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Effects of gonadectomy on the incidence rate of babesiosis and the risk of severe babesiosis in dogs aged 6 months and older at a veterinary academic hospital in South Africa: A case-control and retrospective cohort study

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A R T I C L E I N F O

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A B S T R A C T

Gonadectomy in dogs is associated with changes in risks of a variety of non-infectious health conditions, but few studies have examined its effects on infectious disease outcomes. The objectives of our study were to estimate the causal effect of gonadectomy on the incidence rate of babesiosis diagnosis, and on the risk of severe babesiosis in diagnosed cases, in dogs 6 months and older seen at a veterinary academic hospital in South Africa from 2013 through 2020. To estimate the effect of gonadectomy on the incidence rate of babesiosis diagnosis in dogs, we conducted a case-control study with incidence density sampling of dogs seen through the hospital's primary care service, adjusting for sex, age, breed category and weight. We identified 811 cases and selected 3244 time-matched controls. To estimate the effect of gonadectomy on disease severity in dogs with babesiosis, we conducted a retrospective cohort study among all dogs with a diagnosis of babesiosis (n=923), including these 811 cases and a further 112 referred to the hospital, also adjusting for sex, age, breed category and weight. Gonadectomy substantially reduced the incidence rate of babesiosis (total effect incidence rate ratio [IRR] 0.5; 95 % confidence interval [CI] 0.41–0.60) and the risk of severe babesiosis among diagnosed dogs (total effect risk ratio [RR] 0.72; 95 % CI 0.60–0.86). Tipping point sensitivity analysis shows that these effect estimates are robust to unmeasured confounding bias. There was no evidence for modification of the effect of gonadectomy by sex, with effect estimates qualitatively similar for males and females for both outcomes. Compared to females, males had a higher incidence rate of babesiosis (IRR 1.74; 95 % CI 1.49–2.04) and a higher risk of severe disease (RR 1.12; 95 % CI 0.98–1.28). In conclusion, our study shows a robust protective effect of gonadectomy on the incidence and severity of babesiosis in both male and female dogs 6 months of age and older, and contributes important evidence to the debate on the overall risks and benefits of gonadectomy to dogs in this population.

1. Introduction

Gonadectomy is the surgical removal of the gonads (the testes in males and the ovaries in females). In dogs, gonadectomy is frequently performed to render dogs infertile for purposes of reproductive management and population control, although the frequency of the procedure in owned dogs for this purpose, and its cultural acceptance, differs substantially between countries and owner demographics (Dawson et al., 2019; Glasser, 2021). Gonadectomy in dogs is known colloquially as neutering in both sexes, castration in males and spaying in females.

Gonadectomy is associated with changes in the risk of a variety of non-infectious health conditions in dogs including cancer (Priester, 1979; Hoffman et al., 2013; Norris et al., 1992; Knapp et al., 2000; Ru et al., 1998; Prymak et al., 1988; Ware and Hopper, 1999; Torres de la Riva et al., 2013; Villamil et al., 2009), musculoskeletal (Duval et al., 1999; Slauterbeck et al., 2004; Whitehair et al., 1993; Spain et al., 2004; van Hagen et al., 2005), endocrine (Yoon et al., 2020; Zink et al., 2023) and immune disorders (Sundburg et al., 2016). Fewer studies have examined the effect of gonadectomy in dogs on the risk of infectious

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diseases. In one study of medical records of dogs one year of age or older presenting to North American veterinary academic hospitals from 1984 to 2004, Hoffman et al. (2013) showed that gonadectomy was associated with a substantially decreased risk of death from infectious diseases across both sexes, controlling for age and breed. For five specific infectious etiologies reported, the protective association was seen for parasitic (intestinal parasites, heartworm) and viral infections (canine distemper and parovirus disease), but not for a fungal infection (blastomycosis) (Hoffman et al., 2013).

Results from laboratory studies in rodents suggest that effects of gonadectomy on infectious disease susceptibility may differ by sex and pathogen type. For bacteria, gonadectomy in female mice increased bacterial load of *Coxiella burnetii* (Leone et al., 2004) and of *Mycobacterium avium* (Tsuyuguchi et al., 2001) in tissues, whereas gonadectomy in male mice significantly reduced bacterial load of *M. marinum* in tissues (Yamamoto et al., 1991). A similar pattern for mycobacterial infections was noted in two rare human studies, in which gonadectomy was associated with lower mortality rates to *M. tuberculosis* in males (Hamilton and Mestler, 1969) but increased mortality rates in females (Svanberg, 1981). Similarly, gonadectomy in female but not male mice resulted in increased mortality to another bacterial infection, *Vibrio vulnificus* (Merkel et al., 2001). For parasitic infections, gonadectomy increased the parasitic load of *Taenia crassiceps* in male mice and decreased it in female mice (Morales-Montor et al., 2002); by contrast, gonadectomy in male mice reduced development of liver abscesses following experimental *Entamoeba histolytica* infection but did not have an effect in female mice (Lotter et al., 2013). Gonadectomy in male but not female mice reduced mortality following *Plasmodium chabaudi* infection (Cernetich et al., 2006). Conversely, for the fungal pathogen *Candida albicans*, gonadectomy reduced infection and increased clearance in females but not in males (Rifkind and Frey, 1972). Thus, the effects of gonadectomy on susceptibility to infectious disease and severity of disease outcomes appear to be influenced by host biological sex and pathogen taxa; however, few studies have explored the relevance of this in the context of risks and benefits of gonadectomy in dogs.

The high prevalence of both gonadectomy and infectious diseases in dogs in certain populations provides an opportunity to examine the causal effects of gonadectomy on the incidence and severity of naturally occurring infections due to specific pathogens, through observational epidemiological studies. Such studies must consider potential confounding bias by factors that affect both gonadectomy status and the infectious disease outcome of interest (incidence or severity). These include host factors such as age, body condition score, and breed type (for example, compared to toy breed dogs, working breed dogs may be less likely to undergo gonadectomy, and more likely to be exposed to tick-borne infectious diseases), as well as owner factors that may influence decisions on gonadectomy, veterinary preventive and care-seeking behavior, and therapeutic options (for example, wealth and access to veterinary care). Gonadectomy has also been associated with altered metabolism and a predisposition for weight gain in dogs (Oberbauer et al., 2019), which may partially mediate the effect on disease outcomes.

In this study, we examined the causal effects of gonadectomy on the incidence and severity of babesiosis diagnosed in dogs seen at the Onderstepoort Veterinary Academic Hospital (OVAH) of the University of Pretoria in South Africa. Babesiosis is a tick-borne disease caused by protozoan parasites of the genus *Babesia*. At the OVAH, the overwhelming majority of canine cases of babesiosis are due to infection with *Babesia rossi* Nuttall 1910 (Matjila et al., 2004; Matjila et al., 2008), a virulent, large-form *Babesia* species transmitted by the tick vector *Haemaphysalis elliptica*. It causes a complex multi-systemic disease that can be classified as either uncomplicated or complicated (Jacobson, 2006; Jacobson and Clark, 1994; Leisewitz, Goddard, Clift et al., 2019). Complicated babesiosis occurs when the pathology noted cannot be attributed purely to the anemia or when the anemia becomes severe enough to perpetuate organ dysfunction (Atkinson et al., 2022; Leisewitz, Goddard, Clift et al., 2019). In this paper, we refer to outcomes meeting the definition of complicated babesiosis as ‘severe’. Babesiosis is a common cause of morbidity and mortality of dogs in South Africa with an estimated case-fatality proportion of 10–12 % in admitted cases (Shakespeare, 1995; Mellanby et al., 2011; Jacobson, 2006). Risk factors reported to influence the prevalence for large *Babesia* species (*B. canis*, *B. rossi* and *B. vogeli*) include breed, sex, age, coat length, owner use of acaricides, housing, confinement, and season (Zygner et al., 2023).

The objectives of our study were to estimate the causal effect of gonadectomy on the incidence rate of babesiosis diagnosis, and on the risk of complicated babesiosis in diagnosed cases, in dogs 6 months and older seen at the OVAH.

2. Materials and methods

The research protocol for this study was approved by the Research Ethics Committee of the University of Pretoria (Protocol no: REC144–20). A dataset of babesiosis cases in dogs seen at the OVAH was created. All electronic health records (EHRs) of canine patients seen at any department in the OVAH from 1 February 2013 through 31 January 2021 were searched to identify cases of babesiosis. Cases were identified by searching for the terms “berenil” across all fields in all canine EHRs, as Berenil® (diminazene aceturate) was the sole drug used for the treatment of babesiosis at the OVAH during the period of interest, and was used only for this purpose. Cases were confirmed by examination of additional fields in records identified in the initial search where microscopic identification of *Babesia* piroplasms on peripheral blood smear examination was described.

2.1. Case-control study

To estimate the effect of gonadectomy on the incidence rate of babesiosis diagnosis in dogs, we conducted a case-control study with incidence density sampling using EHRs from the Outpatient Department (OD) of the OVAH. The OVAH OD provides a primary care service for dog owners resident in the surrounding areas. All records of canine OD visits from 1 February 2013 through 30 November 2020 were extracted. Cases of babesiosis diagnosed through the OD were identified by linking the babesiosis case dataset with the OD visit dataset. We thereby excluded cases of babesiosis from other OVAH departments which might have originated outside the case-control catchment population, for example referral cases to the internal medicine department. We restricted eligibility to dogs 6 months of age and older. Recurrent cases of babesiosis, of which there were few, were excluded. Controls were selected from dogs presenting for any other reason to the OD, excluding follow-up appointments related to a previous OD visit. For each babesiosis case, four controls were randomly selected from risk sets of eligible control visits occurring within 7 days of the case visit (incidence density sampling).

Selection of covariates for confounding adjustment was based on causal directed acyclic graphs (DAGs) analyzed with DAGitty version 3.1 (www.dagitty.net, accessed 11 October 2023) (Fig. 1). In the DAG in Fig. 1A, weight1 (weight at the time of gonadectomy) is unmeasured. Under the assumptions in this DAG (DAG1a), the total effect of gonadectomy on babesia diagnosis at the OVAH OD cannot be estimated as no adjustment sets could be found with the measured variables; however, the direct effect of gonadectomy on babesiosis diagnosis (that is, the effect not mediated through weight2, being weight at the time of the OD visit) can be estimated by adjusting for sex, age, breed category, and weight2, assuming no unmeasured confounding (represented by *U* in Fig. 1A). Under these same assumptions, the total effect of biological sex (male vs. female) on babesiosis diagnosis can be estimated without adjustment for any covariates, and the direct effect of biological sex can be estimated by adjusting for gonadectomy, age, breed category, and weight2. We also present a second DAG (DAG1b; Fig. 1B) in which it is

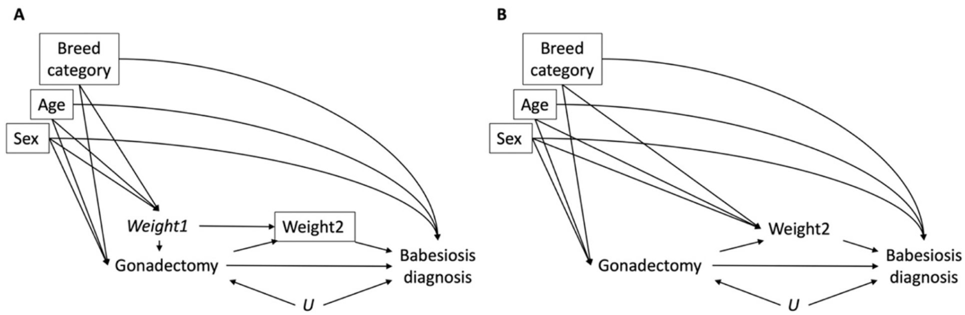

Fig. 1. Causal directed acyclic graphs (DAGs) representing our assumptions of the causal relationships between measured and unmeasured variables in the case-control study. *U:* unmeasured variables potentially confounding the exposure-outcome relationship. Variables in italics are unmeasured. Variables in boxes were controlled for in the analyses.

assumed that weight1 (conditional on sex, age and breed category) had no effect on gonadectomy and was therefore not included. Under these assumptions, the total effect of gonadectomy on babesiosis diagnosis (including any effect mediated by weight2) can be estimated by adjusting for sex, age, and breed category, assuming no unmeasured confounding (represented by *U* in Fig. 1B).

We used the American Kennel Club classification of each breed into one of eight categories: toy, terrier, working, non-sporting, sporting, hound, herding or mixed (www.akc.org/dog-breeds, accessed 5 October 2023). Age was modelled as a second-order polynomial and weight as a third-order polynomial in the regression models, based on visual inspection and comparison of lower-order polynomials using Akaike's information criterion (AIC) and likelihood ratio tests. Records with missing data on gonadectomy status, sex or age were excluded from analysis. Missing data on weight and breed category were imputed using predictive mean matching and polytomous logistic regression, respectively, implemented using the `mice` package in R statistical software (van Buuren and Groothuis-Oudshoorn, 2011; R Core Team, 2023). Effect sizes were estimated from the coefficients of conditional logistic regression models with matched case-control sets as strata and containing covariates for adjustment. With incidence density sampling, exponentiated coefficients of conditional logistic regression models are interpretable as incidence rate ratios (IRR; Labrecque et al., 2021). We tested for effect modification by sex using a likelihood ratio test of the interaction term between sex and gonadectomy, and we conducted a planned subgroup analysis by sex.

2.2. Retrospective cohort study

To estimate the effect of gonadectomy on disease severity in dogs with babesiosis, we conducted a retrospective cohort study among all dogs with a diagnosis of babesiosis identified in the initial search of EHRs from the OVAH. The cohort comprised all dogs 6 months of age and older presenting to OVAH in which a diagnosis of babesiosis was made, between 1 January 2013 and 31 December 2021. Cases were not restricted to the OD and thus included cases arising from outside that catchment population. Recurrent cases of babesiosis were again excluded. Gonadectomy status at the time of diagnosis was the exposure of interest. The outcome was a binary variable of disease severity, classified as severe or not. The outcome was categorized as severe if any of the following conditions were met:

- Death
- Hospitalization of 2 or more days
- Blood transfusion
- Collapsed (unable to walk unassisted)
- Hematocrit $\leq$15 or $\geq$55
- Seizures accompanied by glucose $>$3.3 mmol/L
- Body temperature $\leq$36 °C
- Acute respiratory distress syndrome
- Renal failure

Selection of covariates for confounding adjustment was again based on causal DAGs analyzed with DAGitty version 3.1 (Fig. 2). As with the DAGs presented in Fig. 1 for babesiosis diagnosis, the direct but not total effect of gonadectomy on severity of babesiosis can be estimated by adjusting for sex, age, breed category, and weight at the time of OVAH visit (weight2), assuming no unmeasured confounding (Fig. 2A). Under these same assumptions, the total effect of biological sex (male vs. female) on babesiosis severity can be estimated without adjustment for any covariates, and the direct effect of biological sex can be estimated by adjusting for gonadectomy, age, breed category, and weight2. Assuming no effect of weight1 on gonadectomy and no unmeasured confounding by *U*, the total effect of gonadectomy on babesiosis severity can be estimated by adjusting for sex, age, and breed category (Fig. 2B). The continuous covariates age and weight were not transformed in the model, based on visual inspection and comparison of higher-order polynomials using AIC and likelihood ratio tests. Records with missing data on gonadectomy status, sex, age or outcome status were excluded from analysis. Missing data on weight and breed category were imputed as for the case-control study. Effect sizes (risk ratios; RR) were estimated from the coefficients of log-binomial regression models containing covariates for adjustment. We tested for effect modification by biological sex using a likelihood ratio test of the interaction term between sex and gonadectomy, and conducted a planned subgroup analysis by sex. For log-binomial models that did not converge (the subset models for males and females), we used Poisson regression with robust standard errors.

2.3. Sensitivity analysis for unmeasured confounding

For both studies, we tested the robustness of our estimates to the assumption of unmeasured confounding by *U* (Fig. 1 & 2) by performing a 'tipping point' sensitivity analysis (D'Agostino McGowan, 2022a). We examined the qualities of *U* as a binary unmeasured confounder (for example, owner acaracide use) that would 'tip' the observed effect such that the confidence interval includes the null. Specifically, we set the prevalence of *U* in the gonadectomized group to 0.8 (continuing the example of acaracide use, this assumes 80 % of owners of gonadectomized dogs applied acaracide to their dogs) and examined a range of prevalences in the non-gonadectomized group as well as a range of unmeasured confounder-outcome effect sizes that would tip the limiting bound (the bound closest to the null) of the 95 % confidence interval of the effect of gonadectomy above 1 (that is, to include the null). We applied this method using the 'tip_with_binary' function in the 'tipr' package in R (D'Agostino McGowan, 2022b).

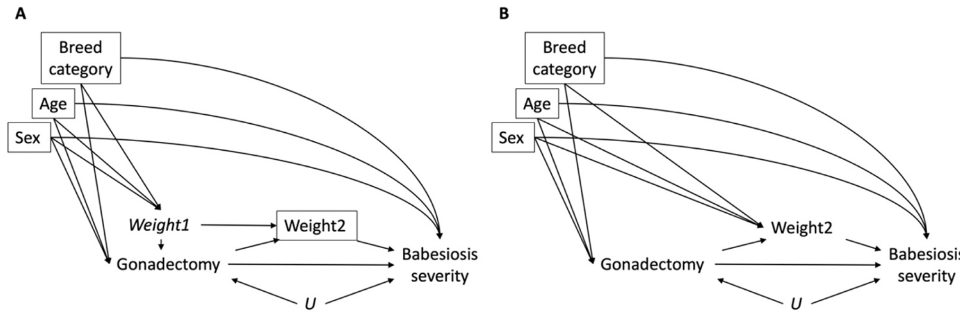

Fig. 2. Causal directed acyclic graphs (DAGs) representing our assumptions of the causal relationships between measured and unmeasured variables in the retrospective cohort study. *U:* unmeasured variables potentially confounding the exposure-outcome relationship. Variables in italics are unmeasured. Variables in boxes were controlled for in the analyses.

3. Results

3.1. Case-control study

The selection of patients attending the OVAH OD into the case-control study based on EHRs is shown in Fig. 3. All 811 babesiosis cases were time-matched (within 7 days of OD visit) with 4 controls each (3244 controls). Of these 4055 records, 67 (1.7 %) had missing values for weight2 only, 167 (4.1 %) had missing values for breed category only, and 8 (0.2 %) had missing values for both covariates. The distributions of exposures and covariates before imputation of missing values are shown in Table 1. Conditional logistic regression results, adjusted for measured confounders, are shown in Table 2 (under assumptions in DAG1a) and Table 3 (under assumptions in DAG1b). There was no evidence of modification by sex of the effect of gonadectomy on babesiosis incidence rate (LRT p-value = 0.41).

3.2. Retrospective cohort study

The cohort comprised 923 dogs 6 months and older diagnosed with babesiosis at the OVAH (811 from the OD and 112 additional cases from other departments). Of these, 470 were categorized as complicated and 453 as uncomplicated. Of the 923 cases, 45 (4.9 %) had missing values for weight2 only, 80 (8.7 %) had missing values for breed category only, and 10 (1.1 %) had missing values for both covariates. The distributions of covariates and outcomes by exposure group before imputation of missing values are shown in Table 4. Log-binomial regression results, adjusted for potential confounders, are shown in Table 5 (DAG2a) and Table 6 (DAG2b). There was no evidence of modification by sex of the effect of gonadectomy on the risk of severe babesiosis (LRT p-value =

Table 1
Distributions of exposures and measured covariates in babesiosis cases and controls.

	Cases (n = 811)	Controls (n = 3244)
Gonadectomy		
No	586 (72.3 %)	1477 (45.5 %)
Yes	225 (27.7 %)	1767 (54.5 %)
Sex		
Female	312 (38.5 %)	1689 (52.1 %)
Male	499 (61.5 %)	1555 (47.9 %)
Sex/gonadectomy		
Female intact	191 (23.6 %)	598 (18.4 %)
Female gonadectomised	121 (14.9 %)	1091 (33.6 %)
Male intact	395 (48.7 %)	879 (27.1 %)
Male gonadectomised	104 (12.8 %)	676 (20.8 %)
Breed category		
Toy	72 (8.9 %)	710 (21.9 %)
Terrier	195 (24.0 %)	745 (23.0 %)
Working	185 (22.9 %)	474 (14.6 %)
Non-sporting	20 (2.5 %)	127 (3.9 %)
Sporting	70 (8.6 %)	270 (8.3 %)
Hound	53 (6.5 %)	383 (11.8 %)
Herding	65 (8.0 %)	189 (5.8 %)
Mixed	68 (8.4 %)	156 (4.8 %)
Missing	83 (10.2 %)	190 (5.9 %)
Mean age in days (sd)	1210 (999)	2088 (1555)
Mean weight in g (sd)	20,757 (13,704)	17,464 (14,532)
Missing weight	20 (4.7 %)	56 (1.7 %)

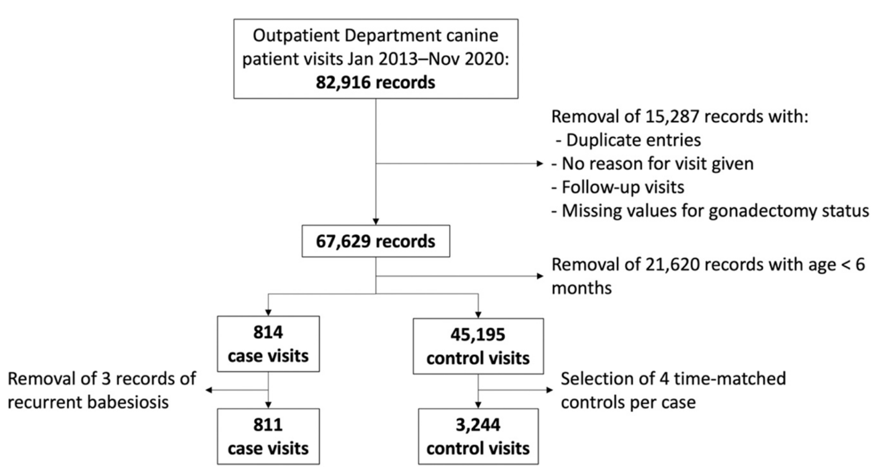

Fig. 3. Flow diagram of electronic health records (patient visits) selected into the case-control study.

Table 2
Results of the analysis of the case-control study, based on assumptions in DAG1a.

Effect estimand (DAG1a)	Cases	Controls	IRR	95 % CI	P-value
Direct effect of gonadectomy on incidence of babesiosis	811	3244	0.49	0.41–0.60	<0.001
Total effect of sex on incidence of babesiosis (male vs. female)	811	3244	1.74	1.49–2.04	<0.001
Direct effect of sex on incidence of babesiosis (male vs. female)	811	3244	1.38	1.16–1.64	<0.001
Direct effect of gonadectomy on incidence of babesiosis in **females**	312	1248	0.59	0.44–0.79	<0.001
Direct effect of gonadectomy on incidence of babesiosis in **males**	499	1996	0.48	0.37–0.62	<0.001

Table 3
Results of the analysis of the case-control study, based on assumptions in DAG1b.

Effect estimand (DAG1b)	Cases	Controls	IRR	95 % CI	P-value
Total effect of gonadectomy on incidence of babesiosis	811	3244	0.50	0.41–0.60	<0.001
Total effect of gonadectomy on incidence of babesiosis in **females**	312	1248	0.59	0.44–0.80	<0.001
Total effect of gonadectomy on incidence of babesiosis in **males**	499	1996	0.49	0.38–0.63	<0.001

Table 4
Distributions of exposures and measured covariates in severe and not severe babesiosis cases.

	Severe (n = 470)	Not severe (n = 453)
Gonadectomy		
No	376 (80 %)	311 (68.7 %)
Yes	94 (20 %)	142 (31.3 %)
Sex		
Female	170 (36.2 %)	188 (41.5 %)
Male	300 (63.8 %)	265 (58.5 %)
Sex/gonadectomy		
Female intact	125 (26.6 %)	107 (23.6 %)
Female gonadectomised	45 (9.6 %)	81 (17.9 %)
Male intact	251 (53.4 %)	204 (45.0 %)
Male gonadectomised	49 (10.4 %)	61 (13.5 %)
Breed category		
Toy	48 (10.2 %)	40 (8.8 %)
Terrier	109 (23.2 %)	113 (24.9 %)
Working	113 (24.0 %)	92 (20.3 %)
Non-sporting	14 (3.0 %)	10 (2.2 %)
Sporting	38 (8.1 %)	43 (9.5 %)
Hound	22 (4.7 %)	37 (8.2 %)
Herding	35 (7.4 %)	39 (8.6 %)
Mixed	44 (9.4 %)	36 (7.9 %)
Missing	47 (10.0 %)	43 (9.5 %)
Mean age in days (sd)	1208 (1018)	1210 (1008)
Mean weight in g (sd)	21,282 (14,133)	20,159 (13,328)
Missing weight	24 (5.1 %)	31 (6.8 %)

Table 5
Results of the analysis of the retrospective cohort study, based on assumptions in DAG2a.

Effect estimand (DAG2a)	Severe	Not severe	RR	95 % CI	P-value
Direct effect of gonadectomy on risk of severe babesiosis	470	453	0.72	0.60–0.86	<0.001
Total effect of sex on risk of severe babesiosis (male vs. female)	470	453	1.12	0.98–1.28	0.10
Direct effect of sex on risk of severe babesiosis (male vs. female)	470	453	1.07	0.94–1.23	0.30
Direct effect of gonadectomy on risk of severe babesiosis in females	149	155	0.65	0.49–0.85	0.002
Direct effect of gonadectomy on risk of severe babesiosis in males	252	232	0.79	0.63–1.00	0.05

Table 6
Results of the analysis of the retrospective cohort study, based on assumptions in DAG2b.

Effect estimand (DAG2b)	Severe	Not severe	RR	95 % CI	P-value
Total effect of gonadectomy on risk of severe babesiosis	470	453	0.72	0.60–0.86	<0.001
Total effect of gonadectomy risk of severe babesiosis in females	149	155	0.65	0.49–0.86	0.002
Total effect of gonadectomy on risk of severe babesiosis in males	252	232	0.79	0.63–1.00	0.05

0.35).

3.3. Sensitivity analysis for unmeasured confounding

Fig. 4a & b show the results of the tipping point sensitivity analysis for an unmeasured confounder *U* for the total effect of gonadectomy on the outcome from the case-control and cohort study, respectively, assuming a prevalence of 0.8 for *U* in the gonadectomized group.

For example, from Fig. 4a, in order to tip the effect of gonadectomy on babesiosis diagnosis to the null, an unmeasured confounder such as ectoparasite prophylaxis application practiced by 80 % of owners of gonadectomized dogs would need to have either

i) a low prevalence in the non-gonadectomized group (1/20 or 5 %) and a strong protective effect on babesiosis incidence (relative risk of 0.48), represented by the top right point in Fig. 4a, or
ii) a higher prevalence in the non-gonadectomized group (1/2.5 or 40 %) but a stronger protective effect (relative risk of 0.29), represented by the lower left point in Fig. 4a).

Similarly, from Fig. 4b, if we consider an unmeasured confounder *U* to be a binary variable such as 'delay/no delay in case presentation' in the cohort study, and assuming that 80 % of owners of gonadectomized dogs present the case without delay, in order to tip the effect of gonadectomy on babesiosis severity to the null, either:

i) a small proportion of owners in the non-gonadectomized group (1/20 or 5 %) present without delay, and timely presentation has a relatively weak protective effect on risk of severe babesiosis (relative risk of 0.82), or

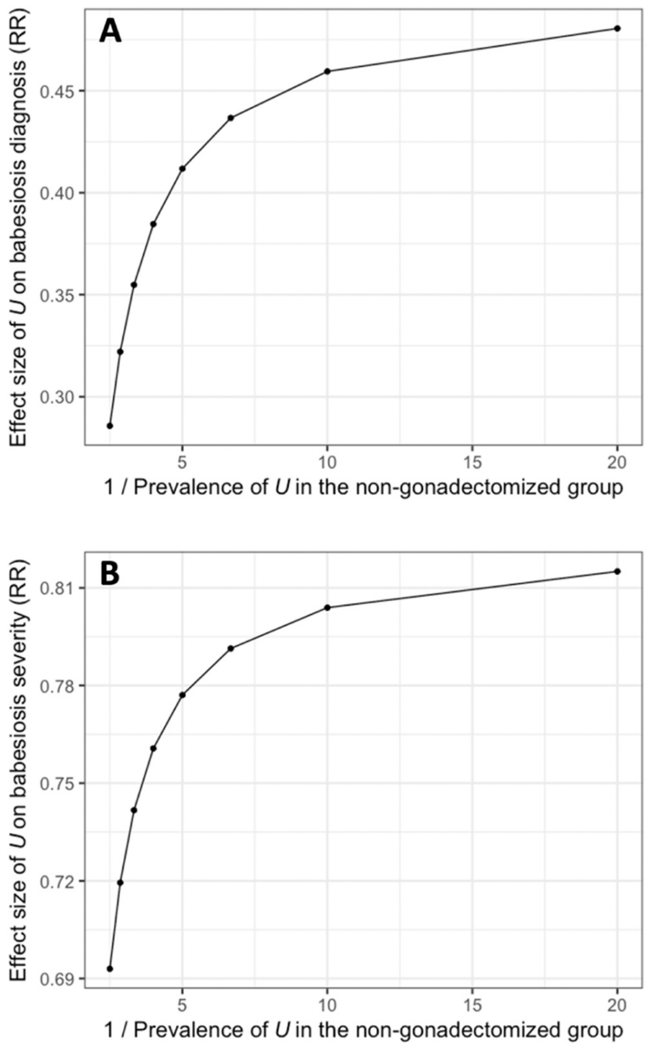

Fig. 4. Results of the tipping point sensitivity analysis for an unmeasured confounder *U* for the total effect of gonadectomy on the outcome from (A) the case-control and (B) the retrospective cohort study, assuming a prevalence of 0.8 for *U* in the gonadectomized group.

ii) a higher proportion of owners in the non-gonadectomized group (1/ 2.5 or 40 %) present without delay, and timely presentation has a stronger protective effect on risk of severe babesiosis (relative risk of 0.69).

To partially control for owner level of care and/or health status of subjects prior to babesiosis diagnosis as potential confounders within the unmeasured set of confounders *U*, we included number of previous visits to the OD in the preceding six months as a covariate in the analysis of the case-control study. This information was not available for dogs in the cohort study whose diagnosis was made in other departments, therefore this analysis was not performed for the cohort study. We found no substantive change in effect estimates when this variable was included (direct effect of gonadectomy on incidence of babesiosis under DAG 1a: RR 0.53, 95 % CI 0.44–0.65; total effect of gonadectomy on incidence of babesiosis under DAG 1b: RR 0.54, 95 % CI 0.44–0.65).

Although we did not have data on the prevalence of ectoparasite use among gonadectomized and non-gonadectomized dogs, we did an exploratory study of the relative prevalence of gonadectomy status of dogs of owners purchasing canine ectoparasitic medications at the OVAH in a 12-month period from November 2022 through to November 2023). We found that non-gonadectomized dogs represented a higher proportion of this population (62.8 %) than of the general population of OD visits in the final 12-month period of our study, from November 2019 through to November 2020 (47.6 %). Although exploratory, these data suggest that owners of non-gonadectomized dogs may be purchasing canine ectoparasitic medications more frequently than owners of gonadectomized dogs.

4. Discussion

We found that gonadectomy substantially reduced the incidence and severity of babesiosis in dogs 6 months and older, across both sexes. For both incidence and severity, the direct and total effect of gonadectomy were almost identical, indicating that the indirect effect of gonadectomy mediated through its effect on body weight is negligible. We also observed that biological sex affected the outcomes: males had higher incidence of babesiosis and higher risk of a severe outcome than females, although in the latter case the effect was not statistically significant. There was no evidence for modification of the effect of gonadectomy by sex, with effect estimates qualitatively similar for males and females for both outcomes.

Our findings that incidence and severity of babesiosis is higher in males than females is consistent with observations of infectious disease susceptibility across species and pathogen taxa. Several studies have reported a higher prevalence of *Babesia* spp. infections in male dogs compared to females (Adaszek et al., 2011; Mellanby et al., 2011; Veneziano et al., 2018), although others report no difference (Obeta et al., 2020; Selim et al., 2022). In humans, incidence and severity of disease is higher in males for diverse bacterial (Muenchhoff and Goulder, 2014), protozoal, nematode, trematode (Muenchhoff and Goulder, 2014; Klein, 2004; Bernin and Lotter, 2014), and fungal (Egger et al., 2022) infections. A similar pattern of increased male susceptibility is observed in rodent models of infections with bacterial, protozoal and fungal pathogens (Gay et al., 2021), and in prevalence of helminth and arthropod pathogens in non-human (largely non-domesticated) mammal populations (Moore and Wilson, 2002). The topic has not been systematically explored in domesticated animal species (Byaruhanga and Knobel, 2022).

Other than body weight, our study did not examine the potential mechanisms through which the effect of gonadectomy on babesiosis outcomes might be mediated. Gonadal sex steroid hormones influence immunological susceptibility to infection and disease, and may also have behavioral or physiological effects that change exposure risk. Gonadal sex hormones affect the response of mice to infection with *Plasmodium* spp. in models of human malaria, a disease with which babesiosis due to *B. rossi* shares commonalities (Reyers et al., 1998; Leisewitz, Goddard, De Gier et al., 2019). Male mice have higher parasitemia and mortality than female mice, and these outcomes in males are reduced by gonadectomy (Cernetich et al., 2006; Cervantes-Candelas et al., 2021) but reversed by administration of testosterone (Kamis and Ibrahim, 1989; Wunderlich et al., 1991). In females, gonadectomy increases parasitemia, as does administration of 17β-estradiol; neither treatment affects female mortality (Cervantes-Candelas et al., 2021). While it is clear that gonadectomy can influence innate and adaptive immune responses to pathogens in a sex-specific manner, further studies are needed to determine the presence and magnitude of this effect in dogs to *B. rossi* infection, as well as other prevalent pathogens.

Roaming behavior in dogs may increase exposure to tick vectors of *B. rossi*, increasing risk of infection and plausibly severity of disease due to higher infectious dose. Studies have shown that gonadectomy is associated with reduced owner-reported roaming behavior in males (Maarschalkerweerd et al., 1997; Neilson et al., 1997; Baquero et al., 2020) and in females (Baquero et al., 2020); however, gonadectomy did not reduce home range size or observed roaming behavior in a study of male dogs (Garde et al., 2016) that followed large numbers of dogs continuously using global positioning system (GPS) technology. Gonadectomy in dogs has also been associated with reduced tick infestation (Raghavan et al., 2007; Smith et al., 2011) and endoparasitic infections

(Kirkpatrick, 1988). It is unclear whether these associations are due to direct effects of sex hormones, secondary behavioural effects, or confounding by owner level of responsibility (Kirkpatrick, 1988).

Our findings are in contrast to those of Mellanby et al. (2011) who reported that, compared to intact female dogs, neutered female dogs had a significantly higher odds of being diagnosed with canine babesiosis at the OVAH OD between 2004 and 2010 (OR 1.32; 95 % CI 1.01–1.71). This difference may be due to the time periods studied (2004–2010 vs. 2013–2020) or differences in the methodologies used (specifically, control selection and confounding control). Substantial changes to the demographics of dog owners seen at the OVAH as well as availability and diversity of acaricides may have occurred between study periods and could potentially explain the discrepant results. Reanalysis of both datasets using comparable methods would be informative.

Our study has several limitations. Body condition score (BCS) may be a more appropriate covariate to include in the DAG, rather than weight; however, BCS is not routinely captured during OD visits at the OVAH. Breeds were determined by assessing the dogs' physical appearances to known breed standards. Although this may result in misclassification of breed (compared to for example evaluation of pedigree records), we think it unlikely to result in meaningful misclassification of breed category, the covariate used in our study. Diagnosis of babesiosis at OVAH is based on microscopy, which does not distinguish between *Babesia* species. While previous studies using molecular methods confirmed that the vast majority of babesiosis cases at OVAH were due to *B. rossi* (Matjila et al., 2004; Matjila et al., 2008), these did not cover the period of our research (2013–2021) and so we cannot preclude changes in relative prevalence of *Babesia* species, which could plausibly modify the effect of gonadectomy on diagnosis and/or severity. Our search strategy may have missed cases of babesiosis that were not treated with Berenil®, resulting in misclassification bias. This bias may be differential if for example cases that were not treated with Berenil® were more likely to have been euthanized prior to treatment due to cost constraints; we assume these constraints would be more prevalent among owners of non-gonadectomized dogs. However, a quantitative sensitivity analysis for misclassification of outcome bias under these assumptions shows that the corrected effect would be even further from the null (that is, a stronger protective effect of gonadectomy on babesiosis incidence) than was observed in the case-control study. Our results may also be biased by unmeasured confounding of the exposure-outcome relationships, for example owner factors affecting access to veterinary care, preventive measures, patient co-morbidities or pathogen exposure (Gates and Nolan, 2010; Sánchez-Vizcaíno et al., 2017; Wisnieski et al., 2023). In particular, owner practices around ectoparasite use and care-seeking could be major unmeasured confounders. While the results of the tipping point sensitivity analysis provide some confidence in the robustness of our estimates to unmeasured confounding, future students would benefit from availability of data on these and other potential confounders in the study population. Our exploratory study of gonadectomy status of dogs of owners purchasing canine ectoparasite medications at OVAH suggested that purchases were more frequent among owners of non-gonadectomized dogs and therefore less likely to confound results. Owners of non-gonadectomized dogs may comprise a heterogenous population including breeders, who may provide a higher level of care than owners of gonadectomized dogs on average, and owners at a lower socioeconomic level who may not have affordable access to routine veterinary services such as spay/neuter or prophylactic care.

In conclusion, our study shows a robust protective effect of gonadectomy on the incidence and severity of babesiosis in both male and female dogs 6 months of age and older, and contributes important evidence to the debate on the overall risks and benefits of gonadectomy to dogs in this population.

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CRediT authorship contribution statement

Darryn Knobel: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Josef Hanekom:** Writing – review & editing, Project administration, Investigation, Data curation. **Maria C. van den Bergh:** Writing – review & editing, Investigation, Data curation. **Andrew L. Leisewitz:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of Competing Interest

None.

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