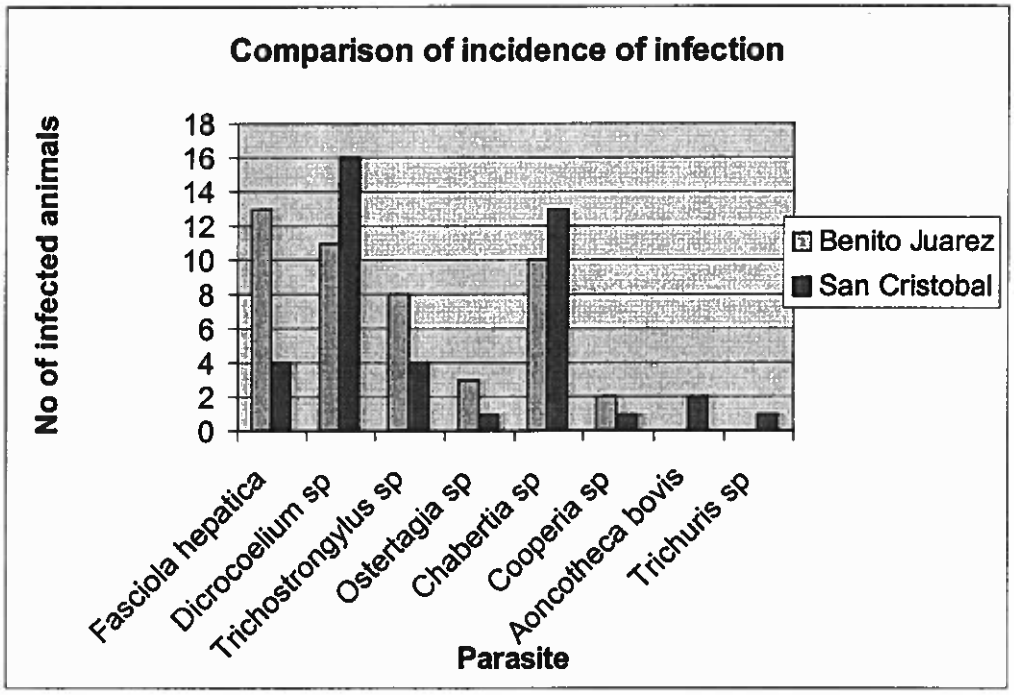
T.colub means *Trichostrongylus colubriformis*

A.bovis means *Aoncotheca bovis.*

Table 2 statistics

Parasite	t value	Level of significance for two-tailed test
F. hepatica	2.333	$p< 0.05$
Dicrocoelium sp	0.505	Not significant
T. colubriformis	1.066	Not significant
Ostertagia sp	1.179	Not significant
Chabertia sp	0.355	Not significant
Cooperia sp	0.588	Not significant
A. bovis	1.453	$p< 0.2$
Trichuris sp	1.000	Not significant

Graph 1 shows the number of animals infected with parasites in both regions



Ziehl-Nielsen Stain

No oocysts could be found in the faecal smears. The positive sample showed bright red oocysts, proving that the staining procedure had been successful (see image 3).

Coproculture

See fig. 1 for drawings and characterisitics used to identify larvae. Only three species of nematode were found in each sample and *Nematodirus sp* was present even though these eggs were too few in number to be detected by the McMaster technique.

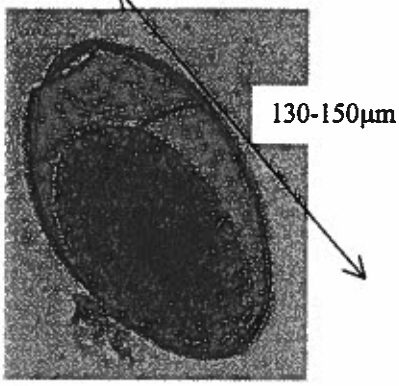


Image 1 *Fasciola hepatica* egg

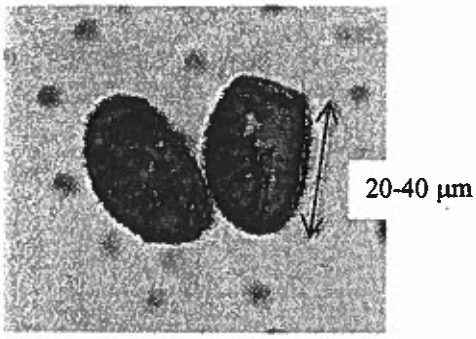


Image 2 *Dicrocoelium sp* egg

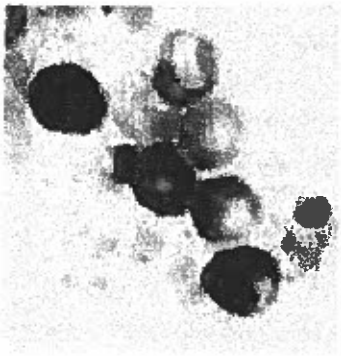


Image 3 *Cryptosporidium sp* oocysts

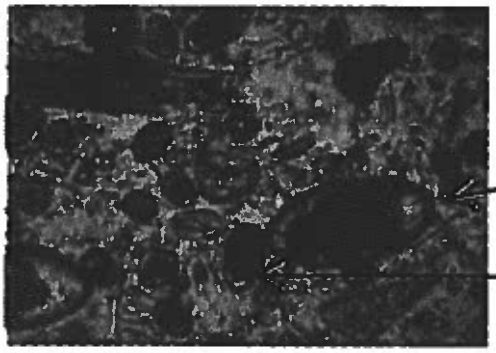


Image 4 Trichostrongyle eggs

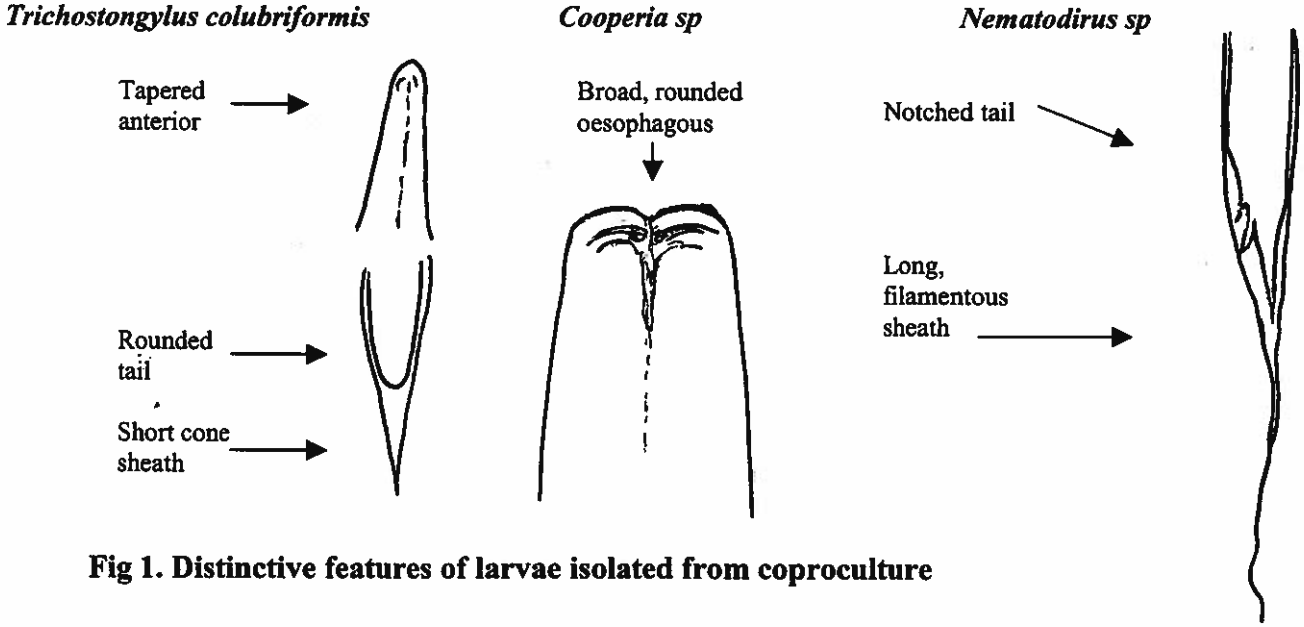


Fig 1. Distinctive features of larvae isolated from coproculture

Discussion

The hypothesis that the marshy nature of the farms in Benito Juarez would propagate a greater number of aquatic snails that are host to the redial stages of the *Fasciola*

parasite, is confirmed by the t test (table 2, $p < 0.05$). The propagation of larval stages of the other parasite species are not particularly affected by waterlogged pastures, as their life cycles do not depend upon it. The statistical test analyses parasitaemia at the group level, rather than that of the individual. As the FEC varies between individuals, graph 1 shows a more representative view of the incidence of infection in the two populations. However, the number of animals used in the study does not reflect the true epidemiology of these parasites. Many of the results were insignificant (table2), suggesting that there was no difference in the prevalence of most parasites between the two populations. This was a surprising result as a difference in burden was expected due to the assumed difference in nutritional status between populations. That is, the supplementation of the diet in San Cristobal animals was expected to introduce higher protein levels to the diet. This practice is known to reduce parasitism via an indirect process of enhanced immunity. Furthermore, animals on Benito Juarez farms have longer grazing time allowances, which one would expect to increase exposure to the infective stages of parasites on the pasture, incurring greater burdens. To rule out the hypotheses stated, another study sampling more animals from each area carried out over a greater time span would have to be performed.

Only one of the subjects out of the 40 sampled had a considerable parasite burden (see *, table 1). The reason for which is unknown, but there are infinite possibilities. It could not have been a consequence of peri-parturient relaxation in immunity as none of the subjects were in-calf, nor did any show any symptoms of disease or plant poisoning. The latter is a common ailment in cattle of this region however all animals were examined by a vet on both visits. Treatment with any of the following anthelminthic therapies was suggested to the university: pro-benzimidazole, benzimidazole, levamisole or ivermectin.

The Ziehl-Nielsen stain yielded no oocysts. However, the subjects were adult and likely to be immune to this disease and would only be expected to release a small number of oocysts in their faeces. Neonatal calves are most at risk of developing a diagnostic infection.

The coproculture technique was not quantitative, but larvae are often more diagnostic in their features than are eggs. It was a useful procedure to confirm that the correct identification of eggs had been made.

References

Greene, J. and D'Oliveira, M. *Learning to use statistical tests in Psychology.* Open University Press, 1982.

Images 1-4 adapted from those in www.parasitology.org.

Acknowledgements

The Weir and Barnson Bequest funds provided adequate financial assistance that covered the costs of flights and living expenses for the time spent in Toluca, whilst carrying out this project.

I would like to thank Dr Mendoza-Vilchis for his assistance in identification of parasites. Dr Arriaga Jordan for his aid in providing contacts within the U.A.E.M. Dr Ortega-Castelan for his organisation of my placement in the lab, accomodation in the city of Toluca, transport to the field and English translations! Thanks also to Benito and Silvia who answered all of my questions enthusiastically.

Itinerary

Tuesday 14th August: Fly from Edinburgh to Mexico City
(Travelled around Mexico until project due to start).
Sunday 2nd September: Arrive in Toluca.
Monday 3rd Septemeber: Meet with Dr Castelan and tour of university campus.
Tuesday 4th and Wednesday 5th September: Practice laboratory techniques.
Thursday 5th Sept: Trip to the field to collect samples.
Friday 6th – Wednesday 13th Sept: Analysis of samples.
Thursday 14th Sept: Trip to field to collect repeat samples.
Friday 15th onwards: Analysis of samples.
Friday 28th Sept: Travel to Mexico City.
Sunday 30th Sept: Flight to Edinburgh.