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ORIGINAL PAPER

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Behavioural dominance and *Taenia crassiceps* parasitism in BALB/c male mice

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Abstract Behavioural dominance relationships in mouse populations are based upon fighting and antagonistic behaviour. Social rank is affected by the physiological states present in the mice. Experimental infection by *Taenia crassiceps* cysticerci induced physiological disorders and disrupted the dominant-subordinate status. Infected male mice showed strong perturbations in territorial behaviour and aggressiveness. Infected dominant male mice did not show a significant reversal of dominance order compared to uninfected mice. In addition, during confrontation between naive infected and healthy mice, infected animals more often assumed a subordinate status than healthy ones. The effects of the infection by *T. crassiceps* were more likely to prevent adult male mice from becoming behaviourally dominant than to reverse existing dominance relationships. The results are discussed on the basis of the parasite manipulation hypothesis and host optimal foraging and decision-making theories.

Introduction

The social system of male mice is based on behavioural dominance and territoriality (Poole and Morgan 1975; Poshivalov 1977), and dominance hierarchies are known to be maintained by fighting and antagonistic behaviour (Beilharz and Beilharz 1975; de Catanzaro and Ngan 1983). In this system, the social rank of a male mouse is dependent on physical, physiological, and immunological capabilities consistent with the adaptive modulation hypothesis and differences in life-history strategy between individuals (Barnard et al. 1994, 1996, 1998). Mice experimentally infected by *Taenia crassiceps* (Zeder, 1800) Rudolphi, 1810 cysticerci are known to exhibit profound physiological (Huerta et al. 1992; Larralde et al. 1995; Morales-Montor et al. 1999; Corbin et al. 2000) and behavioural (Morales et al. 1996; Gourbal et al. 2001) modifications. The present study was undertaken, using both behavioural traits (wounding, urinary marking, and testosterone and corticosterone levels) and behavioural interactions (fighting, submissive posture, retaliation), in order to ascertain whether infection by *T. crassiceps* cysticerci in adult BALB/c male mice interferes with the establishment of dominance or whether the infection could reverse an already established dominance order.

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Materials and methods

Mice and husbandry

The specific pathogen-free BALB/c male mice (Charles Rivers) used were 8-9 weeks old and were maintained in individual cages (250x160x136 mm) under a 12-h light/12-h dark photoperiod at 22 ± 1°C to avoid any social contact (=naive mice). Food (cereal pellets; Usine d'Alimentation Rationnelle, Epinay-sur-Orge, France) and water were given ad libitum.

Parasite and experimental infections

The strain of *Taenia crassiceps* came from cysticerci recovered during the autopsy of a vole, *Microtus duodecimcostatus,* trapped on Saint-Clément-de-Rivière (Hérault, France). Experimental infections of 40 *T. crassiceps* cysticerci were performed via i.p. injection (under anaesthesia) in 0.5 ml of PBS (phosphate buffered saline: 0.15 M NaCl+0.01 M Na_2HPO_4 $7H_2O$, pH 7.2) (Freeman 1962; Righi et al. 1996). Healthy mice were sham-injected using the same protocol except that they received only 0.5 ml of PBS. The anaesthesia of animals was achieved using 10% ketamine-rompun in PBS solution, injected i.p. at a dose of 0.1 ml/10 g body weight.

T. crassiceps cysticerci undergo asexual multiplication in the mouse intermediate host. This implies an increase of parasite load during the course of infection (Gourbal et al. 2001).

Blood collection and steroid radioimmunoassay

Blood samples (300-400 µl) were centrifuged and the plasma (100-150 µl) stored at -20°C until use. All blood samples were collected between 2 p.m. and 4 p.m. in order to control for diurnal fluctuations in circulating steroid levels. Plasma samples were assayed for testosterone and corticosterone according to the methods of Mauget et al. (1.994), in the CNRS laboratory of Chizé. The assay procedure was the same for both testosterone and corticosterone and each was measured without chromatography on ether-extracted samples (50 µl) via radioimmunoassay (RIA) using specific antibodies (Falvo and Nalbandov 1974).

Urinary marking patterns and ultraviolet light visualization

In each experiment, urinary marking was observed to determine the social status of paired animals. Dominant males vigorously mark the entire cage floor with many small urine spots whereas subordinate ones typically void urine in only five to ten pools in the corners of the cages. The number and location of urine spots was used as an indicator of social status (Desjardins et al. 1973). Paired mice were isolated from each other by a transparent and perforated partition; the bottom of the cage was covered with a sheet of Whatman N°3 MM filter paper during overnight testing (8 p.m.-8 a.m.). Urine marks on the filter paper were visualized using an ultraviolet lamp.

Experimental procedures

The experimental procedures are listed in detail in Table 1. "Pairs" indicates two male mice that were kept in one cage. The mice had not interacted socially prior to the experiments. To determine the dominance status in each experiment over the 5 days of confrontation, mice were weighed, the number of tail and rump wounds was counted and the pattern of urine marks was observed. In experiment 2, blood was collected to determine the testosterone and corticosterone levels (1) before the establishment of dominance status, (2) once dominance status was established and (3) at 36 and 90 days post-infection (DPI).

Statistical analysis

A Mann-Whitney U-test was used to analyse the differences observed between urine spots as well as for differences in steroid levels measured via RIA. A paired Student's *t*-test was used to compare differences in weight within a group and non-paired *t*-tests were used to compare wound numbers between different groups. Data from experiments 2 and 3 were analysed using Fisher's exact test. Results were considered significant at $P < 0.05$ (Scherrer 1984; Zar 1996). The Statistica and Statview programs were used for analysis.

Results

Experiment 1

For the dominant male in uninfected pairs (controls), urine deposition (Fig. 1A, Z=-3.97, $P<0.0001$) and weight (Fig. 1C, between day 1 and day 5: t=-5.19, P=0.0004) increased significantly. For subordinate males the wounds were significantly greater than for the dominant mouse (Fig. 1E, day 5: t = -6.08, $P<0.0001$). In infected pairs, dominant mice presented the same urinary marking patterns as subordinate mice (Fig. 1B). The weight of dominant, infected mice increased significantly (Fig. 1D, between day 1 and day 3: t=-3.01, P=0.029) There was no significant difference in the number of wounds between dominant and subordinate infected mice (Fig. 1F).

Experiment 2

During the first 90 days, no retaliation or attack from a when the partitions were removed either for infected or uninfected dominant males. After 90 days, the urinary marking of the mice, the weight and wounds showed that among the six experimental pairs, four dominant males remained dominant and two became subordinate. Among the seven control pairs, four dominant males remained dominant and three became subordinate. There was no significant difference between the reversions observed in the experimental pairs and those ob-served in control pairs (P=0.407).

Table 1. Experimental procedures. *DPI* Days post-infection

		Observation
Experiment 1	Six pairs infected 90 days prior to start of observations	5 days of confrontation
Aggressiveness and expression of dominance	Controls: 11 uninfected pairs	
Experiment 2	Six experimental pairs and seven control pairs, checked for dominance order during 5 days. Then paired animals were separated by a perforated plexiglas partition	For 90 DPI the partition was removed each 3 days, observation time 10 min/day
Reversal of dominance order	Experimental pairs: dominant males were infected, subordinate sham-infected Controls: sham-infection of dominant as well as subordinate male	After 90 DPI, 5 days of confrontation
Experiment 3	14 pairs composed of one male infected 90 days prior to start of observation and one uninfected male	5 days of confrontation
Development of dominance order		

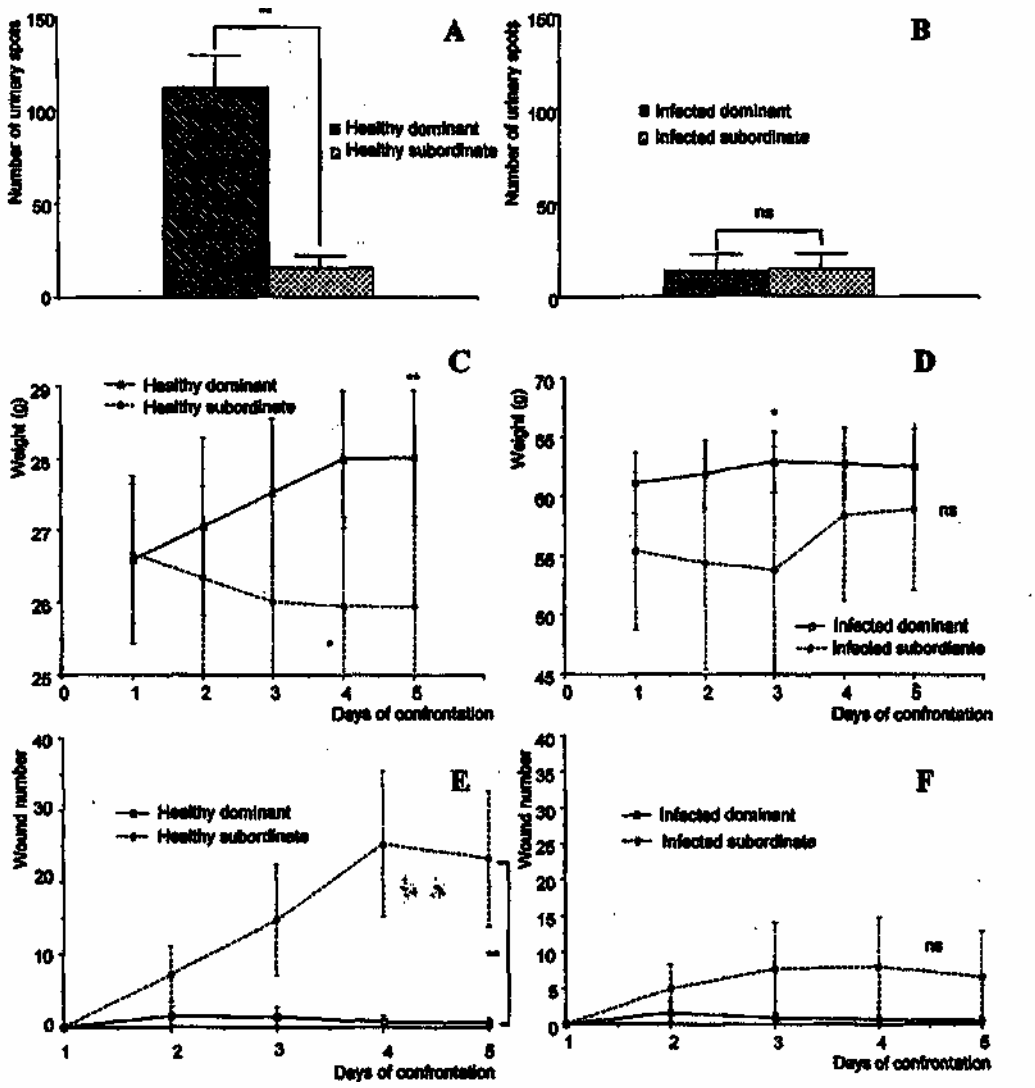

Fig. 1. Number of urine spots, changes in weight and wound number for dominant and sub-ordinate healthy mice (**A, C, E**) (n = 22) and infected mice (90 days post-infection) (**B, D, F**) (n= 12). Data represent means ±SE. *P<0.05; **P< 0.001; *ns* not significant

Experiment 3

Healthy mice deposited significantly more urine spots than infected individuals (Fig. 2A, Z=-2.71, P= 0.0066). There was no significant difference in weight between infected and healthy mice (Fig. 2B). Wounds were significantly more numerous in infected mice than in healthy ones (Fig. 2C, day 3: t=2.67, P=0.02). Healthy mice became dominant significantly more often than infected mice (P=0.024).

Steroids radioimmunoassay

There were no significant differences in testosterone or corticosterone levels between healthy dominant and subordinate mice (Table 22). For infected animals, a significant decrease in testosterone level at 36 and 90 DPI was observed (healthy and 36 DPI infected mice: Z=-2.10, P=0.035; healthy and 90 DPI infected mice: Z=-1.99, P=0.045). The corticosterone levels of infected male mice were the same as healthy animals (Table 2).

Discussion

The data obtained indicate that an experimental infection of BALB/c male mice with *T. crassiceps* cysticerci does not modify an established dominance order. In fact, infected dominant males lost their social status less frequently than did control mice (experiment 2). In experimental pairs in which the dominant-subordinate relationship was firmly established, the uninfected sub-ordinate male always showed a submissive attitude in the face of an infected dominant male. Such an absence of dominance order reversion has been defined as a settled or stable dominance relationship (Blanchard et al. 1988; Haemisch et al. 1994; Berdoy et al. 1995). On the other hand, for "naive" mice, the infection modified the development of the dominance relationship. During paired confrontations, infected mice were more often subordinate than healthy ones (experiment 3). In addition, the infected animals showed deep perturbations in the expression of dominance status. Furthermore, territorial marking behaviour was affected and the

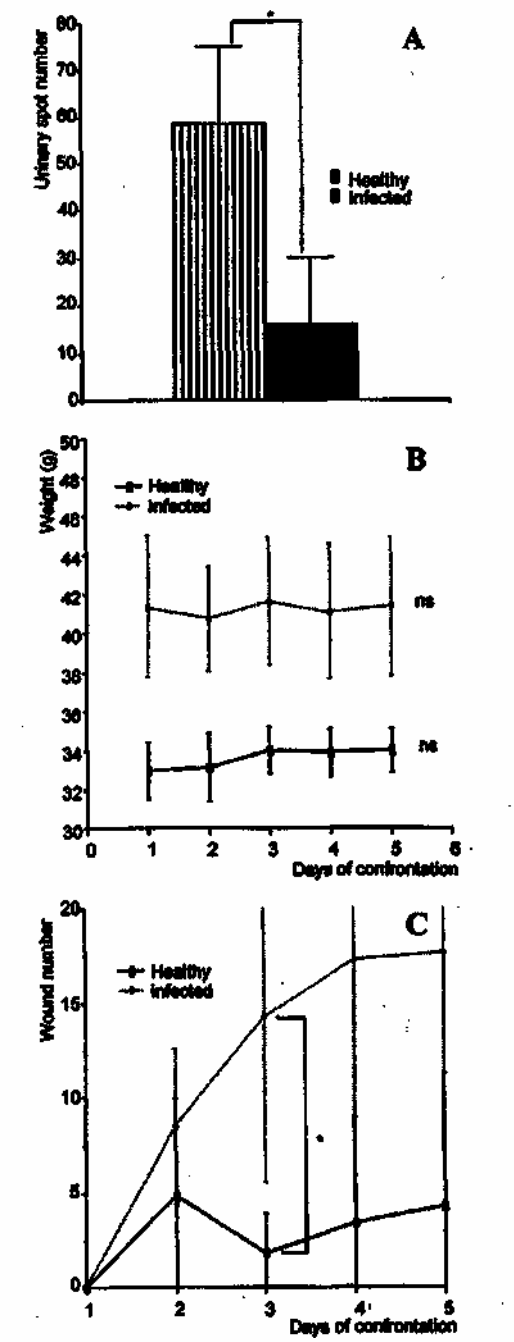

Fig. 2. A Number of urine spots , B weight change and C change in wound number for healthy and infected mice (90 days post-infection). Data represents means ±SE with «=14 mice for each group. *P<0.05; *ns* not significant

aggressiveness of the infected male mice was strongly reduced (experiment 1). In short, infection by *T. crassiceps* was more likely to prevent adult male mice from becoming dominant than to reverse an existing dominance order.

In what ways could the parasite disrupt male mice agonistic behaviour and dominance relationships?

For healthy animals, different studies have reported that dominance rank and social status were associated with the physiological states of mice. Actually, the testosterone levels were often implicated in aggressive encounters between male mice (Kurischko and Oettel 1977; Maruniak et al. 1977; Machida et al. 1981; Compaan et al. 1994). In addition, hormone or energy disorders occurring with aging (Engellenner et al. 1986; Schefer and Talan 1996), and food intake and the cost of fighting (Haller 1991) were implicated in the dominance relationships.

Parasites can disrupt all host health characteristics (Thompson 1983; Thompson and Kavaliers 1994). *T. crassiceps* cysticerci induced disruptive effects on the physiological and energetic states of the mice. In fact, mice infected by *T. crassiceps* metacestodes showed a decrease in testosterone level (Table 2). This decrease was also observed by Larralde et al. (1995). In addition, these authors demonstrated a 200-fold increase of 17 β-oestradiol, involving a feminization of *T. crassiceps*-infected male mice. The cysticerci of *T. crassiceps* could also be involved in the disruption of mouse food intake (Crompton et al. 1985; Corbin et al. 1996; 2000), as well as affecting the immunological system of the mouse host (Huerta et al. 1992; Terrazas et al. 1994, 1999; Villa and Kuhn 1996; Toenjes et al. 1999). Under these circum-stances, it was not surprising that *T. crassiceps* infection led to modification of the antagonistic behaviour and dominance status of male mice.

The influence of infection on the establishment and expression of dominance status could be interpreted in two ways:

1. On the basis of the manipulation hypothesis (Dawkins 1990; Combes 1995), parasites could modify the establishment of behavioural dominance in order to cause a loss of host social status. This has been demonstrated by Rau (1983, 1984) for rodents infected by *Trichinella spiralis*. The consequence of this manipulation is the expulsion of the infected host from the group. The expelled individuals were more susceptible to predation and thus favored the parasite life cycle.

Table 2. Testosterone and corticosterone radioimmunoassay on 50 µl plasma sample for healthy naive, dominant and subordinate mice arid for mice infected for 36 and 90 days. Values are means ± SE (ng/ml). *DPI* Days post-infection

	Healthy naive mice	Healthy dominant mice	Healthy subordinate mice	Infected mice (36 DPI)	Infected mice (90 DPI)
Testosterone (ng/ml)	9.2 ± 7.8	6.7 ± 5.4	11.7 ± 7.0	2.1 ± 2.4	3.8 ± 5.1
Corticosterone	88.1 ± 40.0	72.2 ± 34.7	68.5 ± 27.4	98.7 ± 28.9	83.1 ± 40.2

2. On the basis of optimal foraging theory (Milinski 1990), such behavioral modification could be interpreted as effecting "host decision-making".

In mice infected by *Heligmosomoides polygyrus,* Freeland (1983) observed that infected animals did not invest energy in functions such as social behaviour or sexual competition. This favoured their survival by compensating for both the drain on energy induced by the presence of parasites (Minchella 1985; Hart 1988, 1990; Lozano 1991; Poulin 1998) and the cost of the immune response (Folstad and Karter 1992; Saino et al. 1997).

In conclusion, the effects of a *T. crassiceps* infection on the social behaviour of male mice could be interpreted as a consequence of the disruptions caused by the parasite at two levels. First, on host physiology, including endocrine, neural, immune and neuromodulatory mechanisms and, second, on the host energy budget. In our opinion, these behavioural modifications can not to be considered just consequences of parasite manipulation (Dawkins 1990; Combes 1995) or as a careful use of energy by the host (Milinski 1990), but as the result of the co-evolution of the host-parasite interactions which could be of selective significance for both host and parasite fitness.

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References

Barnard CJ, Behnke JM, Sewell J (1994) Social behaviour and susceptibility to infection in house mice *(Mus musculus):* effects of group size, aggressive behaviour and status related hormonal responses prior to infection on resistance to *Babesia micoti.* Parasitology 108:487-496

Barnard CJ, Behnke JM, Sewell J (1996) Environmental enrichment, immunocompetence, and resistance to *Babesia microti* in male mice. Physiol Behav 60:1223-1231

Barnard CJ, Behnke JM, Gage AR, Brown H, Smithhurst PR (1998) The role of parasite-induce immunosupression, rank and social environment in the modulation of behaviour and hormone concentration in male laboratory mice *(Mus musculus).* Proc R Soc Lond B Biol Sci 265:693-701

Beilharz RG, Beilharz VC (1975) Observations on fighting behaviour of male mice *(Mus musculus* L.). Z Tierpsychol 39,: 126-140

Berdoy M, Smith P, Macdonald DW (1995) Stability of social status in wild rat colonies: age and the role of settled dominance. Behaviour 132:193-212

Blanchard RJ, Flannelly KJ, Blanchard DC (1988) Life-span studies of dominance and aggression in established colonies of laboratory rats. Physiol Behav 43:1-7

Combes C (1995) Interactions durables: écologie et évolution du parasitisme. Masson, Paris

Compaan JC, Hutchison JB, Wozniak A, De Ruiter AJH, Koolhaas JM (1994) Brain aromatase activity and plasma testosterone levels are elevated in aggressive male mice during early ontogeny. Dev Brain Res 82:185-192

Corbin I, Blackburn BJ, Wolowiec T, Novak M (1996) Metabolic profile of the liver of mice infected with cysticerci of *Taenia crassiceps.* Parasitol Res 82:273-275

Corbin I, Novak M, Blackburn BJ (2000) Changes in hepatic lipids of mice infected with cysticerci of *Taenia crassiceps.* J Helminthol 74:209-214

Crompton DWT, Arnold SE, Walters DE, Whitfield PJ (1985) Food intake and body weight changes in mice infected with metacestodes of *Taenia crassiceps.* Parasitology 90:449-456

Dawkins R (1976) The selfish gene. Oxford University Press, London

De Catanzaro D, Ngan ET (1983) Dominance in intermale encounters and subsequent sexual success in mice. J Comp Psychol 97:269-278

Desjardins C, Maruniak JA, Bronson FH (1973) Social rank in house mice: differentiation revealed by ultraviolet visualisation of urinary marking patterns. Science 182:939-941

Engellenner WJ, Burright RG, Donovick PJ (1986) Lead, age and aggression in male mice. Physiol Behav 36:823-828

Falvo RE, Nalbandov AV (1974) Radioimmunoassay of peripheral plasma testosterone in males from eight species using a specific antibody without chromatography. Endocrinology 95:1466-1468

Folstad I, Karter AJ (1992) Parasite, bright males, and immunocompetence handicap. Am Nat 139:603-622

Freeland WJ (1981) Parasitism and behavioural dominance among male mice. Science 213:461-462

Freeman RS (1962) Studies on the biology of *Taenia crassiceps* (Zeder, 1800) Rudolphi, 1810 (Cestoda). Can J Zool 40:969-990

Gourbal BEF, Righi M, Petit G, Gabrion C (2001) Parasite-altered host behaviour in the face of a predator: manipulation or not? Parasitol Res 87:186-192

Haemisch A, Voos T, Gärtner K (1994) Effects of environmental enrichment on aggressive behavior, dominance hierarchies and endocrine states in male DBA/2J mice. Physiol Behav 56:1041-1048

Haller J (1991) Biochemical cost of a fight in fed and fasted *Betta splendens.* Physiol Behav 49:79-82

Hart BL (1988). Biological basis of the behavior of sick animals. Neurosci Biobehav Rev 12:123-137

Han BL (1990) Behavioral adaptations to pathogens and parasites: five strategies. Neurosci Biobehav Rev 14:273-294

Huerta L, Terrazas LI, Scuitto E, Larralde C (1992) Immunological mediation of gonadal effects on experimental murine cysticercosis caused by *Taenia crassiceps* metacestodes. J Parasitol 78:471-476

Kurischko A, Oettel M (1977) Androgen-dependent fighting behaviour in male mice. Endokrinologie 70:1-5

Larralde C, Morales J, Terrazas I, Govezensky T, Romano MC (1995) Sex hormone changes induced by the parasite lead to feminization of the male host in murine *Taenia crassiceps* cysticercosis. J Steroid Biochem Mol Biol 52:575-580

Lozano GA (1991) Optimal foraging theory: a possible role for parasite. Oikos 60:391-395

Machida T, Yonezawa Y, Noumura T (1981) Age-associated changes in plasma testosterone levels in male mice and their relation to social dominance or subordinance. Horm Behav 15:238-245

Maruniak JA, Desjardins C, Bronson F.H (1977) Dominant-subordinate relationships in castrated male mice bearing testosterone implants. Am J Physiol 233:E495-E499

Mauget R, Jouventin P, Lacroix A, Ishii S (1994) Plasma LH and steroid hormones in king penguin *(Aptenodytes patagonicus)* during the onset of the breeding cycle. Gen Comp Endocrinol 93:36-43

Milinski M (1990) Parasite and host decision-making. In: Barnard CJ, Behnke JM (eds) Parasitism and host behaviour. Taylor and Francis, London, pp 95-116

Minchella DJ (1985) Host life-history variation in response to parasitism. Parasitology 90:205-216

Morales J, Larralde C, Arteaga M, Govezensky T, Romano MC, Morali G (1996) Inhibition of sexual behavior in male mice infected with *Taenia crassiceps* cysticerci. J Parasitol 82:689-693

Morales-Mentor J, Gamboa-Dominguez A, Rodriguez-Dorantes M, Cerbon MA (1999) Tissue damage in the male murine reproductive system during experimental *Taenia crassiceps* cysticercosis. J Parasitol 85:887-890

Poole TB, Morgan HD (1975) Aggressive behaviour of male mice *(Mus musculus)* towards familiar and unfamiliar opponents. Anim Behav 23:470-479

Poshivalov VP (1977) Group behavior of mice in population cages. Zh Vyssh Nervn Deyat Im I P Pavlova 27:1011-1019

Poulin R (1998) Evolution and physiology of behaviour manipulation of insect hosts by parasites. Parasitology 116:S3-S11

Rau ME (1983) Establishment and maintenance of behavioural dominance in male mice infected with *Trichinella spiralis.* Parasitology 86:319-322

Rau ME (1984) Loss of behavioural dominance in male mice infected with *Trichinella spiralis.* Parasitology 88:371-373

Righi M, Cibrelus P, Gabrion C (1996) Réalisation expérimentale du cycle biologique de *Taenia crassiceps* (Cestoda, Taeniidae). Bull Soc Fr Parasitol 14:71-78

Saino N, Bolzern AM, Moller AP (1997) Immunocompetence, ornamentation and viability of male barn swallow *(Hirundo rustica).* Proc Natl Acad Sci U S A 94:549-552

Schefer V, Talan MI (1996) Oxygen consumption in adult and aged C57 BL/6j mice during acute treadmill exercise of different intensity. Exp Gerontol 31:387-392

Scherrer B (1984) Biostatistique. Gaëtan Morin, Paris

Terrazas LI, Bojalil R, Govezenski T, Larralde C (1994) A rote for -17 β-estradiol in immunoendocrine regulation of murine cysticercosis *(Taenia crassiceps).* J Parasitol 80:563-568

Terrazas LI, Cruz M, Rodriguez-Sosa M, Bojalil R, Garcia-Tamayo F, Larralde C (1999) Thl-type cytokines improve resistance to murine cysticercosis caused by *Taenia crassiceps,* Parasitol Res 85:135-141

Thompson SN (1983) Biochemical and physiological effects of metazoan endoparasites on their host species. Comp Biochem Physiol 748:183-211

Thompson SN, Kavaliers M (1994) Physiological bases for parasite induced alterations of host behaviour. Parasitology 109:8119-S138

Toenjes SA, Spolski RS, Rooney KA, Kuhn RE (1999) γδT cells do not play a major role in controlling infection in experimental cysticercosis. Parasitology 119:413-418

Villa OF, Kuhn RE (1996) Mice infected with the larvae of *Taenia crassiceps* exhibit a Th2-like immune response with concomitant anergy and down regulation of Thl-associated phenomena. Parasitology 112:561-570

Zar JH (1996) Biostatistical analysis. Prentice-Hall, London