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SYSTEMATIC REVIEW

The effect of neutering on the risk of mammary tumours in dogs – a systematic review

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A commonly-stated advantage of neutering bitches is a significant reduction in the risk of mammary tumours, however the evidence for this has not previously been assessed by systematic review. The objectives of this study were to estimate the magnitude and strength of evidence for any effect of neutering, or age of neutering, on the risk of mammary tumours in bitches. A systematic review was conducted based on Cochrane guidelines. Peer-reviewed analytic journal articles in English were eligible and were assessed for risk of bias by two reviewers independently. Of 11,149 search results, 13 reports in English-language peer-reviewed journals addressed the association between neutering/age at neutering and mammary tumours. Nine were judged to have a high risk of bias. The remaining four were classified as having a moderate risk of bias. One study found an association between neutering and a reduced risk of mammary tumours. Two studies found no evidence of an association. One reported "some protective effect" of neutering on the risk of mammary tumours, but no numbers were presented. Due to the limited evidence available and the risk of bias in the published results, the evidence that neutering reduces the risk of mammary neoplasia, and the evidence that age at neutering has an effect, are judged to be weak and are not a sound basis for firm recommendations.

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INTRODUCTION

The decision of whether, and when, a bitch should be neutered is highly complex. It may have implications not just for the bitch's own health, but also her working potential or suitability as a pet, dog population dynamics and subsequently the numbers of unwanted dogs and strays (Jagoe and Serpell 1988, Kustritz 2002, 2007, Howe 2006). Not only are veterinarians expected by the public to advise on when and if neutering should be performed, but in the Guide to Professional Conduct, the Royal College of Veterinary Surgeons (RCVS) states that owners should be informed of the "significance and risks" of procedures before obtaining consent (Royal College of Veterinary Surgeons 2011). Despite the obvious need for clear guidance, there is considerable disparity amongst veterinarians in the advice given to owners. Whilst approximately 54% of dogs (male and female combined) in the UK are neutered (Diesel and others 2010) the practice is seen as unethical in some countries (Salmeri and others 1991, Kustritz 2007). Even within the UK veterinary population there is a huge range of opinion, with only 72% of vets always recommending spaying of bitches not used for breeding (compared to mostly, sometimes or rarely). There is even more disparity in the opinions on optimal age of spaying, with approximately 16% recommending spaying before the first season "all of the time" and the remaining veterinarians recommending this, "mostly", "sometimes", "rarely" or "never" in almost exactly equal proportions (Diesel and others 2010).

A common justification for early neutering of bitches is that it protects against mammary neoplasia. However, many frequently cited references are over 40 years old (Frye 1967, Dorn and others 1968, Schneider and others 1969), and this evidence has not been scrutinised with the benefit of recent developments in epidemiological methods and knowledge of potential confounders of the association between mammary masses and neutering, such as age, breed and treatment with synthetic derivatives of ovarian steroids (Priester 1979, Misdorp 1988).

Systematic review methods have been used frequently in the medical field (Greenhalgh 1997), and to a lesser extent in veterinary literature (Lean and others 2009), to evaluate the strength of evidence for an association by assessing the findings of different studies as objectively as possible. They have highlighted the importance of publication bias (although they are not necessarily immune to it) (Eyding and others 2010).

As part of a larger project to develop evidence-based guidelines for neutering bitches, the objectives of this study were to evaluate the strength of evidence for the association between mammary tumours (of any histological type) and neutering, or age at neutering, and to estimate the magnitude, and confidence interval, of the effect of neutering, or age at neutering, on the frequency of mammary tumours (of any histological type) in bitches.

MATERIALS AND METHODS

Protocol

A systematic review was conducted using a predefined protocol (available on request) based on Cochrane guidelines (Higgins and Green 2009). Where the protocol was modified during the review process, this is indicated in the description below.

Search strategy

Three databases (CAB Direct 2011, ISI Web of Knowledge 2011, U.S. National Library of Medicine 2011) were searched using the following search terms:

1. Dog OR dogs OR bitch* OR canis* OR canine* OR canid* OR "Dogs"[MAJR]
2. Spey* OR Spay* OR neuter* OR ovariohysterectom* OR ovariectom* OR gonadect* OR gonad OR gonads OR "Ovariectomy/veterinary"[MAJR]
3. mammar* OR breast* OR "Mammary Glands, Animal"[MAJR]
4. tumour* OR tumor* OR cancer* OR neoplas* OR mass OR masses OR lump* OR "Neoplasms/veterinary"[MAJR]

(#1 AND #2) OR (#1 AND #3 AND #4)

(Medical Subject Heading (MeSH) terms used in PubMed only)

The search was conducted on November 5, 2010. No limits were set. In addition, two references were found during the screening process that had not been identified during the search but were eligible for review and were also included.

Screening process

All references were imported into Endnote x4 (Thomson Reuters), and duplicates were deleted using the automatic function, based on matching title, author and reference type. Duplicates that were not detected by the software, due to differences in abbreviations or spelling errors, were deleted manually by the primary author based on the same criteria.

The remaining references were screened by the primary author to eliminate any that did not fulfil eligibility criteria one to four, shown in Table 1. In summary, only original journal articles containing data concerning the association between neutering and mammary masses, or age of neutering and mammary masses, were included. A random sample of 40 of the references was screened by the primary author and a PhD student in veterinary epidemiology as a pilot-test.

Table 1. Eligibility criteria for inclusion in the systematic review

Eligibility criteria
1. EITHER the frequency of mammary masses (of any classification) has been measured in both neutered and entire female dogs (or animals neutered at different ages) OR the frequency of neutering (or neutering at different ages) has been measured in both female dogs with and without a history of mammary masses (of any classification)
2. The frequencies given in criterion 1, or the results of analysing these frequencies, have been stated in the report
3. The "neutered" dogs were neutered by ovariectomy or ovariohysterectomy or by an unstated method
4. The report is an original research article*
5. The report is published in a peer-reviewed section of a journal (according to details on the journal's website)*
6. The full text of the report is available in English*

*These criteria were added in the course of the review process because of practical constraints

Full text was retrieved for the remaining papers, which were then re-screened by the primary author to eliminate any that did not fulfil the eligibility criteria. It was decided, at this stage in the review process, that only peer-reviewed articles in English would be included because of practical and financial constraints.

Data extraction and assessment of bias

The remaining papers were reviewed by two veterinary epidemiologists: the primary author, and one of two other authors. A preformed data extraction form was used for this purpose, which had been pilot-tested on 2 papers by approximately 12 researchers in the Veterinary Epidemiology and Public Health group at the Royal Veterinary College during an interactive workshop and adjusted based on their responses.

The form was based on questions suggested in the Cochrane Handbook (Higgins and Green 2009) and included the following sections: study design and outcomes, bias, results, applicability of results and miscellaneous questions. All results were extracted for each study. If there was no measure of association [risk ratio, odds ratio (OR) or rate ratio], confidence interval or corresponding P value (for the association between neutering or age at neutering and mammary tumours) reported, these were calculated by reviewers where possible.

The risk of bias for each study outcome (e.g. effect of age of neutering on risk of malignant mammary tumours) was assessed using adapted versions of the Cochrane tool (for trials) (Higgins and Green 2009), the Newcastle Ottawa tool (for cohort and case control studies) (Ottawa Hospital Research Institute 2011) and the Dobson and Black tool (for all other study designs) (Downs and Black 1998). The latter two tools have been identified as the most useful for systematic review of

non-randomised studies (Deeks and others 2003, Higgins and Green 2009). Tools were modified as required to achieve the following objectives:

- To clarify the questions in terms of the topic of this systematic review (i.e. mammary masses and neutering in female dogs).
- For cohort studies only, an additional question was included (How was neuter status defined and ascertained?) to judge whether the study classified the temporal relationship between neutering and onset of neoplasia (and excluded dogs which were neutered as a consequence of mammary neoplasia – this is commonly thought to improve the prognosis).
- During the review process, it was decided for case control studies to simplify selection and definition of controls to one question: "could cases have become controls had they not had the outcome of interest and vice versa?"
- Where possible, the question format was changed so that the reviewer was asked to describe a feature of the study and then to answer a closed question to which the answers *yes, no* or *unclear* determined whether a criterion was met or not, or if sufficient information was lacking.
- The criteria for *yes*, *no* or *unclear* were clearly defined in the guidance notes.

Initially, studies would be excluded if there were any *no* or *unclear* responses; however, this would have resulted in all of the studies being excluded. The risk of bias for each study outcome was therefore classified according to the SIGN (Scottish Intercollegiate Guidelines Network) "levels of evidence" system (Scottish Intercollegiate Guidelines Network 2008). This was modified to include cross-sectional studies, as has been done previously (University of Liverpool 2011), and an intermediate category "2" was defined to accommodate studies with a "moderate risk of confounding or bias".

Extracted data were entered into a spreadsheet (Microsoft Excel 2007). Any disagreement between reviewers was resolved by consensus. Where further information was required to complete the form, an attempt to contact the primary author was made and any new information acquired was included in the review.

Data analysis

Studies were grouped according to study outcomes (e.g. malignant mammary tumours). The study designs and measures of frequency used were considered to be sufficiently heterogeneous that it would not be meaningful to conduct a statistical test of heterogeneity or calculate summary measures of effect.

The overall strength of evidence for each outcome was assessed using the SIGN system (Scottish Intercollegiate Guidelines Network 2008), which is designed to rate the strength of recommendations for medical interventions from A (strongest) to D (weakest). Disagreements amongst the reviewers were resolved by consensus.

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) guidelines were followed where possible (Liberati and others 2009).

RESULTS

The searches retrieved 11,147 references, as shown in Fig 1. In addition, two papers identified solely through references within reports that were screened were included. A total of 7557 papers remained after the elimination of duplicates and 340 remained after the first screening, which eliminated any reports that clearly did not address the research questions (eligibility criteria 1 to 4, Table 1). Of the 340 remaining reports, 6 were eliminated when the full text could not be obtained via resources available at the British Library, Royal Veterinary College or accessed freely online. Ninety-six were eliminated because they did not address the research question (eligibility criterion 1), 75 because they were not original research articles (criterion 4), 5 because they were not peer-reviewed (criterion 5) and 140 as the full text was not available in English (criterion 6). Five articles in which the research question of interest was addressed by the study were excluded because the relevant results were not reported (criterion 2).

Of the 13 studies that addressed the research questions and were published in peer-reviewed English-language journals, 9 were judged to be at high risk of confounding or bias, according to the SIGN system. The remaining four were assigned a level of 2: "Case control, cohort and cross-sectional studies with a moderate risk of confounding or bias".

Table 2 summarises the risk of bias assessment for each of the nine studies excluded because of bias, all but one of which were observational, rather than intervention, studies. The only intervention study had been designed primarily to assess the effect of synthetic progesterone on the risk of mammary tumours. The

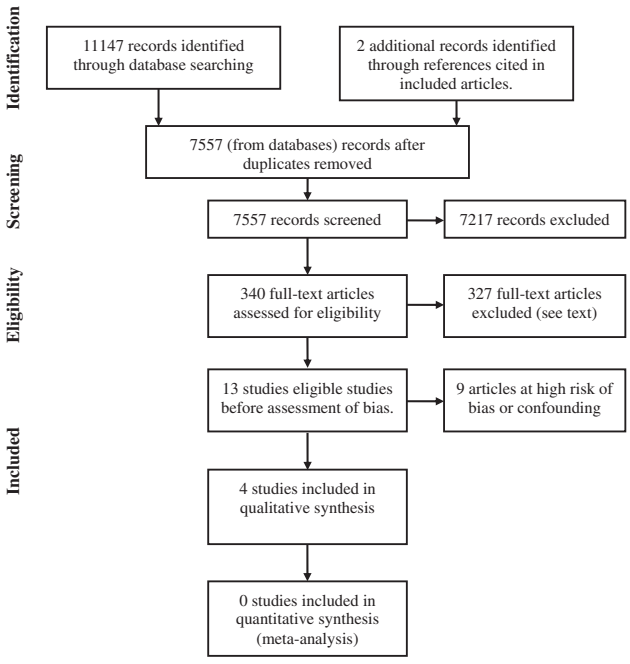
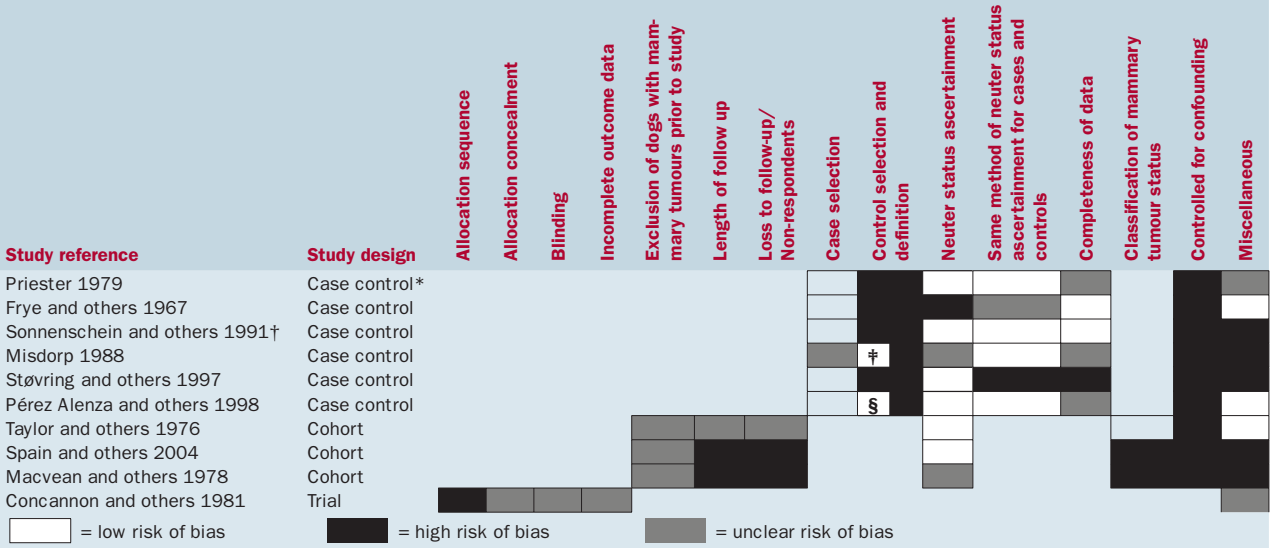


FIG 1. Flowchart showing numbers of reports at each stage of the screening process (template provided by PRISMA)

Table 2. Studies excluded because of potential bias, showing reasons for exclusion, according to prespecified criteria



The following areas were at low risk of bias in all of the studies, and are not included in the table: case control studies: case definition; cohort studies: selection of cohorts; trials: selective reporting

*Unclear – it appears that the results presented relate only to the case series and not to the reference population at all, and are therefore non-analytic

†The controls appear to have been matched to cases on neuter status (since this was not a primary exposure of interest) which could cause bias to the association with neuter status

‡Different control groups

§Different control groups; data for hospital control group included, data for healthy control group excluded

spayed dogs had received high doses of synthetic progesterone and treatment groups had not been randomly allocated, so the results were not applicable to the general dog population and were also judged to be at high risk of bias (Concannon and others 1981).

One study was not clearly reported and appeared to present results of a case series only, and was therefore not strictly analytic despite describing case-control methodology (Priester 1979). Another study, the primary aim of which was to investigate the effect of diet on the risk of mammary tumours, appeared to have matched controls to cases on neuter status, thus preventing the analysis of a relationship between neutering and mammary tumours. Although age at neutering was recorded, the measures of association (ORs) were calculated using the odds of mammary tumours amongst entire bitches as a baseline. This suggests that the results of comparing dogs neutered at different ages could also be biased by the matching process (Sonnenschein and others 1991). Reasons for a high risk of bias in the other studies included the selection of controls from a different time period or veterinary hospital from the cases or an insufficiently long follow-up time for cohorts. (The present study criteria for "low risk of bias" for "length of follow up" in cohort studies was that follow-up included at least one year of life during which the dogs were at least seven years old.)

The key features of the four included studies are shown in Table 3. All four addressed the association between neutering and mammary neoplasia, and in addition one of these (Schneider and others 1969) addressed the association between age at neutering and mammary neoplasia. All except one were case control studies of between 144 and 2270 dogs, in which cases and controls had been selected either from laboratory records of histopathology results or the veterinarian-attending population. The remaining cohort study was an analysis of a control group within a trial, the primary purpose of which had been to investigate the effect of radium therapy on mammary neoplasia in beagles.

The risk of bias assessment for included studies is summarised in Table 4. None of the studies controlled for all three confounders that we considered important: age, breed and previous treatment with synthetic derivatives of ovarian steroids. Schneider and others (1969) matched individually for age and breed, and controlled for age in the analysis. It was unclear if breed had been controlled for in the analysis. This would be necessary in order to obtain valid results, because cases and controls were individually matched for breed. Pérez Alenza and others (1998) had not controlled for any of our prespecified potential confounders. Richards and others (2001) did not control for previous treatment with synthetic derivatives of ovarian steroids, but did consider age and breed for a multivariable model of variables associated with mammary neoplasia with a univariable P value of less than 0·25 although the variables included in the final model were not reported. Bruenger and others (1994) did not control for age or treatment with synthetic derivatives of ovarian steroids, although breed was accounted for by restriction (only beagles were included).

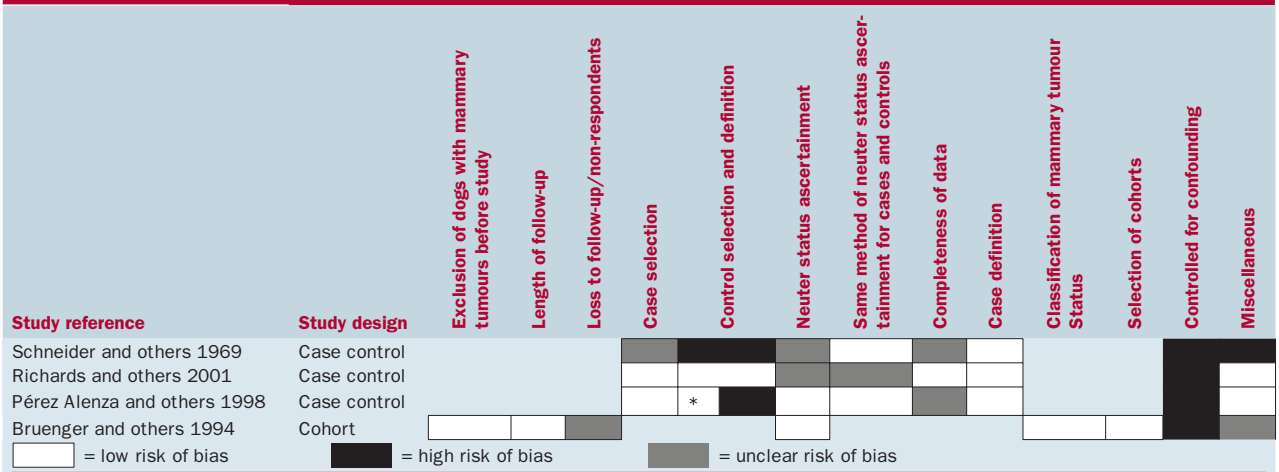
Pérez Alenza and others (1998) used two control groups: "hospital controls" were selected from dogs presenting with various diseases whereas "healthy controls" were selected from dogs attending veterinary practices for preventive treatment. Only the analysis for the "hospital controls" was included in this review, as the "healthy controls" met the prespecified criteria for high risk of bias. In Schneider and others (1969), controls and cases had been drawn from the registry in different time periods. This was judged to lead to moderate risk of bias. A lack of clarity

Table 3. Key features of all included studies in a systematic review to address the association between neutering/age of neutering and frequency of mammary masses, subdivided by outcome

Study	Period of observation	Country	Study design	Number of dogs	Study population*	Age of dogs/ years	Age at neutering/ years
Association between neutering† and malignant mammary tumours‡							
Schneider and others 1969	1963-1966	USA	Case control	174	Private practice cases	10±3§	Before first oestrous to >2·5
Association between neutering* and all mammary tumours† (benign or malignant)							
Bruenger and others 1994	1952-1970	USA	Cohort	65	Beagles bred for research	"young adult" to death	10 to 12§
Richards and others 2001	1986-1998	UK	Case control	2270	Private/charity practice cases	Not stated	Not stated
Association between neutering* and all mammary masses† (neoplastic or non-neoplastic)							
Pérez Alenza and others 1998	1992-1993	Spain	Case control	144¶	University/private practice cases	5-13	Not stated
Association between age at neutering* and malignant mammary tumours†							
Schneider and others 1969	1963-1966	USA	Case control	172	Private practice cases	10±3§	Before first oestrous to >2·5

*All dogs included in this systematic review were female, although some studies presented results on both genders separately
†The method of neutering was not fully described except for Bruenger and others (1994) who used the term "complete ovariectomy". The terms "neutered", "spayed" or "ovariohysterectomised" were used in the remaining reports
‡Schneider and others (1969) includes only mammary adenocarcinomas and mixed mammary neoplasias. Bruenger and others (1994) is not explicit but appears to assess benign tumours, malignant tumours and both together. Pérez Alenza and others (1998) includes dysplastic, benign and malignant masses as one category. Richards is not explicit but appears to include any neoplastic diagnosis
§Approximate values
¶Only analysis using hospital controls was used, as the analysis using healthy controls did not meet the eligibility criteria

Table 4. Risk of bias assessment in included studies



*Different control groups; data for hospital control group included, data for healthy control group excluded

concerning the statistical calculations undertaken by Schneider and others (1969), and whether matching had been accounted for, was another source of moderate risk of bias. Other areas of uncertainty are indicated in Table 4.

The effect of neutering on the risk of mammary tumours

Schneider and others (1969) found a strong protective (approximately 10-fold) effect of neutering on the risk of malignant mammary tumours (Table 5). However, no confidence interval or P value was presented (although it was stated that the results were significant at the 5% level), and the results are only generalisable to animals from which samples are submitted for histopathological diagnosis.

Bruenger and others (1994) reported "some protective effect" (no numerical data presented) of neutering on the risk of mammary tumours (benign and malignant combined) in beagles but concluded that the evidence was "inconsistent". Richards and others (2001) found no signifcant (P>0·1) evidence of an association between neutering and the proportion of mammary tumour submissions that were neoplastic. However, this was only generalisable to dogs from which mammary samples were submitted for histology. Pérez Alenza and others (1998) found no evidence of an association between neutering and mammary masses of any histopathological type (neoplastic or non-neoplastic) [OR 0·7 (0·2 to 2·1) P=0·6, unadjusted for potential confounders; calculated by review authors] amongst veterinarian-attending dogs.

Table 5. Results of included studies

Study	Measure of frequency	Exposures compared	Number of cases (number neutered)	Number of controls (number neutered)	Measure of association (95% CI)*	P-value*
Association between neutering and malignant mammary tumours						
Schneider and others 1969 (n=174)	Odds that a histological submission is diagnosed as a malignant mammary tumour†	Neutered/entire	87 (24)	87 (64)	"Relative risk" 0·1†§(¶)	¶
Association between neutering and all mammary tumours (benign or malignant)						
Bruenger and others 1994 (n=65)	Rate of new tumours per dog; mean number of tumours per dog	Neutered/entire	**	**	††	††
Richards and others 2001 (n=2270)	Odds that a mammary histological submission is diagnosed as neoplastic†	Neutered/entire	2018	252	††	>0·1§§
Association between neutering and all mammary masses (neoplastic or non-neoplastic)						
Pérez Alenza and others 1998 (n=144)	Odds that a dog presenting at a veterinary hospital has a diagnosis of at least one mammary mass	Neutered/entire	102 (11)	42 (6)	Odds ratio 0·7 (0·2, 2·1)¶¶	0·6¶¶
Association between age at neutering and malignant mammary tumours						
Schneider and others 1969 (n=174; missing = 2)	Odds that a histological submission is diagnosed as a malignant mammary tumour)†	Neutered before first oestrous/entire	87 (1)	85 (26)	"Relative risk" 0·005†§ (¶)	¶
		Neutered before second oestrous/entire	87 (3)	85 (11)	"Relative risk" 0·08†§ (¶)	¶
		Neutered after second oestrous/entire	87 (20)	85 (25)	"Relative risk" 0·26†§ (¶)	¶
		Neutered after second oestrous and before 2·5 years of age/entire	87 (2)	85 (10)	"Relative risk" 0·06†§ (¶)	¶
		Neutered after second oestrous and after 2·5 years of age/entire	87 (18)	85 (15)	"Relative risk" 0·4†§ (***)	***

CI Confidence interval
*For the association between neutering (or neutering at a certain age) and mammary masses
†Indirectly measured, using case control logic
†It is not clear, and cannot be verified that this can be interpreted as a relative risk (otherwise known as a risk ratio) or that it has taken into account the matching which was done
§Controlling for age; unclear if breed controlled for
¶Not stated but author states P value is significant at the 5% level
**55 entire; 10 neutered. Numbers of tumours per dog unknown
††No values given. Author states that results are "inconsistent"; "some protective effect"
††No values given. Author states "no association"
§§Adjusted for age and breed
¶¶Calculated by review authors as values not given in report; not adjusted for confounders
***Not stated but author states that P value is not significant at the 5% level

The effect of age of neutering on the risk of mammary tumours

Schneider and others (1969) reported a significant association between the risk of malignant mammary tumours and neutering before first oestrous ["relative risk" (RR) 0·005], second oestrous (RR 0·08), and after second oestrous but before 2·5 years of age (RR 0·06), compared with entire dogs. However, cases and controls were not directly comparable (as discussed above), there was a lack of clarity in how the relative risk was calculated, no confidence intervals were reported and results were again only directly generalisable to bitches from which samples are submitted for histopathological diagnosis. The author stated that there was no significant association between malignant mammary tumours in entire dogs and those spayed after 2·5 years.

Strength of evidence assessment

The SIGN grades of evidence, where A is the strongest and D is the weakest, were classified as D for associations between neutering and the risk of malignant mammary tumours, mammary neoplasia (of any histological type), mammary masses (of any histological type, including non-neoplastic) and for the effect of age at neutering on the risk of malignant mammary tumours (Table 6). The key reasons for this classification were that there were only two studies that found an association between neutering bitches and mammary tumours, although in one of those it was not clear if it was a statistically significant association, and there were considerable risks of bias in both studies.

DISCUSSION

The objectives of this study were to estimate the strength of evidence, and effect measure, for an association between (age of) neutering and the risk of mammary masses. Our results suggest that there is some evidence in one study that neutering is associated with a reduction in the risk of malignant mammary tumours (approximately 10-fold), amongst dogs from which samples had been submitted for histopathology, although there was no evidence that neutering after 2·5 years of age is associated with any change in the risk of malignant mammary tumours.

Table 6. Summary of findings of a systematic review of the effect of neutering on the risk of mammary tumours (of any type) in female dogs

Measure of association (95% confidence interval)	Number of dogs in each study	Grade of recommendation (on scale A-D)	Comments
Association between neutering and malignant mammary tumours			
"Relative risk" 0·12	Schneider and others 1969:174	D	No P values or confidence intervals given, although text states that the relative risk is significant at the 5% level Did not control for potential confounder: previous treatment with synthetic ovarian steroids Not clear, or possible to verify, that matched analysis was done (in which case results may be biased) Not clear that the measure of association calculated can be correctly interpreted as an estimation of relative risk (also known as a risk ratio) Unclear if cases were selected randomly or not Controls were histopathology submissions from different time periods to cases Study population only included dogs from which samples had been submitted for histopathology Missing data not described Unclear if neuter status before onset of mammary neoplasia was reliably ascertained
Association between neutering and all neoplastic mammary tumours (benign and malignant combined)*			
None given	Bruenger and others 1994: 65 Richards and others 2001: 2270	D	Bruenger and others 1994: "inconsistent, although some protective effect [of neutering] seemed to be present" Did not control for potential confounders: age, previous treatment with synthetic ovarian steroids (this is unlikely to have caused under-estimation of effect) Unclear if dogs were lost to follow-up Richards and others 2001: Assessed risk that a histology sample was neoplastic vs. non-neoplastic "No significant difference in [risk of diagnosis of neoplasia] in mammary gland samples from entire or neutered females" Did not control for potential confounder: previous treatment with synthetic ovarian steroids (this is unlikely to have caused under-estimation of effect) Unclear if neuter status before onset of mammary neoplasia was reliably ascertained
Association between neutering and all mammary masses (neoplastic and non-neoplastic combined)			
OR†: 0·7 (0·2-2·1)	Pérez Alenza and others 1998‡: 144	D	Did not control for potential confounders: age, breed, previous treatment with synthetic ovarian steroids Missing data are not described
Association between age at neutering and malignant mammary tumours			
"Relative risk" comparing entire dogs to neutering: before first oestrous: 0·005 before second oestrous: 0·08 after second oestrous: 0·26 after second oestrous but before 2·5years of age: 0·06 after second oestrous and after 2·5 years of age: 0·4	Schneider and others 1969: 172	D	See previous comments The report states that the relative risks are significant at the 5% level except the value relating to dogs spayed after 2·5 years of age

*It is unclear if the results in Bruenger and others (1994) relate to benign and malignant tumours combined, or separately
†This was calculated by the review authors as there was no OR or risk ratio presented in the report
‡Only analysis using hospital controls was used, as the analysis using healthy controls did not meet the eligibility criteria

However, our findings suggest that there are considerable risks of bias in this study. The statistical methodology was unclear, and insufficient data were reported to be able to verify the values presented as estimates of "relative risk", particularly since cases were individually matched to controls, and this was not clearly taken account of in the analysis. In addition, other sources of potential bias make estimating the magnitude and direction of overall bias very difficult.

No evidence of an association between neutering and mammary tumours was observed in studies which considered all types of mammary tumour as a single category, with the exception of one study that reported "inconsistent, but some evidence of a protective effect" of neutering on the risk of mammary tumours (all histological types combined). However, no data were presented to support this conclusion. The single study that measured the risk that a mammary mass was neoplastic (compared to non-neoplastic) also found no association with neutering (Richards and others 2001). There were considerable risks of bias in all of the studies. These issues, combined with the heterogeneity amongst studies, contributed to the overall strength of evidence for each outcome being assigned a level of D (weakest).

Of 13 studies that addressed the research question and were in peer-reviewed English-language journals, we found that 9 were at high risk of bias, according to SIGN criteria. Some key sources of potential bias, which should be taken into account in future studies, are as follows. With the exception of two studies,

none accounted for the effect of previous treatment with synthetic derivatives of ovarian steroids, which has the potential to explain a large proportion of the association between neutering and mammary neoplasia. Age and breed were also inadequately controlled for in many studies. These are likely to be important confounders of any association, although it is less clear if the effect of neutering would be under- or over-estimated.

Most studies did not account for time at risk or age at the time of neutering. This could have introduced measurement error and caused an under-estimation of the effect of neutering, as well as mis-classification of bitches neutered after the onset of neoplasia.

In case control studies there was a tendency to report "relative risk" or "R" but it was not always made clear how this was calculated. It is now generally considered to be inappropriate to use risk ratios in case control studies. The OR is preferred because it accounts for the artificially determined ratio of cases to controls. Unfortunately, sufficient detail was often not given in the reports to verify or adjust calculations and this is something that future authors should consider doing.

Potential sources of bias

There were some limitations to our methods. Practical and time constraints meant that only published, peer-reviewed English-language articles were included, resulting in the exclusion of 140 papers on the basis of language alone. Given the large proportion of English language papers eliminated at the second screening (187 out of 200) and excluded after the risk of bias assessment (9 out of 13), it would seem reasonable to assume that the majority of these 140 foreign language papers would also have been excluded because of other reasons, if the full text had been available in English. To have any effect on our conclusions, any evidence missed by our methods would need to have exceeded the quality of the evidence identified, in terms of measurement of confounders and minimising bias. If further studies at high risk of bias were included in the review, they would be unlikely to improve our estimation of any effect.

The inclusion in this review of only published, peer-reviewed papers may have introduced publication bias. However, it has not been definitively established that bias in systematic reviews of observational studies is reduced by extensive searches and there has been some suggestion that including non-peer-reviewed literature may increase the risk of bias due to the large volume of poor-quality unpublished observational studies (Higgins and Green 2009).

We found some evidence of selective reporting amongst the studies that we screened. Five studies were excluded because no results were reported or discussed, although the necessary parameters appear to have been measured. This under-reporting could have biased our conclusions. There is also an indication of selective outcome reporting in the results we have presented for included studies: only the author who found a strong effect reported a measure of association (OR, rate ratio or risk ratio), while the other three authors did not report any measure of association, although one did report a P value.

Producing summary effect measures was not feasible because of the heterogeneity in study outcomes, units of measurement, study design, study population, control of confounding and potential bias. In addition, some studies did not report sufficient detail, so that P values, effect estimates and confidence intervals could be calculated, making comparisons difficult.

CONCLUSION

There is some evidence to suggest that neutering bitches before the age of 2·5 years is associated with a considerable reduction in the risk of malignant mammary tumours, and that this risk may be reduced further by neutering before the first oestrous. However, our study, which involved screening over 10,000 articles in any language but reviewed only the English literature in detail, demonstrated that the strength of this evidence was weak because of the paucity of published studies that adequately address this issue. Two of the four included studies found no evidence that neutering bitches was associated with a reduced risk of mammary tumours; however, there were also potential biases in these results. This information should be balanced with other available information on the risks and benefits of neutering, including the potentially broader impact of unwanted pregnancies, for example.

Further research on an association between mammary tumours and neutering should focus on recording age, breed and previous exposure to synthetic derivatives of ovarian steroids as potential confounders, ideally in the general dog population, and should take into account both the age that the bitch has been neutered at and the amount of time which she has been neutered for.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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