

Differential association between circulating testosterone and infection risk by several viruses in natural cat populations: a behavioural-mediated effect?

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(Received 29 March 2012; revised 11 July 2012; accepted 3 October 2012; first published online 3 January 2013)

SUMMARY

Testosterone is involved in the development and expression of physiological, morphological and behavioural traits. High levels are often associated with high infection risk and/or intensity, suggesting a trade-off between sexual traits and immunity. Classically invoked mechanisms are immunological or behavioural, i.e., testosterone increases susceptibility or resistance to parasites via an impact on immunity or modulates behaviours involved in parasite transmission. However, studies report contrasted patterns. Given its modes of action and the diversity of host-parasite interactions, testosterone should not act similarly on all interactions. To reduce host and context diversity, we studied 3 viruses in the same cat population: the aggressively transmitted Feline Immunodeficiency virus (FIV), and the Feline Calicivirus (FCV) and Herpesvirus (FHV) both transmitted during friendly contacts. Testosterone had a strong effect on the probability of being positive to FIV whereas its effect was significantly weaker on FCV and FHV. These findings demonstrate that testosterone can be differentially associated with parasites of the same type (viruses). The difference we observed was consistent with a behavioural-mediated effect (increased aggressiveness), supporting the idea that the testosterone effect on infection risk is at least partially driven by behavioural mechanisms in our system. Further investigations (e.g., individual immunity measures) are required to confirm this hypothesis.

Key words: aggressively-transmitted parasites, multi-parasitism, Feline calicivirus, Feline herpesvirus, Feline immunodeficiency virus.

INTRODUCTION

Steroid hormones such as testosterone are involved in the development and expression of physiological, morphological and behavioural traits. Testosterone seems in particular to play an important role in individuals' risk of infection. A positive correlation between testosterone levels and parasite prevalence and/or infection intensity has been reported in a wide range of taxa (Zuk and McKean, 1996), including helminthes (e.g. Poulin, 1996), arthropods (e.g. Olsson *et al.* 2000; Cox and John-Adler, 2007), protozoa (e.g. Hughes and Randolph, 2001) and viruses (Easterbrook *et al.* 2007). Despite the importance of this topic for parasitologists and the community of disease ecologists as a whole, the underlying mechanisms of this relationship remain relatively unclear.

Two types of mechanisms are usually invoked to explain the effect of testosterone on infection. It may have immunological effects, i.e., increase

susceptibility to parasites by impairing the immune system, and/or behavioural effects, i.e., increase the exposition to parasites by modulating behaviours involved in parasite transmission. On the one hand, field and laboratory studies have linked sex differences in immune function with circulating steroid hormones (Zuk and McKean, 1996; Klein *et al.* 2000; Roberts *et al.* 2001) and several experimental approaches have shown that testosterone can suppress immune function (e.g., Hirota *et al.* 1976; Grossman, 1985; Casto *et al.* 2001). The underlying physiological mechanisms remain nonetheless unclear. Sex hormones may inhibit the immune response directly by binding to steroid receptors on lymphocytes (Sullivan and Wira, 1979; Tanriverdi *et al.* 2003) or indirectly (Owen-Ashley *et al.* 2004) by redistributing energy reserves away from the costly immune response (Sheldon and Verhulst, 1996) during stress (Apanius, 1998) or reproductive events (Wedekind and Folstad, 1994).

On the other hand, testosterone has been shown to have important effects on transmission-relevant behaviours. Male competitive behaviour during the breeding season has been linked to elevated testosterone concentrations across a wide variety of bird (Wingfield *et al.* 2000; Smith *et al.* 2005), reptile

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(Klukowski and Nelson, 1998) and mammal species (e.g. Mehlman *et al.* 1997; Brockman *et al.* 1998; Cavigelli and Pereira, 2000; Buck and Barns, 2003). In particular, aggressiveness (Frank *et al.* 1985; Marler and Moore, 1988; Wingfield *et al.* 1990; Nelson, 2005) and territoriality (e.g., Negro *et al.* 2010; Edler *et al.* 2011) are known often to be testosterone-mediated and to increase individuals' exposition to certain parasites (e.g. Salvador *et al.* 1996; Easterbrook *et al.* 2007; Negro *et al.* 2010).

However, most studies dealing with the effect of testosterone on parasitism only focus on a single parasite and are largely biased towards ectoparasites and intestinal worms. These have led to different patterns, ranging from an absence of association (e.g., blood parasites of honey-eaters, Buttemer and Astheimer, 2000), to positive (e.g. helminthes, Poulin, 1996) or even negative correlations (e.g. ecto- and hemoparasites in wall lizards, Oppliger *et al.* 2004) between testosterone and parasite load. Such discrepancies appear both between and within parasite taxa (e.g. positive effect on worm burden in rats, Kamis *et al.* 1992 but a negative effect in mice, Nakazawa *et al.* 1997; more references in Klein, 2004). It is important to consider that testosterone may differentially affect different types of parasites (e.g. Roberts *et al.* 1996; Veiga *et al.* 1998; Fuxjager *et al.* 2011). A previous meta-analysis suggested, for instance, that testosterone was more likely to increase the abundance of ectoparasites compared to that of endoparasites (Roberts *et al.* 2004). Considering several parasites in the same host population, Fuxjager *et al.* (2011) revealed a positive effect of testosterone on mite loads, but negative or null effects on nematodes and little on blood-borne endoparasites in free-living mountain spiny lizards (*Sceloporus jarrovi*). Considering the parasites one by one may also lead to misinterpretations because numerous parasites co-circulate within natural populations. As an example, studies focusing on ectoparasites do not take into account the fact that they may contribute to the transmission of bacteria or viruses they harbour, which can introduce important confounding factors. More generally, host species, populations and/or individuals experiencing different life conditions, diverse multi-infections and exhibiting different social and mating systems may show different abilities to resist parasite infection (Sheldon and Verhulst, 1996) and are likely to express differently trade-offs between immunocompetence and reproduction (Norris and Evans, 2000; Schmid-Hempel, 2003; Lee, 2006). To understand the discrepancies between studies, it is therefore necessary to investigate the testosterone effect on different types of parasites in a given socio-environmental context. Although experimental protocols may help to tease apart physiological and behavioural effects in certain cases (Mougeot *et al.* 2005), it cannot be applied in all natural populations. Investigating

the relationship between testosterone and several parasites inducing close immune responses (e.g. several viruses, several nematode species or several blood parasites), but with different transmission modes (e.g. the exposition of the host involving different behaviours), and circulating in the same host population(s) may help, in a less invasive way, to better understand the nature of the relationship between testosterone and parasitism.

In this paper, we propose to investigate the effect of circulating testosterone levels on the infection risk by 3 feline viruses with contrasted transmission modes in natural populations of domestic cats (*Felis silvestris catus*). The cat-viruses system is particularly appropriate to investigate such questions. First, cat natural populations have been extensively studied from a behavioural point of view (Liberg, 1980; Pontier *et al.* 1998, 2009; Say *et al.* 1999; Natoli *et al.* 2005; Hellard *et al.* 2011). Second, its main viruses are well known due to their veterinary implications and because they are easy to survey in the field. It enables the study of the effect of testosterone on viruses, a type of parasite that is largely under studied up to now (but see Friedman *et al.* 1972; Lindstrom *et al.* 2001; Easterbrook *et al.* 2007). Interestingly, their transmission also implies different behaviours. The feline immunodeficiency virus (FIV) is transmitted through a direct horizontal mode (Sparger, 1993) by bites during aggressive or sexual contacts and induces a life-long chronic disease (Courchamp and Pontier, 1994; Bendinelli *et al.* 1995). By contrast, the feline herpesvirus (FHV) and calicivirus (FCV) are transmitted through 'amicable' close and prolonged contacts, by oral, nasal and ocular secretions, causing acute infections often followed by latent infections (Povey and Johnson, 1970; Gaskell and Povey, 1982). During latency, FHV can be reactivated by a stress (Gaskell and Povey, 1977). Such a context offers the opportunity to investigate the effect of testosterone in a natural system and to determine whether it acts similarly on different viruses. By studying multiple parasites in a same host population, we avoid potential confounding effects linked to host and context diversity. In addition, thanks to the contrasted characteristics of the studied viruses, we can test whether data are coherent with a behavioural-mediated effect of testosterone.

MATERIALS AND METHODS

Cat populations and sampling

Three rural cat populations were sampled in April and May 2007 (i.e. during cats' breeding season), in North-Eastern France (Fouchet *et al.* 2010; Hellard *et al.* 2011). The populations are located in a mixed habitat of deciduous forests and pasturelands. Cat densities are low (≈ 200 cats/km²) as in other rural areas (Liberg *et al.* 2000). Males live in large home

ranges overlapping those of females (Corbett, 1979; Pontier and Natoli, 1996; Liberg *et al.* 2000). The mating system has been shown to be polygynous in our area (Say *et al.* 1999). Males enter into intense agonistic interactions with other males during the reproductive period to monopolize the access to receptive females (Liberg, 1981, 1983; Davies, 1991; Yamane *et al.* 1996).

Cats were captured using baited traps or directly caught by the owner and anaesthetized. For each captured cat, sex, age and body mass were recorded. Age was given by the owner or estimated according to Pascal and Castanet (1978) for un-owned cats. They all received an electronic passive integrative transponder (pig-tag) to allow individuals to be identified in case of re-capture during the same session. Blood samples were taken from the jugular vein before cats were released. An exhaustive survey was carried out to determine whether cats were vaccinated, neutered and had an owner. This enabled us to remove any neutered or vaccinated cat from the study as this would interact with testosterone levels and serological status. On the whole, 41 males (34 feral and 7 free-to-roam owned males) were kept in the analysis.

Circulating testosterone assay

After each capture, the serum was extracted from blood samples by centrifugation (5000 *g* for 5 min) and stored at -20°C until assayed. The circulating testosterone levels were then determined spectrophotometrically (Xenius MP96, SAFAS, Monaco), using an Enzyme Immuno-Assay commercial kit (Testosterone EIA kit, Cayman Chemical). The test is based on the competition between testosterone contained in the sample and a T-acetylcholinesterase (AChE) conjugate for a limited amount of testosterone antiserum. Concentrations of testosterone (expressed in pg/ml) were calculated against a standard curve. Cross-reactivity with the other main steroid hormones was considered negligible (Androsterone: 0.05%, Estradiol: <0.01%).

Serological data

The serological statuses for the feline immunodeficiency virus (FIV), herpesvirus (FHV) and calicivirus (FCV) were determined from blood samples. FIV-antibodies were immediately searched for with a commercial kit using the ELISA method (SNAP Combo +, Idexx), whereas specific antibodies against FHV or FCV were measured by a specific blocking ELISA (Poulet, 2007).

Statistical analyses

Determination of the most appropriate model to describe T levels. First, a model selection procedure was applied to determine the most appropriate model

describing the observed testosterone levels in males. The aim was to identify factors being potentially confounding to the association between testosterone levels and serological statuses. Four factors were considered; 2 continuous variables: age (AGE) and body mass (MASS) and 2 factors with 2 modalities: way of life (WoL; feral or domestic) and orange phenotype (PHENO; orange or non-orange). This last factor was included because cats with an orange fur colour are sometimes suspected to be more aggressive than other fur coat colours (Pontier *et al.* 1995, 1998). Testosterone level, Mass and Age were standardized to make their effects dimension-free and comparable to those of the other factors. All possible models with simple effects and double interactions were generated. A square transformation was applied to ensure the normality of the residuals.

The most appropriate model was selected using the Akaike Information Criterion adjusted for small sample size (AICc, Anderson *et al.* 1994). The models were ranked according to the smallest AICc differences (denoted ΔAICc) between the focal model and the lowest AICc model. When ΔAICc was smaller than 2 for more than one model, those models were combined to ensure the inclusion of a maximum of potential confounding factors in the second part of the analysis.

The parameters of the selected linear model were estimated using Bayesian inference. Flat priors were used (in practice independent Gaussian laws with very large variances for each parameter). Samples from the posterior distribution of the model parameters were derived from a MCMC algorithm using JAGS and the package rjags in R software (R Development Core Team, 2009). To get rid of the effect of initial condition, the 10 000 first elements of the chain were burnt up. To avoid auto-correlation only 1 element of the chain out of 100 was kept.

Testosterone effect on serological statuses. Second, selected variables were included in logistic regression models to describe each serological status. The testosterone level was added as a continuous variable in each model. Since quantitative variables were standardized, the odds-ratios now represent the effect of an increase of testosterone level, mass or age of 1 standard deviation on the probability of infection. For each virus, model parameters were estimated using Bayesian inference as described previously.

A fundamental question is to determine whether testosterone affects the different viruses similarly. Answering this question requires to test the plausibility of the hypothesis according to which the coefficient of the testosterone effect is the same for the different viruses. In mathematical terms, let us call b_V the coefficient of testosterone for virus V and $\delta = b_{V1} - b_{V2}$ the differential effect of testosterone on 2 viruses. Testing whether two viruses are similarly affected by testosterone is made by comparing

Table 1. Best linear regression models according to their AICc to describe testosterone levels

(T, testosterone levels; WoL, way of life (with or without an owner); PHENO, orange phenotype (orange or non-orange fur). Only the 5 best models are shown. The selected model combines the 2 models in bold.)

Model	n ^a	k ^b	AICc	ΔAICc
sqrt(T) ~ AGE	41	3	334.415	0
sqrt(T) ~ AGE + PHENO	41	4	335.826	1.411
sqrt(T) ~ MASS	41	3	336.171	1.756
sqrt(T) ~ AGE + MASS	41	4	336.352	1.937
sqrt(T) ~ AGE + WoL	41	4	336.859	2.444

^a Sample size.

^b Number of parameters.

H0: $\delta = 0$ against H1: $\delta \neq 0$. Now, if we assume that the serological statuses of the two viruses are independent, then sampling from the posterior distribution of δ is easily obtained by subtracting the chains obtained for b_{V1} and b_{V2} . Hypothesis H0 is then simply tested by looking at whether zero is a plausible value for δ . *P*-values testing coefficients (i.e., regression coefficients or δ) are estimated by $P = 2\min(p, 1 - p)$ where *p* is the ratio between the number of simulated values below zero plus one and the total chain length plus one.

RESULTS

The most appropriate model to describe T levels

According to the AICc, we selected the model explaining the testosterone levels by the age, body mass and phenotype of the cats (Table 1). The effects of these explanatory variables were not significant (Table 2) but all were kept to remove potential confounding factors when investigating the relationship between testosterone and the serological statuses of the 3 viruses. Similarly, although age and body mass were correlated ($\rho = 0.56$), meaning that it is likely that only one of the two variables was biologically relevant (i.e., the other being linked to T through a confounding effect), both were kept in the analysis.

T levels and risks of infection

After correction by age, mass and phenotype, the testosterone levels of rural cats were significantly linked to their FIV serological status (*P*-value = 0.18×10^{-3} , Table 3). The risk of being seropositive to FIV strongly increased with testosterone level (Fig. 1). When the testosterone level increased of one standard deviation, the risk to be infected increased 105 times (Odds-ratio = 104.90, CI_{95%} = [8.14; 10259.92], Table 3). This value is not plausible from a biological point of view; however, it

Table 2. Selected linear regression model: testosterone levels in relation to age, body mass and orange phenotype

(Parameter estimates were obtained by Bayesian inference; the median of each MCMC chain is shown. *P*-values (*P*) were calculated using $P = 2\min(p, 1 - p)$ where *p* is the ratio between the number of simulated values below zero plus 1 and the total chain length plus 1.)

	Estimate	CI95% ^a	<i>P</i>
Intercept	0.56	[-0.39; 1.52]	0.227
AGE	0.27	[-0.10; 0.63]	0.154
MASS	0.20	[-0.19; 0.59]	0.295
PHENO (orange)	-0.43	[-1.15; 0.26]	0.201

^a Credibility interval.

may be due to the small sample size. What is important is to look at the lower bound of the credibility interval; as it is 8.14, we are confident in saying that testosterone effect is marked. On the contrary, no significant relationship was evidenced between testosterone levels and the other serological statuses (FHV: *P*-value = 0.052; FCV: *P*-value = 0.174, Table 3, Fig. 1).

Furthermore, the comparison of the coefficients of testosterone revealed that the effect of the circulating hormone was different on the status to FIV than on the status to the other viruses (Table 4, after Bonferroni correction: *P*-value = 0.014 for FIV-FHV, *P*-value = 0.015 for FIV-FCV, but *P*-value = 0.996 for FHV-FCV).

DISCUSSION

This work investigated the relationship between testosterone level in male cats and their risk of being seropositive to 3 feline viruses with different transmission modes. Contrary to most studies, which focus on a single parasite and a single mechanism (i.e. immunological or behavioural) we argue that considering several parasites in the same host population (i.e. in a given socio-environmental context), may help deciphering some of the underlying mechanisms.

Applied here to 3 viruses infecting cats, this approach revealed different relationships between testosterone and males' serological status, depending on the considered virus. High levels of testosterone were significantly associated with high infection risk by FIV but not with that of the other viruses. It does not mean that testosterone has no effect at all on FHV and FCV but indicates that the plasma hormone is much more associated with FIV.

Understanding the differential effects of testosterone

Behaviours exposing cats to infection by the studied viruses, and the effects testosterone has on these

Table 3. Logistic regression models to test for an effect of testosterone level on each serological status

(Parameter estimates were obtained by Bayesian inference; the median of each MCMC chain is shown. *P*-values were calculated using $P = 2\min(p, 1 - p)$ where *p* is the ratio between the number of simulated values below zero plus 1 and the total chain length plus 1.)

	Estimate	CI95% (estimate) ^a	<i>P</i>	OR ^b	CI95% (OR) ^a
(a) FIV					
Intercept	-6.52	[-14.01; -1.63]	0.005	1.47×10^{-3}	$[0.1 \times 10^{-5}; 0.20]$
AGE	1.29	[-0.32; 3.44]	0.124	3.61	[0.73; 31.09]
MASS	1.30	[0.72; 3.99]	0.230	3.68	[2.05; 53.95]
PHENO (orange)	3.25	[-0.19; 8.05]	0.065	25.74	[0.83; 3133.79]
TESTO	4.65	[2.10; 9.24]	0.18×10^{-3}	104.90	[8.14; 10259.92]
(b) FHV					
Intercept	2.05	[-0.45; 4.83]	0.102	7.75	[0.64; 124.99]
AGE	0.52	[-0.41; 1.60]	0.264	1.68	[0.66; 4.94]
MASS	0.43	[-0.55; 1.50]	0.402	1.54	[0.58; 4.48]
PHENO (orange)	-1.14	[-3.11; 0.69]	0.212	0.32	[0.04; 1.98]
TESTO	0.89	$[-0.29 \times 10^{-2}; 2.03]$	0.052	2.43	[1.00; 7.59]
(c) FCV					
Intercept	2.97	[0.23; 6.35]	0.034	19.46	[1.25; 574.67]
AGE	1.32	[0.19; 2.78]	0.018	3.76	[1.21; 16.05]
MASS	-0.07	[-1.20; 1.03]	0.888	0.93	[0.30; 2.81]
PHENO (orange)	-1.30	[-3.56; 0.69]	0.211	0.27	[0.03; 2.00]
TESTO	0.75	[-0.31; 2.05]	0.173	2.11	[0.74; 7.80]

^a Credibility interval.

^b Odds-ratio.

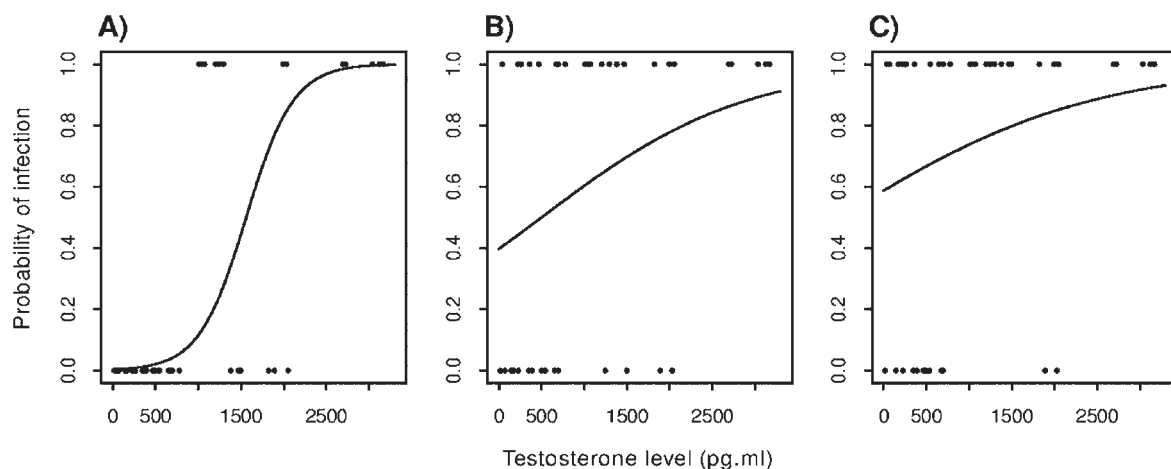


Fig. 1. Infection risk by each virus according to males' testosterone levels. Predicted (line) and observed (dots) infection risks for FIV (A), FHV (B) and FCV (C).

behaviours are well known. FHV and FCV are transmitted by close and prolonged contacts (Povey and Johnson, 1970; Gaskell and Povey, 1982), which are not known to be testosterone-dependent behaviours. Conversely, FIV is transmitted by bites, between males during fights (females do not bite each other) and from male to female during coitus (when the male bites the female at the neck) (Yamamoto *et al.* 1989; Sparger, 1993; Bendinelli *et al.* 1995; Courchamp *et al.* 1998). Aggressive males are more exposed to this virus than non-aggressive ones, as evidenced by the higher vulnerability to FIV of bold (pro-active) individuals (Courchamp *et al.* 1998,

2000; Natoli *et al.* 2005). Since aggressiveness is known to be testosterone-mediated in cats (Rosenblatt and Aronjon, 1958; Hart and Barrett, 1973; Brown and Bradshaw, 1996) as in many other species (e.g., Frank *et al.* 1985; Marler and Moore, 1988; Wingfield *et al.* 1990; Nelson, 2005), the higher FIV-infection of males with high testosterone levels found in this study is consistent with a higher exposition to FIV through increased aggressiveness.

In addition, mean levels of circulating testosterone measured in our polygynous rural populations (1085.34 ± 892.42 pg/ml) were significantly higher

Table 4. Test of a differential effect of testosterone level on the serological statuses

(The differential effect of testosterone on 2 viruses is given by $\delta = b_{V1} - b_{V2}$ where b_V is the coefficient of testosterone for virus V (obtained by Bayesian inference). P -values are calculated using $P = 2\min(p, 1-p)$ where p is the ratio between the number of simulated values of δ below zero plus 1 and the total chain length plus 1.)

	FIV	FHV
FHV	0.005	–
FCV	0.005	0.849

($F = 22.43$, D.F. = 1, $P = 1.10 \times 10^{-5}$) than in a much less aggressive and promiscuous urban population (335.67 ± 204.91 pg/ml; La Croix-Rousse, Lyon, France, unpublished data; see also Say *et al.* 1999; Say and Pontier, 2004). Those results are consistent with unpublished data suggesting that the type of mating system significantly affects testosterone levels and cats' aggressiveness (D. Pontier, O. Hubert, L. Say and C. Duchamp, *personal communication*). Although more data are necessary to generalize, this would be in accordance with the challenge hypothesis (Wingfield *et al.* 1990) that states that increases in testosterone levels of male animals during the breeding season are directly related to the extent of intrasexual competition for resources or mates that they experience. Accordingly, the prevalence of FIV in natural cat populations is higher in polygynous cat populations than in promiscuous urban ones where FIV can be absent (Xemar, 1997; Courchamp *et al.* 1998, 2000; Hellard *et al.* 2011).

Furthermore, we cannot exclude subtle differences in the immune responses induced by the studied viruses and/or a differential effect of testosterone on such responses. As we work with serological data, we are looking at a possible link between testosterone levels and the establishment of the infection, i.e., on the immune response occurring before seroconversion. FHV and FCV are transmitted through body secretions and first need to pass epithelial barriers to infect a new host. By contrast, FIV is transmitted by bite and thus bypasses epithelial barriers more easily and more rapidly ends up in the circulatory system. The relative importance of the different components of the immune system may not be the same for these two types of virus entry. The innate and cell-mediated immune responses are likely to play an important role in the infection success of FHV and FCV, as supported by the existence of some cats protected against FHV and FCV infection but with no detectable antibody levels (Knowles *et al.* 1991; Radford *et al.* 2007), whereas FIV may be quickly exposed to the cellular actors of the immune system. Such differences in the immune responses remain nonetheless hypothetical.

It is also interesting to note that cats' status to FHV tends to be influenced by testosterone levels as well ($P = 0.052$), even if this link is much weaker than for FIV ($P = 0.005$). Considering that contrary to FCV this virus can be reactivated, it raises the question of a potential link between testosterone and the reactivation of latent infections.

Taken together, our results support the hypothesis that the strong relationship between testosterone levels and FIV status suggests a behavioural action of testosterone. Knowing the effect testosterone has on cats' aggressiveness (Rosenblatt and Aronjon, 1958; Hart and Barrett, 1973; Brown and Bradshaw, 1996), this hormone is likely to increase the exposition to this aggressively transmitted microparasite. We cannot exclude an additional immunological action of testosterone, but this would need further (experimental) investigations. A better knowledge of testosterone's impact on the different components of the immune system, as well as of cats' immune response following infection by each of the studied viruses (i.e. key molecular or cellular actors and if they are influenced by testosterone) would be valuable. Besides, the immunosuppressive effects of the hormone were investigated on birds and small mammals (i.e. rabbits, rodents) and we cannot exclude Felids/domestic cats' particularities in terms of sensitivity to testosterone effects on the immune system.

Towards epidemiological and evolutionary consequences

This study supports the idea of a crucial role of behaviour and behavioural variability in the dynamics and evolution of host-parasite systems (Bahi-Jaber *et al.* 2008; Barber and Dingemanse, 2010; Hawley *et al.* 2011). If testosterone increases hosts' aggressiveness, it may fuel the spread of any aggressively transmitted parasite, and individuals with high testosterone levels may accumulate such parasites. This is an important point as aggressive encounters are hypothesized to be a mode of transmission for several pathogens, such as, for example, the simian immunodeficiency virus in non-human primates (Nerrientet *et al.* 1998; Fouchet *et al.* 2012) or hantaviruses in rodents (Glass *et al.* 1988; Klein, 2000). If immunological and behavioural effects co-occur, testosterone may also serve as a common driver of superspreading phenomena for aggressively transmitted parasites.

More generally, the question of the role of testosterone in infection risk and intensity is of great interest for many human and animal pathogens (e.g. *Toxoplasma gondii*, Flegr *et al.* 2008; Kankova *et al.* 2011; *Leishmania donovani*, Zhang *et al.* 2001). It is particularly true for those whose transmission depends on behaviours that can be influenced by circulating testosterone. In certain cases, determining

whether a correlation between testosterone and infection is due to a testosterone-mediated increase in host exposition or to a manipulation of host hormone level by the parasite is also an important challenge (Flegr *et al.* 2008; Kankova *et al.* 2011).

Finally, our approach is general and could be applied to any type of parasite. While more studies are still needed to define (i) the precise effects of testosterone on the immune system, (ii) the specific immune response developed by hosts toward each parasite and parasite type, and (iii) the interactions between testosterone and other hormones (e.g. corticosterone, glucocorticoids, see for example Fuxjager *et al.* 2011), this study suggests an important role for testosterone in the circulation of an aggressively transmitted parasite. The search for the possible underlying mechanisms also confirms the complexity of the interactions existing between immunity, behaviour and physiology. If, as suggested here, the relationship between testosterone and infection risk differs when only 3 viruses are considered – 3 closely related viruses studied within the same host population – important variations are certainly expected at a larger scale, when more parasite types and/or host species are involved. Finally, our work highlights the need for interdisciplinary and integrative approaches if one wants to understand the complex interplays that exist both within and between the endocrine and immune systems, and their consequences for the evolution of parasite-host interactions, mating systems and epidemiological patterns in a world with multiple parasites.

ACKNOWLEDGEMENTS

The authors would like to thank Guillaume Leblanc for serological data collection in the field (Accreditation number 692660703 delivered to the UMR-CNRS 5558 for the program); Béatrice Tarin, Vincent Badol and Christine Coupiér for laboratory analyses. They are also grateful to Professor Patrice Debré for fruitful discussions.

FINANCIAL SUPPORT

This work was supported by Lyon-Biopôle (FIV-VAX program).

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