Changes in gastro-intestinal helminth species diversity in lambs under mixed grazing on irrigated pastures in the tropics (French West Indies)

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Abstract – The development of gastro-intestinal helminth diversity was monitored in lambs grazing alone or grazing with heifers in the ratio one heifer to four lambs. Five successive cohorts of lambs were studied from January 1994 to May 1996. Each cohort of lambs grazed irrigated pastures of Pangola grass for 4 months (from weaning to 6 months of age). A total of 50 lambs was necropsied and their worms counted and identified at the end of each grazing period. Four heifers were also necropsied on one occasion. Special attention was dedicated to the identification of the most pathogenic worm, i.e. *Haemonchus* spp. Malate dehydrogenase polymorphism in *H. contortus* was studied in order to evaluate changes between cohorts and between grazing managements. The species diversity was estimated by Shannon diversity indices (main species or all species). It was higher in the mixed grazing group than in the lambs that grazed alone. Diversity increased in successive cohorts. This was due in part to the acquisition of *Cooperia* spp. of cattle origin. The increase in diversity in the mixed grazing lambs corresponded to the lower faecal egg excretion and better weight gains recorded previously in that group. There seemed to be no cross-transmission of *H. similis* found in heifers and *H. contortus* harboured by lambs. The latter species was not morphologically or genetically different in the lambs grazed alone or with heifers, indicating that the presence of cattle did not modify qualitatively the transmission of *H. contortus*. © Inra/Elsevier, Paris.

***Haemonchus* / *Trichostrongylus* / *Cooperia* / species diversity / mixed grazing**

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Résumé – Modifications de la diversité spécifique des helminthes chez des agneaux élevés en pâturage mixte avec des bovins sur des prairies irriguées sous les tropiques (Antilles Françaises). L'évolution de la diversité des infestations helminthiques a été suivie chez des agneaux élevés au pâturage, soit seuls ou avec des génisses (une génisse pour quatre agneaux). Cinq cohortes successives d'agneaux ont été étudiées de janvier 1994 à mai 1996. Chaque cohorte d'agneaux a pâturé des prairies irriguées de Pangola durant quatre mois (du sevrage à 6 mois). Au total, cinquante agneaux ont été autopsiés, les vers ont été identifiés et dénombrés à la fin de chaque période de pâturage. Quatre génisses ont été également autopsiées au cours de l'expérimentation. Une attention particulière a été portée aux vers du genre *Haemonchus* qui constituent les vers les plus pathogènes dans la région. L'étude d'une enzyme, la malate déshydrogénase, a été réalisée car elle constitue un bon marqueur de la variabilité et permettait d'évaluer des changements éventuels entre cohortes ou type de pâturage. La diversité spécifique a été estimée par l'indice de Shannon, portant soit sur les espèces principales ou sur l'ensemble des espèces. La diversité était plus élevée chez les agneaux bénéficiant du pâturage mixte que chez les agneaux élevés seuls. La diversité helminthique a augmenté au cours des cohortes successives. Cela était dû en partie à l'acquisition par les ovins de *Cooperia* spp. d'origine bovine. L'accroissement de la diversité helminthique chez les agneaux en pâturage mixte s'accompagnait d'excrétion d'œufs réduite et de gains de poids supérieurs, comme l'indique une étude antérieure pour ces deux dernières mesures. Il ne semble pas y avoir eu de transmission croisée d'*Haemonchus similis* rencontré chez les génisses et d'*H. contortus* hébergés par les agneaux. Les *H. contortus* des agneaux pâturant seuls ou avec les bovins ne montrent pas de différence majeures, tant au plan de la morphologie que de la diversité génétique évaluée par les isoenzymes.

***Haemonchus* / *Trichostrongylus* / *Cooperia* / diversité spécifique / pâturage mixte**

1. INTRODUCTION

Mixed and alternate grazing have been proposed as methods to increase productivity and reduce worm burdens. The productivity benefit of mixed sheep and cattle grazing has been evaluated positively in temperate areas [13, 20, 23]. This was attributed to the complementarity of grazing behaviour of sheep and cattle that allowed for a better use of pastures. A ratio of four lambs to one heifer was considered as optimal [20]. Only two productivity studies are available for tropical humid areas [4, 19]; these showed that mixed grazing increased significantly the body weight gains of lambs, whereas no significant increase was recorded in heifers.

Alternate grazing has been evaluated in temperate areas to decontaminate cattle pastures by grazing with sheep [5, 6] or to decontaminate sheep pastures by grazing with cattle [27]. Decontamination of sheep pastures by cattle grazing resulted in reduction of *Haemonchus* spp. and *Trichostrongylus colubriformis* in lambs [27]. Mixed grazing and alternate grazing might modify digestive-tract strongyle infection differently. A previous study in Martinique (French West Indies) did show that strongyle egg excretion was significantly reduced in permanent lambs [19]: the reduction was particularly important during the first 2 months and remained modest at the end of the grazing period, as if the host–parasite equilibrium had been attained. Two other works relate to mixed grazing of sheep and cattle in the tropical humid climate of Brazil [1, 25]. They found that cattle in mixed grazing harboured *Haemonchus placei* and *H. similis* (adapted to cattle) as well as a few *H. contortus* (adapted to sheep). The identification of the main parasite nematode in the tropics, *Haemonchus* spp., has been simplified [14]. Cross-transmission between cattle and sheep has been shown [15, 27]. A previous report from Guadeloupe (French West Indies) stated that *H. placei* was found in cattle [2] but our unpublished data showed that *H. similis* was also present; no data on nematode species are available for Martinique (French West Indies). It has been shown that the latter species rarely parasitises sheep grazing with cattle [25], whereas *H. placei* might infect sheep [15, 25]. The species found in sheep grazed alone or grazed with heifers might then be different; then the evaluation using faecal egg counts could be misleading as nematode fecundity strongly differs between species of nematodes.

The aim of the present work was to compare the species richness harboured by sheep grazed either alone or with cattle in a tropical humid area (Martinique) where it was shown to be beneficial for lamb productivity. Diversity has been also associated with stable communities (e.g. there is no risk of worm community increase to the point at which it is harshly detrimental to the host) in cattle (unpublished data) and we were interested in investigating this hypothesis in sheep. A particular attention was given to *Haemonchus* spp., the most pathogenic nematode encountered in tropical areas, using morphology and isoenzymes, and for which adaptation to cattle or sheep is not totally obvious, and which might then infest sheep to an unpredictable extent. All evaluations were performed at the end of the grazing season, e.g. when the parasite–host interaction was possibly at equilibrium.

2. MATERIALS AND METHODS

The hosts were Martinik lambs (aged 85 days and weighing 15 kg at the beginning of experiment) and Brahman heifers (aged 11 months and weighing 200 kg at the beginning of experiment). The lambs were treated with an anthelmintic before their inclusion in the experimental design; the calves were not treated as they were only lightly infected. The lamb/heifer ratio was 3.7 (ram lambs) to 4 (ewe lambs). The experimental design has been previously described by Mahieu et al. [19]. Briefly, four groups were studied: ewe or ram lambs grazing alone, ewe or ram lambs grazing with heifers. These four groups grazed their own pastures for 4 months. The stocking rates were similar as expressed in metabolic weight (weight$^{0.75}$): 488, 526, 536 and 534 kg/ha, respectively. The lambs were drenched with levamisole every 6–7 weeks, whereas heifers remained untreated. Five cohorts of lambs were investigated from January 1994 to May 1996 using the same protocol. The Pangola grass (*Digitaria decumbens*) irrigated pastures were used on a rotational system (five paddocks for each group) with regrowth of 28 days. The irrigation was organised in such a manner that an equivalent of a minimal 30 mm rainfall/week was reached. Fertilisation was achieved with 250 N, 80 P and 160 K kg/ha/year and was distributed on a one-and-a-half-month basis.

Five lambs were necropsied at the end of a 4-month grazing period in each group and for each cohort. The selection of the rams was not random and we chose the most infected on the basis of faecal egg counts in order to obtain enough worms for the diversity study. This was particularly true in the mixed grazing group which had lower egg counts. The abomasum and small intestine washings were sieved on a 71-µm mesh sieve. One tenth to one twentieth were examined. The immature worms from the abomasal mucosae were extracted after incubation for 4 h at 39 °C. The total number of worms in large intestine was recorded. The worms were identified by species; the identification of *Haemonchus* spp. was based on spicule morphology according to Jacquiet et al. [14, 15]. Females of *H. contortus* were classified by their vulvar appearance as knobbed, smooth or linguiform. On one occasion, four heifers either grazed alone (two) or with lambs (two) were necropsied and *Haemonchus* spp. collected for identification.

Diversity was assessed using the number of species (species richness) or the diversity index of Shannon [26] established on natural logarithm of worm counts [16]. The Shannon index takes into account not only the number of species but also their representation within the nematode community.

In cohorts 2, 3 and 5 the genetic variability of *H. contortus* was assessed in order to test for differences between *H. contortus* in sheep grazing alone or grazing with cattle. Malate dehydrogenase (MDH E.C. 1.1.1.37) was used as it was polymorphic and showed patterns clearly interpretable in terms of putative alleles. The processing of individual samples and staining procedure has been previously described [11]. Isoelectrofocusing on polyacrylamide gels was used to separate products of putative alleles [8]. The genetic data were analysed using an exact test for differentiation [22] or F_{is} (variance within populations) or F_{st} (variance between populations) as described in Weir and Cockerham [28]. The significance of F_{is} and F_{st} was assessed using resampling based on permutations (alleles within genotypes for F_{is} and individual genotypes between populations for F_{st}) [7]. The *Haemonchus* spp. found in cattle were also identified on several occasions.

Univariate statistical procedures were Fisher's exact probability test for the proportions of female morphs, Pearson or non-parametric Spearman correlations, Kruskall-Wallis analysis of variance, using Simstat software [21].

3. RESULTS

3.1. Intensity of infection by the different species (*table I*)

The influence of cohort was due to low infection by *H. contortus* in cohorts 2 and 5 and low infection by *Cooperia* sp. in cohorts 1, 2 and 5. The infection of lambs by *Cooperia* sp. was nearly nil in sheep grazed alone, whereas it was important in mixed grazing with cattle. The influence of season was only found in *T. colubriformis*, with higher burdens during the dry season. Ram lambs were more heavily infected than ewe lambs. The intensity of infection was not very different in lambs grazed alone or lambs grazed with heifers.

3.2. Species diversity (*table II*)

Succession of cohorts ($P < 0.01$) and season ($P < 0.01$) were the significant modificators of diversity as assessed by the Shannon index. Diversity was higher during the dry season, when *T. colubriformis* was more abundant, *H. contortus* (lambs grazed alone or with heifers) and *Cooperia* sp. (lambs grazed with heifers) being recorded whatever the season. Diversity was positively related to the total number of worms (Spearman r = 0.51; $P = 0.001$). The number of species (all species) was higher in lambs grazed with heifers. This number of species was also positively correlated with the total number of worms (Spearman r = 0.47; $P = 0.01$).

Table I. Number of worms in lambs at the end of each grazing period in relation to cohort, season, gender of host and mixed grazing with cattle.

Parasitological parameters	Environment and host			
	Cohort (1 in 1994 to 5 in 1996)	Season(dry, intermediate or rainy)	Gender (male or female lambs)	Lambs alone or mixed grazing with cattle
Haemonchus contortus	1 343; 234; 2 192; 2 229; 345*	1 269; 1 343; 1 232	1 501; 920*	1 296; 1 241
Cooperia punctata and *Cooperia pectinata*	121; 79; 210; 377; 42	126; 121; 228	183; 140	3; 329**
Trichostrongylus colubriformis	8; 0; 151; 21; 55*	103; 8; 11	44; 51	69; 25
Strongyloides papillosus	1 224; 137; 161; 198; 48	105; 1 224; 167	494; 144	518; 189
Oesophagostomum nodules	1.8; 3.4; 0.9; 3.0; 3.2	2; 1.8; 3.2	2.2; 2.9	2; 3
Moniezia sp.	1.0; 0.3; 1.8; 5.6; 2.2	2; 1; 3	2.8; 1.3	2.4; 2

* Significant at $P < 0.05$ or ** $P < 0.01$ (Kruskall-Wallis test).

Table II. Diversity (Hr, Shannon index H', based on the three major nematode species, *H. contortus*, *T. colubriformis* and *Cooperia* spp.; Ht, Shannon index H' based on all the species) and species richness (S, all helminths) at the end of each grazing period in relation to cohort, season, gender of host and mixed grazing with cattle.

Diversity indices	Environment and host characteristics			
	Cohort (1 in 1994 to 5 in 1996)	Season (dry, intermediate or humid)	Gender (male or female lambs)	Lambs grazed alone or mixed grazing with cattle
Shannon Hr (H' for Trichostrongyles)	0.19; 0.32; 0.36; 0.29; 0.50	0.43; 0.19; 0.31	0.37; 0.28	0.21; 0.45*
Shannon Ht (H' for all helminths)	0.30; 0.31; 0.36; 0.54; 0.65*	0.51; 0.30; 0.43	0.47; 0.39	0.30; 0.57*
Species richness (all helminths)	3; 2; 3.3; 4.1; 3.4*	3.4; 3.0; 3.1	3.43; 2.75	2.48; 3.84**

* Significant at $P < 0.05$ or ** $P < 0.01$ (Kruskall-Wallis test).

3.3. Morphologic and genetic variability of the main species, *H. contortus*

All the males studied (44 worms in seven lambs grazed with heifers and 58 worms in five lambs grazed alone) were typical *H. contortus* based on spicule morphology, except two that were *H. placei* type in the lambs grazed with cattle (data not shown). In necropsied heifers only *H. similis* were found. Male morphometrics were consistent from one host to another (*table III*). The proportions of female (*table IV*) morphs were similar ($P = 0.12$ for smooth and $P = 0.25$ for linguiform type, using Fisher's exact-test) in lambs grazed alone or lambs grazed with cattle.

Eight distinct phenotypes could be observed at the more rapid migrating locus of MDH: four single-banded phenotypes classified as homozygotes (coded 11, 22, 33 and 44, the latter having the more rapid migration) and four three-banded phenotypes characteristic of heterozygotes for dimeric enzymes (12, 13, 23 and 24). The distribution of the different putative genotypes is shown in *table V*. The fourth allele was rare. A departure from Hardy-Weinberg equilibrium was found in five populations out of ten; a deficiency of heterozygotes being found at the beginning of the genetic investigation (two in cohort 2, one in cohort 3), whereas an excess of heterozygotes was recorded later in two cohorts (one in cohort 3, one in cohort 5), the equilibrium being the rule for the remaining populations in cohorts 3 and 5. The values of F_{is} (0.018) and F_{st} (0.014) were low and only F_{st} was significantly different from zero ($P = 0.01$, using permutations between individuals). This indicates that the entire set of *H. contortus* populations was in Hardy-Weinberg equilibrium and that although there were differences among populations, they were small. The differences (F_{st}) between lambs alone and lambs grazed with heifers remained small in ewe lamb batches (cohorts 2, 3 and 5: 0.03, 0.03 and 0.04, respectively) and non-existent in ram lambs (cohorts 3 and 5: –0.06 and –0.004, respectively). No significant difference was found between *H. contortus* collected from the hosts (ram or ewe lambs) maintained under both types of grazing ($F_{st} = 0.005$; $P = 0.39$

Table III. Morphologic characteristics of male *Haemonchus* spp. recovered in lambs and heifers grazed alone or mixed.

Shape of dorsal ray	*H. contortus* type		*H. similis* type	
	Lambs alone (three lambs)	Lambs with heifers (three lambs)	Heifers alone (two heifers)	Heifers with lambs (two heifers)
Length of spicules in µm	381 ± 6[a] (7)[b] 379 ± 13 (10) 385 ± 10 (6)	395 ± 20 (9) 394 ± 11 (9) 394 ± 12 (14)	333 ±13 (19) 333 ± 10 (12)	332 ± 12 (19) 331 ±10 (10)
Distance from the hook to the tip of the right spicule	35 ± 1 (7) 36 ± 2 (10) 35 ± 2 (6)	36 ± 2 (9) 36 ± 2 (9) 36 ± 2 (14)	65 ± 5 (19) 65 ± 4 (12)	65 ± 5 (19) 67 ± 3 (10)
Distance from the hook to the tip of the left spicule	17 ± 2 (7) 18 ± 2 (10) 17 ± 1 (6)	18 ± 1 (9) 18 ± 2 (9) 17 ± 2 (14)	49 ± 3 (19) 50 ± 4 (12)	50 ± 3 (19) 50 ± 5 (10)

[a] Length ± standard deviation; [b] number of worms.

Table IV. *Haemonchus* spp. female morphotypes recovered in lambs and heifers grazed alone or mixed.

Female morphotypes (%)	Lambs alone (seven lambs) (110 worms)	Lambs with heifers (20 lambs) (165 worms)	Heifers alone (two heifers) (165 worms)	Heifers with lambs (2 heifers) (126 worms)
Linguiform	39.2 ± 10.2[a]	38.8 ± 12.5	100	100
Smooth	48.1 ± 10.5	46.1 ± 12.8	0	0
Knobbed	12.7 ± 7.0	15.1 ± 9.2	0	0

[a] Confidence interval at $P = 0.05$.

using permutations of genotypes between the two types of grazing system).

4. DISCUSSION

H. contortus was the main parasite and *H. placei* was found only twice at very low numbers in lambs grazing with heifers. This could be due to the absence of evidence of cattle infection with *H. placei* although it can be transmitted easily in lambs [14, 17], and that it is found in natural infections at similar sites [14, 24]. A new fact in our studied area was that *H. similis* was the only worm found in heifers, either grazed alone or with lambs in Martinique. This could be due to irrigation, as *H. similis* requires more humidity than *H. placei*. This makes our trial very different from the Brazilian ones, where *H. placei* was also present in cattle [1, 25] and from the Australian alternate grazing assays [6, 27]. No reduction in the *H. contortus* worm burden was noted in lambs grazed with heifers, probably owing to our sampling procedure (e.g. selecting the most infected lambs in the lambs grazed with heifers). Our study did not allow us to determine whether cattle ingested *H. contortus* without successful subsequent development, although that could explain why infestation was lower in mixed grazing as assessed from faecal egg counts [19]. *H. contortus* was very similar (morphologi-

Table V. Malate dehydrogenase genotypes of *Haemonchus contortus* harboured by ten lambs grazed either alone (LO) or with heifers (LH) in successive cohorts.

Cohort	Lamb gender	Grazing type	Genotypes								Departure from Hardy-Weinberg equilibrium (deficiency or excess of heterozygotes)
			11	22	33	44	12	13	23	14	
2 (rainy season 1994)	F	LH	3	16	2	0	6	1	13	0	deficiency ($P = 0.0001$)
	F	LO	3	12	0	0	4	13	5	0	deficiency ($P = 0.003$)
3 (dry season 1995)	F	LH	0	18	8	1	16	3	14	2	deficiency ($P = 0.002$)
	F	LO	2	15	2	0	19	3	2	0	equilibrium
	M	LH	0	7	0	0	5	0	1	0	excess ($P = 0.04$)
	M	LO	0	3	0	0	2	0	0	0	equilibrium
5 (dry season 1996)	F	LH	1	10	0	0	17	1	3	0	equilibrium
	F	LO	1	8	4	0	8	2	5	0	equilibrium
	M	LH	1	13	2	0	19	2	6	0	excess ($P = 0.0003$)
	M	LO	0	11	2	0	12	2	7	0	equilibrium

cally and genetically) in lambs grazing alone or with heifers, probably indicating that heifers did not interfere much in the transmission of the parasite. No important genetic difference was observed between the nematodes under the two types of grazing; however, this was assessed with only one polymorphic enzyme and was probably not powerful enough to assess differences between populations. The deficiency in heterozygotes recorded at the beginning of experiment and which disappeared in the 5th cohort is probably due to a Wahlund effect (false deficiency in heterozygotes due to mixed populations with different allelic frequencies). It should be noted that the ecotype (based on the proportions of three vulvar morphs) from Martinique did not correspond well to any ecotype recorded by Das and Whithlock [10]. The ecotype *H. contortus utkalensis* was expected in the tropical humid climate of Martinique but it did not correspond to the morph frequencies we recorded, as very few knobbed morphs were recovered.

Species diversity depended on environment and host. Seasonal variations in the proportions of the different helminth species were expected [2, 3] as previously shown in the area. However, the total number of worms at the end of the grazing period was not significantly modified in relation to season, except for a rise in *T. colubriformis*. The seasonal variability was then reduced in our conditions probably owing to irrigation but also to repeated treatments that might have reduced the increase in helminth infections. The gender of lambs influenced the intensity of infection as previously shown in another investigation on Barbados Blackbelly sheep [29], which are very similar to the Martinik sheep. The higher infection of males has also been recorded in other breeds and under different climatic conditions [18]. The number of species recovered was more important in mixed grazing groups (due to the acquisition of *Cooperia* species originating from cattle). A similar finding was evidenced in one Brazilian study [1]. From the data in the latter study, we calculated that the Shannon index increased from 0.41 in the sheep grazed alone compared with 0.50 in the sheep grazed with cattle. There was also a clear increase in indices of diversity with time, from the 1st to the 5th cohort. This was understandable for Trichostrongyles (acquisition of *Cooperia* sp.); the increase was even more marked when all helminth species were considered. This could be due to the fact that lambs might have a greater opportunity to acquire species with increasing time spent grazing on the same pastures, somehow similar to increased diversity reported in larger natural pastures [12]. Diversity indices were higher in the mixed grazing group which had lower egg excretion during the whole grazing period and presented higher weight gains [3], giving support to our hypothesis that higher diversity corresponded to better controlled worm community, which in turn permitted better production. We used cardinal indices of diversity (Shannon index: all species are equal) and species abundance distribution might have seemed a better alternative (all species are different), which corresponds to an ordinal index of diversity as stated by Cousins [9]. The species abundance distributions could not be fitted to any interpretable distribution (data not shown) and we present, therefore, only the cardinal index.

The helminth fauna of lambs was richer in mixed grazing conditions due in part to the acquisition by sheep of cattle nematodes, such as *Cooperia* spp. The species diversity was not fully investigated in this study, as the *Oesophagostomum* or *Moniezia* were not identified to species level and further studies could give information on the eventual acquisition of cattle species by sheep. The results from the present and previous work on mixed grazing indicates that the benefit from mixed grazing could be due to an increase in species diversity that would allow a better control of the size of the helminth community either through species interactions or/and host response.

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