

Short note

**Responses of *Fasciola hepatica* infected sheep
to various infection levels**

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(Received 2 June 2000, accepted 12 September 2000)

Abstract – The response to *Fasciola hepatica* was studied in sheep infected with 5, 30, 150 metacercariae. The animals were necropsied 12 weeks post-infection (p-i) for counting and measuring flukes. Cellular and humoral responses were detected by peripheral eosinophil count, peripheral blood lymphocyte proliferation with excretory-secretory products (FhESP) and ELISA. All sheep were infected at necropsy except one sheep which was infected with 5 metacercariae. Mean parasitic intensities were 40%, 44% and 27% of the infection dose in sheep infected with 5, 30, 150 metacercariae respectively. FhESP-specific lymphocyte responses of the 3 infected groups were significantly enhanced in weeks 3 and 4 p-i ($p < 0.05$). The kinetics of the specific humoral response were similar for the 3 infected groups but the antibody level was significantly lower in animals infected with 5 metacercariae than in the 2 other infected groups from week 5 p-i to week 12 p-i ($p < 0.05$). Peripheral eosinophil count was significantly enhanced ($p < 0.05$) in infected groups. The numbers of peripheral eosinophils were significantly different between the 3 infected groups in week 3, 4 and 6 p-i and were related to infection level. These results confirm that sheep are highly susceptible to *F. hepatica* infection, even when infection pressure is very low. Peripheral eosinophilia was dependent of the infection level. The immune response was similar in sheep infected with various numbers of flukes.

***Fasciola hepatica* / eosinophil / lymphocyte / antibody level / experimental infection**

Résumé – Réponses du mouton à différents niveaux d'infestation par *Fasciola hepatica*. La réponse à *Fasciola hepatica* a été étudiée chez des moutons infestés par 5, 30 ou 150 métacercaires. Les animaux ont été autopsiés 12 semaines post-infestation (p-i) et les douves ont été dénombrées et mesurées. Les réponses cellulaires et humorales ont été évaluées par dénombrement des éosinophiles circulants, prolifération des lymphocytes circulants avec des produits d'excrétion-sécrétion de *F. hepatica* (FhESP) et ELISA. À l'autopsie, tous les moutons étaient infestés sauf un mouton infesté par 5 métacercaires. L'intensité parasitaire moyenne était de 40%, 44% et 27% de la dose infestante.

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chez les moutons infestés respectivement par 5, 30 et 150 métacercariae. La réponse lymphocytaire spécifique des FhESP était significativement augmentée en semaines 3 et 4 p-i ($p < 0,05$). Les cinétiques de réponse humorale étaient similaires pour les 3 groupes infestés mais le taux d'anticorps était significativement inférieur chez les animaux infestés par 5 métacercariae de la semaine 5 à la semaine 12 p-i ($p < 0,05$). L'éosinophilie périphérique était significativement augmentée ($p < 0,05$) dans les groupes infestés mais le nombre d'éosinophiles était significativement différent entre les 3 groupes infestés en semaine 3, 4 et 6 p-i en fonction du niveau d'infestation. Ces résultats confirment que le mouton est très sensible à l'infestation par *F. hepatica*, même quand la pression d'infestation est très limitée. L'éosinophilie périphérique était dépendante du niveau d'infestation. La réponse immunitaire était comparable chez les moutons infestés avec un nombre variable de douves.

Fasciola hepatica / éosinophile / lymphocyte / taux d'anticorps / infestation expérimentale

1. INTRODUCTION

The clinical expression of *Fasciola hepatica* infection is dependent on infection level [1]. Only a few studies have investigated the humoral immune response against *F. hepatica* infection after various infection doses; the main purposes of these studies were to propose diagnosis methods which could predict the infection level of the animals [11]. In different hosts (sheep, cattle, rats), immune response against *F. hepatica* is characterised by a precocious increase of antibody level, an increase of peripheral eosinophil count and a precocious and transient parasite-specific lymphoproliferation [3, 4, 7–9]. Usually, these experiments are done after experimental infection with a number of metacercariae which induce chronic fasciolosis. In the present study, we investigated the cellular and humoral response to low infection levels in sheep, the natural sensible host.

2. MATERIALS AND METHODS

Twenty 12 month old cross-breed "Vendéen × Belle Islois" male sheep were randomly divided into 4 groups of 5 animals. Group A was the uninfected control group. The animals of groups B, C and D were infected by oral administration of a gelatin capsule containing 5, 30 or 150 *F. hepatica* metacercariae respectively.

F. hepatica metacercariae were 8 weeks old; they were initially pooled and divided in aliquots to obtain homogenous infection material. The humoral response was investigated by ELISA with excretory-secretory products of *F. hepatica* (FhESP) as previously described [2, 3]. Total peripheral leukocytes were counted from week 0 to week 6 p-i using a Malassez cell and the proportion of the different leukocytes was evaluated on stained blood smears. Every week during the first 6 weeks, peripheral blood mononuclear cells (PBMC) were isolated from blood using a density gradient (Ficoll d = 1.077); and cultured in vitro with FhESP (1.25 and 5 µg per well) for 5 days as previously described [3, 7]; proliferation was evaluated by incorporation of tritiated thymidine and the Stimulation Index (S.I.) was calculated: S.I. = mean CPM triplicate cultures of cells with antigen / mean CPM triplicate cultures of cells with medium. Statistical analysis was done using non-parametric tests (Kruskal-Wallis and Mann-Whitney tests). At the end of the experiment, all animals were necropsied and their livers dissected for recovery and measurement of flukes.

3. RESULTS

In sheep infected with 30 or 150 metacercariae, all animals were well infected. In sheep infected with 5 metacercariae, flukes

Table I. Number of flukes recovered from the infected animals.

Animal	Number of metacercariae		
	5	30	150
1	0	5	17
2	4	14	33
3	2	12	57
4	2	16	62
5	2	19	30
Mean number of flukes	2	13.2	39.8
Mean level of infection (%)	40	44	27

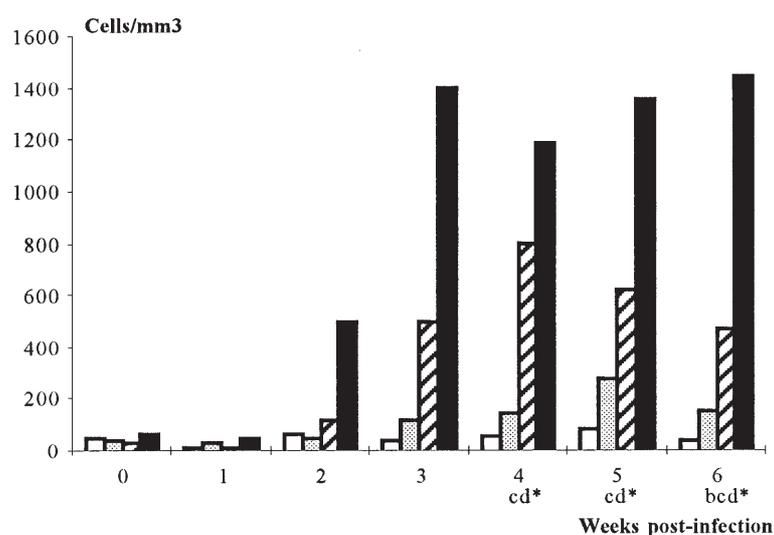


Figure 1. Eosinophil counts in the blood of *F. hepatica* infected animals (group B (5 metacercariae): ▤; group C (30 metacercariae): ▨; group D (150 metacercariae): ■) and non-infected animals (group A: □). Letters (b: group B; c: group C; d: group D) indicate statistical differences between the infected group and the control group as determined by the Mann-Whitney test ($p < 0.05$). Stars (*) indicate statistical differences between the infected groups (B < C < D) as determined by the Kruskal-Wallis and Mann-Whitney tests.

were recovered in 4 animals (Tab. I). Most of the parasites were mature flukes (90%, 83% and 79% of flukes measured more than 16 mm in sheep infected with 5, 30 or 150 metacercariae respectively).

Peripheral eosinophil (Fig. 1) counts were significantly enhanced ($p < 0.05$) in week 6 p-i for sheep infected with 5 metacercariae and in weeks 4, 5 and 6 p-i for sheep infected with 30 or 150 metacercariae. The

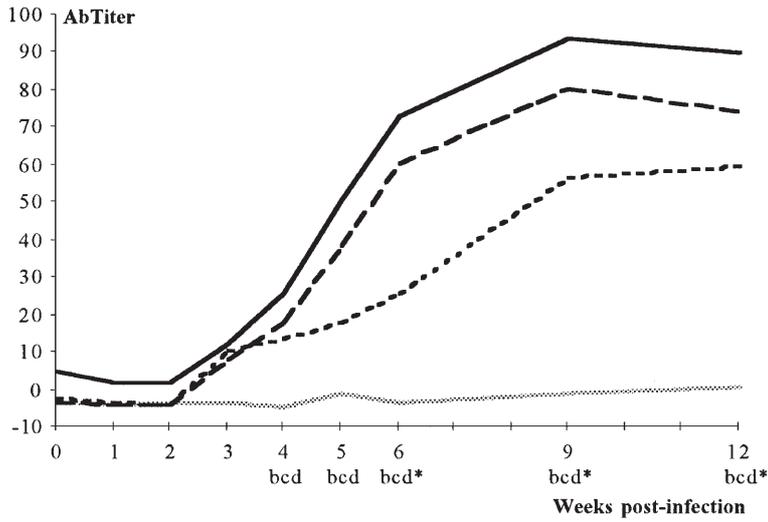


Figure 2. Kinetics of FhESP-specific antibodies in *F. hepatica* infected animals (group B (5 metacercariae):; group C (30 metacercariae): — —; group D (150 metacercariae): —) and non-infected animals (group A: —). Letters (b: group B; c: group C; d: group D) indicate statistical differences between the infected group and the control group (as determined by the Mann-Whitney test ($p < 0.05$)). Stars (*) indicate statistical differences between the infected groups (B < C; B < D) as determined by the Kruskal-Wallis and Mann-Whitney tests.

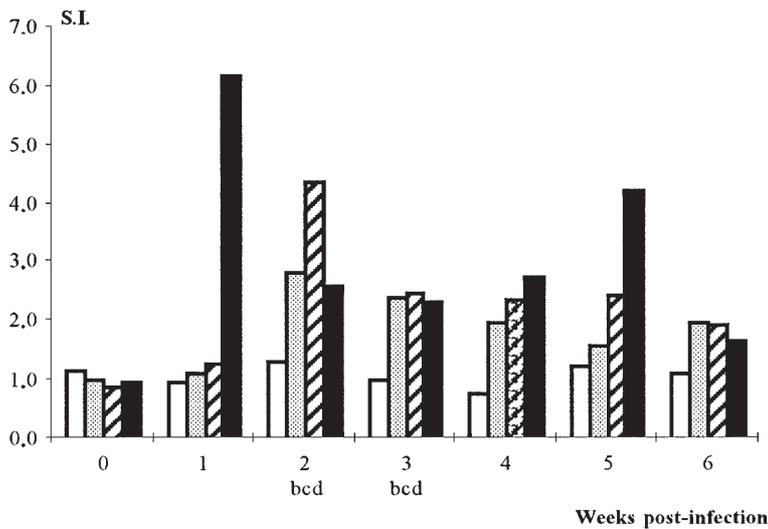


Figure 3. Proliferative responses of PBMC to FhESP in *F. hepatica* infected animals (group B (5 metacercariae): ▨; group C (30 metacercariae): ▩; group D (150 metacercariae): ■) and non-infected animals (group A: □). Letters (b: group B; c: group C; d: group D) indicate statistical differences between the infected group and the control group (as determined by the Mann-Whitney test ($p < 0.05$)).

numbers of peripheral eosinophils were significantly different between the 3 infected groups in weeks 3, 4 and 6 p-i and were related to the infection level.

FhESP-specific antibody responses (Fig. 2) were significantly enhanced in week 3 and 4 p-i ($p < 0.05$). Antibody level was significantly lower ($p < 0.05$) in sheep infected with 5 metacercariae than in the 2 other infected groups from week 6 to week 12 p-i. The antibody level increased more rapidly when animals were more heavily infected.

FhESP-specific PBMC response (Fig. 3) of the 3 infected groups were significantly enhanced ($p < 0.05$) in weeks 3 and 4 p-i. The kinetics and intensity of the cellular response were similar for the infected sheep of the 3 infected groups except for one sheep of group D (150 metacercariae) which showed a high proliferative response in week 1 p-i (D5; IS = 26).

4. DISCUSSION

These results confirm that sheep are highly susceptible to *F. hepatica* infection, even when infection pressure is as low as 5 metacercariae. Infection rates seemed higher with low levels of infections as previously observed in cattle [11] and in sheep [5]. The humoral immune response seemed similar in the sheep infected with various numbers of metacercariae. A significantly lower antibody response was observed only in the group infected with 5 metacercariae. This variation could not be used to evaluate the infection intensity in vivo because the kinetics of antibodies induced more variability than the infection intensity. The kinetics and the intensity of cellular response were similar to those previously observed with 150 to 250 metacercariae [3, 7] and they seemed independent of the infection level. In contrast, eosinophilia was correlated with the intensity of the infection. The regulation of this mechanism, notably by IL-5 secretion should be further investi-

gated. Previous studies have show that flukes from primary infection facilitate the migration of flukes from secondary infection [3, 6, 10], and that, after secondary infection, the immune response is decreased: in sheep, the humoral response and the level and the duration of the FhESP-specific lymphocyte proliferative response is reduced [3] and, in cattle, no IFN γ response could be observed [4]. Clery et al. [4] suggested that primary infection induce a tolerance to reinfection because of a Th2 regulation of the immune response. In France, two periods of infection are usually described, one of low intensity in the spring and one of high intensity in the autumn; the role of the low intensity spring infection in the installation of flukes in autumn should be further investigated.

ACKNOWLEDGMENTS

We thank Anne-Marie Marchand and Cécile Roux (ENVN) for their technical assistance and Florence Carrera (INRA PAP) for providing metacercariae.

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