



Life course analysis of the impact of mammary cancer and pyometra on age-anchored life expectancy in female Rottweilers: Implications for envisioning ovary conservation as a strategy to promote healthy longevity in pet dogs



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ABSTRACT

Mammary cancer and pyometra are important health hazards associated with ovary conservation in pet dogs. Early ovariohysterectomy may reduce the incidence of these two diseases, but an estimate of the extent to which the development of mammary cancer or pyometra adversely influences overall longevity is missing. As a first step toward addressing this knowledge gap, the results of a historical cohort study of Rottweilers that lived in North America are reported. Questionnaires completed by owners and veterinarians were used to obtain lifetime health and medical information on 242 female Rottweilers, including years of lifetime ovary exposure, age at death, and cause of death. To determine the extent to which longevity was shortened in females that developed these ovary-associated diseases, age-anchored life expectancy—defined as the median number of remaining years until death for females alive at specified ages during the life course—and years of life lost, a measure of premature mortality, were estimated.

Mammary carcinoma was diagnosed in 19 (7.9%) females; median age at diagnosis was 8.5 years; case fatality was 37%. Pyometra was diagnosed in 16 (6.6%) females; median age at diagnosis was 5.4 years; case fatality was 7%. Median lifetime ovary exposure for the study population was 4.3 years. Although risk for developing both diseases increased with longer ovary exposure, longer ovary exposure (≥ 4.3 years) was also associated with an overall longevity advantage—a 33% decrease in mortality, living 17 months longer than females with shorter ovary exposure ($P=0.002$). Analysis of age-anchored life expectancy showed that at no time points during the life course was the current or future diagnosis of mammary carcinoma or pyometra associated with shortened survival compared to females who never developed these conditions. This lack of longevity disadvantage is an expected result for diseases with late-onset, moderate (<50%) case fatality (mammary carcinoma) or low (<10%) case fatality (pyometra). These findings fail to support the notion that a strategy, such as elective ovariohysterectomy, implemented to reduce the incidence of mammary carcinoma and pyometra will beneficially impact overall longevity. It follows that future efforts to find and implement effective longevity-promoting interventions should look beyond reducing the incidence of a particular disease to considering trade-offs.

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Introduction

As pet owners and veterinarians become increasingly focused on optimizing healthy longevity, investigators will be counted upon to decipher the complex interplay between aging and disease. A richer and more complete understanding of how

lifestyle choices and interventions, particularly those occurring early in life, alter the physiological trade-offs that impact overall longevity will become a rising priority (Waters, 2014). Reaching this goal will demand efforts not only directed toward the discovery of new longevity-promoting strategies (e.g. anti-aging agents), but also toward a solid reappraisal of interventions that are currently advocated for their health-promoting effects. In an age of specialization, investigators with an eye on promoting longevity will need to look beyond the impact that an intervention exerts on the incidence of a particular disease, and instead focus on

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trade-offs (Waters, 2014)—the avoidance of mortality attributable to a disease within a context of mortality from competing causes.

One such early life intervention that has been credited with health-promoting effects is elective ovariohysterectomy (Root Kustritz, 2007, 2012). Yet removal of ovaries disturbs endocrine output and has the potential to reset physiological networks in significant and unforeseen ways, including the apparent susceptibility to disease conditions ranging from cancer to orthopedic diseases to urinary incontinence (Thrusfield et al., 1998; Cooley et al., 2002; Torres de la Riva et al., 2013; Zink et al., 2014; Zwida and Kutzler, 2015).

Proponents of the health- and longevity-promoting impact of elective ovariohysterectomy point to the protection that this intervention provides against the development of two health hazards, namely mammary neoplasms and pyometra. Indeed, the long-renowned case-control study of Schneider et al. (1969) suggests early ovariohysterectomy exerts a strong suppressive effect on mammary cancer development. And because ovariohysterectomy entails the surgical removal of the uterus, this intervention protects against the advent of pyometra. On the surface, these ideas combine to lend solid support for envisioning elective ovariohysterectomy as a strategy to promote healthy longevity in pet dogs. Yet, a critical question remains unaddressed: To what extent does the development of mammary cancer or pyometra adversely influence overall longevity?

Research on canine longevity by our group provided the first evaluation of the association between the number of years of lifetime ovary exposure and highly successful aging (Waters et al., 2009). The research in Rottweilers showed that keeping ovaries longer was associated with an increased likelihood of achieving exceptional longevity. Our work pointed to a new line of thinking: Ovaries are part of a system that promotes longevity (Waters, 2011). On the surface, this conclusion seems to contradict the above-mentioned stance because ovary removal, rather than ovary conservation, would promote protection against two notable health hazards. But using longevity as an end-point goes well beyond considering the incidence of a particular disease. *Longevity reflects both the incidence and mortality of every disease*—not just mammary cancer and pyometra—as well as the rate of aging, thereby representing a more integrated outcome measure of life-long health.

It would be reasonable to assert that if a particular disease condition—such as mammary cancer or pyometra—adversely impacts longevity, then reducing the incidence of that disease might merit serious consideration as a core principle of any wellness program intended to optimize overall longevity. Alternatively, it is possible that after one accounts for factors such as age at onset and competing causes of mortality, a diagnosis of mammary cancer or pyometra may not cut short life expectancy. To date, no such analysis of these trade-offs is available to guide informed decision-making.

A rapidly growing number of studies in the veterinary literature have explored prognostic factors in dogs diagnosed with mammary cancer, reporting the survival of cases after mammary cancer diagnosis (Chang et al., 2005; Matos et al., 2012; Santos et al., 2013). What is lacking are breed-specific analyses that compare the overall longevity of females that develop mammary cancer versus females who never develop the disease. As a first step toward addressing this knowledge gap, we studied the impact on longevity of mammary cancer and pyometra—the two disease conditions in pet dogs considered the most important health hazards associated with ovary conservation. By calculating time to death at specified ages throughout the life course, we estimated the extent to which females that were ever diagnosed with these two diseases experienced a longevity disadvantage compared to females that never developed these diseases. Here, we report the results of the first breed-specific life course analysis of the impact of these two

diseases on age-anchored life expectancy and premature mortality in 242 female Rottweilers.

Materials and methods

Study population and data collection

A historical cohort study was conducted of Rottweilers in the pet population living in North America (1994–2006). Information was collected from questionnaires mailed to 1500 owners of Rottweilers identified through Rottweiler specialty clubs. Only purebred dogs were eligible for study. With the assistance of a veterinarian, pet owners completed a 12 page questionnaire providing lifetime health and medical information. No incentives for participation were provided. Categories of information gathered included: geographical location of residence, purpose of dog ownership, general dog information (e.g. date of birth, age at neutering, vital status, date of death, cause of death); diet and dietary supplement usage; vaccination history and environmental exposures; health conditions confirmed by a veterinarian. Seven hundred thirty completed questionnaires were returned for evaluation, representing 389 females and 341 males. Fifty-five percent of these dogs were alive at the time of the original questionnaire. Follow-up telephone interviews were conducted to update relevant health information and to ascertain age at death. After completion, this procedure generated data on 261 females that were deceased. After excluding 19 of these females, the current report consists of an analysis of 242 female Rottweilers with age at death ranging from 4.6 to 12.9 years. Five females were censored from this analysis because their age at death was less than 4.3 years. This censoring enabled construction of a study sample in which all females lived a duration that equaled or exceeded the median duration of lifetime ovary exposure (4.3 years), which facilitated an analysis of how survival is influenced by ovary exposure and ovary-driven disease conditions. Fourteen females who lived longer than 12.9 years were excluded because the hypothesis generated by our previous investigation (Waters et al., 2009) on the relationship between lifetime ovary exposure and exceptional longevity in Rottweilers ≥ 13 years old motivated an analysis of females that did not achieve exceptional longevity. For each of the 242 females, the following information was available: duration of lifetime ovary exposure (age at ovariohysterectomy); checklist of 40 health conditions (13 cancer, 27 non-cancer related) which included age at diagnosis and clinical outcome of females diagnosed with mammary cancer or pyometra; age at death. Cause of death (or reason for euthanasia) was determined for 223 females based upon information provided by veterinarians and owners; information was insufficient to assign cause of death in 8% of females.

Ascertainment of mammary carcinoma and pyometra cases

A diagnosis of mammary carcinoma was reported in 19 females. In all cases, diagnosis was made by veterinary pathologists who examined mammary tissue specimens for the presence of characteristic features of malignant epithelial neoplasms. No histology slides were available for independent, centralized review. In each case, pathologic diagnosis was based on examination of tissue specimens obtained at the time of mammary tumor excision performed by primary care veterinarians or at necropsy. Two females with non-epithelial (mesenchymal) mammary cancer were not included among the 19 cases of mammary carcinoma. Cause of death was classified as attributable to mammary cancer if there was clinical evidence that the dog's death or the owner's decision to euthanase was directly related to local or distant mammary tumor disease confirmed by cytology, histology, or thoracic radiography.

A diagnosis of pyometra was reported in 16 females. All cases were confirmed at the time of surgical treatment (ovariohysterectomy). Cause of death was classified as attributable to pyometra if there was clinical evidence that the dog's death or euthanasia was directly related to pyometra complications within a 5-day perioperative period.

Statistical analysis

Data were handled and analyzed in SAS version 9.4. Statistical significance was defined as $P < 0.05$ and all tests were two-sided. Kaplan–Meier survival curves were constructed to analyze longevity differences between females with shorter versus longer lifetime ovary exposure (cut point, median exposure of 4.3 years) in the entire study sample and after exclusion of females who developed mammary carcinoma or pyometra. Survival curves were compared for difference using log rank test. Cox proportional hazards regression was used to estimate the difference in mortality risk between the two categories of ovary exposure.

Risk estimates for mammary carcinoma were made after stratification of females into four categories of ovary exposure: < 2.5 years; 2.5–4.9 years; 5.0–7.4 years; and ≥ 7.5 years. For each ovary exposure category, the number of cases with mammary carcinoma that developed per 100 mammary carcinoma-free years at risk was calculated and rate ratios and 95% confidence intervals (CI) were determined. Because the 79 females in the lowest ovary exposure category (< 2.5 years) did not include any cases of mammary carcinoma, females within the 2.5–4.9 years of ovary exposure category were used as the reference group

(risk = 1.0). To specifically test the proposition of Schneider et al. (1969) that additional ovary exposure after 2.5 years of age has no significant impact on lifetime mammary cancer risk, Cox proportional hazard regression using years of ovary exposure as a continuous variable was used to compare the extent to which the risk of mammary carcinoma increased per year of additional ovary exposure within the entire study sample versus the risk of mammary carcinoma in only those females that had ≥ 2.5 years ovary exposure.

Among females with mammary carcinoma, the median and 95% CI for each time-related measurement (i.e., age at diagnosis, duration of lifetime ovary exposure, age at death and length of post-diagnosis survival) were determined using Kaplan–Meier estimator. Mann Whitney *U* test was used to compare: median age at diagnosis, duration of lifetime ovary exposure, and age at death in females with or without mortality attributable to mammary cancer; lifetime ovary exposure between mammary carcinoma cases and females who never developed mammary carcinoma. To analyze the extent to which pyometra risk increased with increasing age, the number of pyometra cases that developed per 100 uterus-years at risk was determined for three categories of age at diagnosis: <3.9 years; 4.0–7.9 years; and ≥ 8.0 years. Rate ratios were calculated using the <3.9 years category as the reference group.

To determine the extent to which longevity was cut short in females that developed mammary carcinoma, age-anchored life expectancy of cases and non-cases were analyzed. To obtain non-arbitrary time points that would reflect the distribution of age at diagnosis during the life course, age-anchored life expectancy was calculated at four different time points corresponding to the age at which 25%, 50%, 75%, and 90% of mammary carcinoma cases were diagnosed in the study sample. For each anchor, life expectancy was defined as the median number of years of life remaining (i.e., median time to death), which was calculated by subtraction: age at death minus age at anchor. For each anchor, life expectancy and the CI of life expectancy for mammary carcinoma cases and non-cases were calculated using Kaplan–Meier estimator; *P* values were generated using Mann Whitney *U* test. Because life expectancy represents median time to event (death), the difference between life expectancy of cases versus non-cases represents the difference between two medians. The difference (in years) between age-anchored life expectancy in cases and non-cases was estimated using Hodges–Lehmann estimation of location shift, which provides a more robust estimate than is provided by arithmetic difference of two medians obtained by subtraction (Han, 2008). Using the Hodges–Lehmann method, a 95% confidence interval that includes zero indicates the difference in life expectancy between cases and non-cases is non-significant ($P > 0.05$). From this life course analysis, it could be determined whether females alive at a particular age who would ever be diagnosed with mammary cancer displayed a life expectancy disadvantage compared to females who would never be diagnosed with mammary cancer. To further explore the possibility of disease-specific longevity disadvantages, the impact of three other disease conditions—pyometra (a health hazard of sexually intact females) and appendicular bone sarcoma and lymphoma (selected because of their high case fatality)—on life expectancy anchored at the age at which 25%, 50%, 75%, and 90% cases were diagnosed in the study sample was calculated and compared to non-cases. To more directly compare the impact of these four disease conditions—mammary cancer, pyometra, appendicular bone sarcoma, lymphoma—differences in age-anchored life expectancy between cases and non-cases at 4, 6, 8, and 10 years of age were compared.

To further illustrate the relative impact of these four diseases on premature mortality, years of life lost (YLL) was calculated for each disease. Years of life lost provides a measure of premature mortality that can be used to describe the impact of deaths occurring at younger ages by comparing age at death of cases to an external standard of life expectancy (Fontaine et al., 2003; Brown et al., 2009; Carter and Nguyen, 2012). Two different cut points for premature death were used (premature mortality defined as death prior to 10 years or death prior to 9 years) because they represent the most reliable estimates of adult Rottweiler lifespan from this and other studies (Michell, 1999; Proschowsky et al., 2003; O'Neill et al., 2013). Using 10 years as the standard for life expectancy, YLL was calculated for each disease as the sum of YLL (10 minus age at death) contributed by each case whose death prior to age 10 years was attributable to that disease. Proportion of total YLL in the study sample attributable to each disease was expressed as a percentage, providing a description of the relative burden of each of the four diseases. For each of the four diseases, YLL per diagnosis (termed years of life lost per incident by Carter and Nguyen, 2012), a description of the average years of premature mortality per case, was calculated as years of life lost attributable to case fatality divided by number of diagnoses.

Results

Study population and Kaplan–Meier survival analysis

Pertinent life history and medical data for the study population are presented in Table 1. Median age at death for 242 female Rottweilers was 9.9 years (range 4.6–12.9, 95% CI 9.6–10.2). As expected, cancer was the most frequent cause of death in this

Table 1
Characteristics of study population.

Data	Result
Number of female Rottweilers	242
Number of households	187
Residence	
United States (38 states)	229 (94.6%)
Canada	13 (5.4%)
Age at death (years), median (range)	9.9 (4.6–12.9)
Lifetime ovary exposure (years), median (range)	4.3 (0.2–12.9)
Proportionate mortality, ^a (%)	
Cancer	64
Non-cancer	36
Gastrointestinal	6
Musculoskeletal	6
Neurological	6
Urogenital	5
Cardiovascular	4
Other	9

^a Proportionate mortality represents the percentage of deaths in the study population attributable to each category of disease. Cause of death (or reason for euthanasia) was ascertained for 223 females.

cancer-prone breed, accounting for 64% of total deaths. The most frequent causes of non-cancer deaths were: gastrointestinal (6%), neurological (6%), musculoskeletal (6%), urogenital (5%) and cardiovascular (4%) conditions. Less than 5% of females underwent necropsy and cause of death (or reason for euthanasia) could not be assigned in 8% of females. Overall, median lifetime ovary exposure was 4.3 years (range 0.2–12.9, 95% CI 3.8–5.0). Kaplan–Meier survival analysis showed that females with ≥ 4.3 years lifetime ovary exposure had a longevity advantage over females who had shorter ovary exposure (Fig. 1A). Longer ovary exposure was associated with a 33% decrease in mortality risk (hazard ratio [HR] 0.67; 95% CI 0.52–0.86), which translated into living an average of 17 months longer ($P = 0.002$). The percentage of females diagnosed with mammary carcinoma or pyometra—disease conditions associated with ovary exposure—was 7.9% and 6.6%, respectively. If these two ovary-driven conditions were associated with shortened longevity, exclusion of females with mammary carcinoma and pyometra from Kaplan–Meier survival analysis would be expected to increase (widen) the survival advantage associated with keeping ovaries longer. However, when females with mammary carcinoma and pyometra were excluded from survival analysis, no such widening was found (Fig. 1B). In fact, after exclusion of females with mammary cancer and pyometra, longer ovary exposure was associated with a slightly diminished reduction in mortality risk (32%). This unexpected result warranted a deeper analysis of the extent to which the development of these ovary-driven conditions was associated with an adverse impact on overall longevity.

Mammary carcinoma

Nineteen of 242 (7.9%) females were diagnosed with mammary carcinoma (a total of 22 carcinomas). Median age at mammary carcinoma diagnosis was 8.5 years (95% CI 7.3–9.7). Median age at death was 11.4 years (95% CI 10.6–12.0). Eighteen of 19 (95%) cases were treated with surgical excision of their tumors; a dog with pulmonary metastases at the time of mammary carcinoma diagnosis did not receive surgery. No dogs received cytotoxic chemotherapy. There were no cases of inflammatory carcinoma. Seven dogs died of mammary carcinoma. Case fatality (also called case fatality risk), defined as the proportion of females diagnosed with mammary carcinoma that had mortality attributable to their

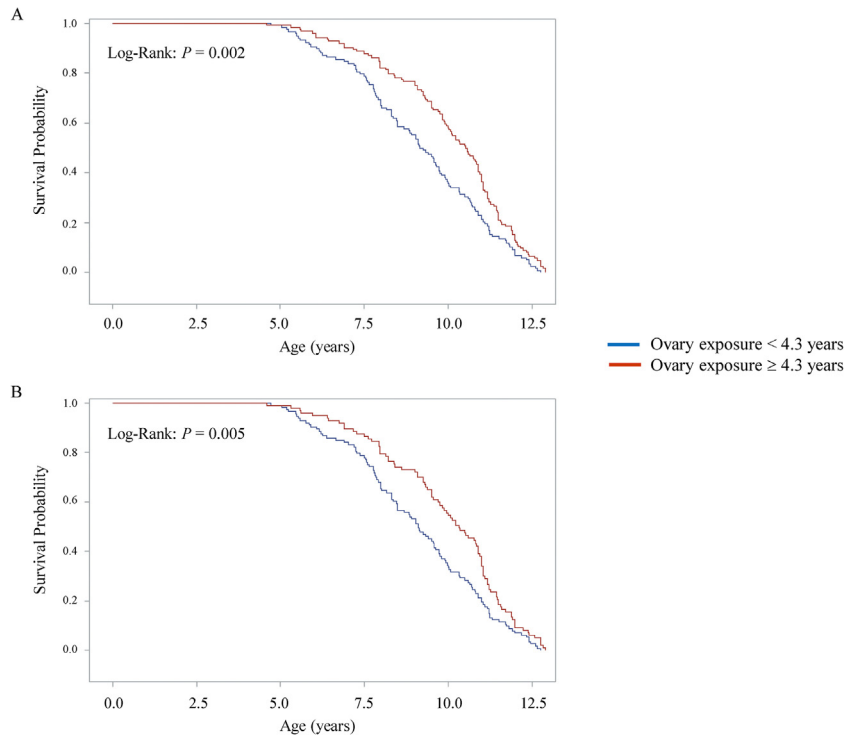


Fig. 1. Kaplan–Meier plot of the probability of survival comparing females with short versus long ovary exposure (cut-point, median ovary exposure 4.3 years) in: (A) entire study sample ($n=242$ females); and (B) subgroup of the study sample that never developed mammary carcinoma or pyometra ($n=210$ females).

mammary cancer (Kelly and Cowling, 2013), was 37%. Proportionate mortality, defined as the proportion of all deaths in the study population attributable to a particular disease, was 2.9% for mammary carcinoma. There was no evidence that females with earlier mammary carcinoma diagnosis had more lethal disease. Females with mammary cancer-related mortality had later age at diagnosis (median 10.0 years, 95% CI 7.3–11.9) and shorter survival (median 0.4 years, 95% CI 0.2–1.5) than females diagnosed with mammary carcinoma that died of other causes (median age at diagnosis 7.5 years, 95% CI 5.0–9.2, $P=0.03$; median survival 3.8 years, 95% CI 0.04–6.0, $P=0.05$). Age at death of females with mortality attributable to mammary carcinoma was not significantly different from the age at death for females with mammary carcinoma that died of other causes (median 10.7 years, 95% CI 10.0–12.2 vs. 11.7 years, 95% CI 9.8–12.1), respectively ($P=0.22$).

Lifetime ovary exposure, mammary carcinoma diagnosis, and mammary carcinoma mortality

Median duration of ovary exposure in dogs with mammary carcinoma was 6.3 years (95% CI 5.0–7.8), which was significantly longer than non-cases (4.0 years, 95% CI 3.5–4.7, $P<0.0001$). There

was no evidence that longer lifetime ovary exposure was associated with an accelerated onset of mammary carcinoma. The tertile of females with mammary carcinoma who had the shortest (<5.8 years) ovary exposure had a median age at mammary carcinoma diagnosis of 7.9 years (95% CI 2.5–10.3), compared to median age at diagnosis of 9.1 years (95% CI 6.0–9.8) in the tertile of females with the longest (>7.5 years) ovary exposure ($P=0.27$). Six females that were sexually intact at the time of mammary cancer diagnosis had median age at diagnosis of 7.5 years (95% CI 2.5–9.8) compared to 8.5 years (95% CI 7.6–10.0) in females that were spayed prior to mammary cancer diagnosis ($P=0.23$).

The risk for mammary carcinoma diagnosis stratified by lifetime ovary exposure is shown in Table 2. Ovary exposure of less than 2.5 years was associated with an apparent mammary cancer-sparing effect. None of the 79 females that were spayed during the first 2.5 years of life subsequently developed mammary carcinoma. Analysis of the number of mammary carcinoma cases developing per 100 dog-years at risk showed the annual risk for developing mammary cancer was low (0.82%) in females spayed at 2.5–4.9 years (four cases per 487 dog-years at risk) (Table 2). Compared to females with 2.5–4.9 years ovary exposure, rate ratios

Table 2
Lifetime ovary exposure and mammary carcinoma risk in 242 female Rottweilers.

Duration of ovary exposure (years)	Dogs with mammary carcinoma (n)	Dogs without mammary carcinoma (n)	Total dog-years ^a	Rate of mammary carcinoma per 100 dog-years (95% CI)	Rate ratio (95% CI)
Total cohort	19	223	2272	0.84 (0.53–1.31)	
Category					
<2.5	0	79	714	0	
2.5–4.9	4	50	487	0.82 (0.25–1.79)	1.0 (ref)
5.0–7.4	8	57	618	1.30 (0.73–2.50)	1.6 (0.5–5.2)
≥7.5	7	37	453	1.54 (0.63–3.66)	1.9 (0.6–6.4)

95% CI, 95% confidence intervals.

^a Represents the sum of mammary carcinoma-free years in cases and non-cases.

for mammary carcinoma in females with 5.0–7.4 years or ≥ 7.5 years ovary exposure were not significantly increased (Table 2). To directly test the prevailing proposition of Schneider et al. (1969) that additional ovary exposure after 2.5 years has no significant impact on lifetime mammary cancer development, Cox regression was used. In the entire sample, there was a significant 16% increase in risk for mammary carcinoma associated with each year of ovary exposure averaged throughout the life course (HR 1.16, 95% CI 1.02–1.34, $P=0.03$). However, when females with less than 2.5 years of ovary exposure were excluded, the risk for mammary carcinoma associated with longer ovary exposure was no longer significant (HR 1.09, 95% CI 0.92–1.30, $P=0.31$), supporting the proposition that additional ovary exposure after 2.5 years was not associated with a further increase in mammary cancer development.

With respect to mortality, there was no significant difference in lifetime duration of ovary exposure (median 6.5 years, 95% CI 4.9–8.0) in females with mammary carcinoma that had mortality attributable to their mammary cancer versus those cases that died of other causes (median 6.0 years, 95% CI 4.5–8.4) ($P=0.27$). All of the mortality attributable to mammary carcinoma was observed in females with ovary exposure of at least 4 years.

Life course analysis of the impact of mammary carcinoma on age-anchored life expectancy

Age-anchored life expectancy, defined as the median number of years of life remaining after a specified age, was calculated at four anchored time points during the life course, representing the age at which 25%, 50%, 75%, and 90% of mammary carcinoma cases were diagnosed. For the 19 cases of mammary carcinoma in this study population, these anchored time points corresponded to 7.3, 8.5, 9.7, and 10.3 years of age, respectively. Comparison of age-anchored life expectancy between 19 mammary carcinoma cases and 223 non-cases showed that at none of these time points during the life course was a mammary carcinoma diagnosis or the future diagnosis of mammary carcinoma associated with shortened life expectancy (Table 3). For example, at 8.5 years of age—the age at which 50% of mammary carcinomas had already been diagnosed—the life expectancy of alive females that would ever be diagnosed with mammary carcinoma at any time during their lifetime was 2.9 years, which was 7 months longer than the life expectancy of those females who would never be diagnosed with mammary

carcinoma ($P=0.048$). Overall, females that developed mammary carcinoma displayed a pattern of preservation of life expectancy compared to the overall study population, which is a result that would be expected for a late-onset disease with moderate (<50%) case fatality.

Pyometra

Sixteen of 242 (6.6%) females were diagnosed with pyometra. Median age at pyometra diagnosis was 5.4 years (range 2.4–10.2, 95% CI 3.5–6.4). Median age at death was 10.6 years (range 6.0–12.1, 95% CI 9.0–11.5). Among intact females, the annual risk for developing pyometra was very low (0.57%) during the first 4 years of life (four cases per 706 uterus-years at risk) then increased more than five-fold between 4 and 8 years of age (10 cases per 321 uterus-years at risk) resulting in a rate ratio (95% CI) of 5.5 (1.7–17.5) ($P=0.003$) (Table 4). Two cases of pyometra were diagnosed at ≥ 8 years among 31 females who were intact at 8 years of age (two cases per 51 uterus-years at risk; rate ratio 6.9, 95% CI 1.3–37.7) ($P=0.06$). All dogs diagnosed with pyometra underwent surgical treatment, consisting of ovariohysterectomy. One of 16 had perioperative mortality attributable to pyometra (7% case fatality). Overall, median survival of 16 females after the diagnosis of pyometra was 5.0 years (95% CI 1.9–6.3).

Life course analysis of the impact of pyometra on age-anchored life expectancy

The anchored time points for pyometra—the age at which 25%, 50%, 75%, and 90% of pyometra cases were diagnosed—were 3.8, 5.1, 6.4, and 7.0 years, respectively. Comparison of age-anchored life expectancy in 16 pyometra cases and 226 non-cases showed that at none of these time points during the life course was the diagnosis or the future diagnosis of pyometra associated with shortened life expectancy (Table 5). At 5.1 years—the Anchor₅₀ for pyometra—the life expectancy of alive females that would ever be diagnosed with pyometra during their lifetime was 5.5 years, which was 9 months longer than the life expectancy of those females who would never be diagnosed with pyometra ($P=0.32$). Overall, females with pyometra displayed a pattern of preservation of life expectancy compared to the overall study population, which

Table 3
Life course analysis of age-anchored life expectancy in mammary carcinoma cases versus non-cases.

	Time points (anchors) that reflect the distribution of age at diagnosis of mammary carcinoma in this study population ^a			
	Anchor ₂₅	Anchor ₅₀	Anchor ₇₅	Anchor ₉₀
Age at anchor (years)	7.3	8.5	9.7	10.3
Life expectancy in years ^b (CI)				
Case (<i>n</i>) ^c	4.0 (3.3–4.7) (19)	2.9 (2.1–3.5) (19)	1.8 (0.9–2.3) (18)	1.5 (0.3–1.7) (15)
Non-case (<i>n</i>)	2.8 (2.5–3.3) (186)	2.3 (1.9–2.5) (150)	1.4 (1.3–1.6) (114)	0.9 (0.8–1.2) (89)
Difference between cases and non-cases in years (CI) ^d	+1.1 (0.4–1.8)	+0.6 (0.01–1.2)	+0.2 (–0.3 to 0.8)	+0.2 (–0.3 to 0.7)
<i>P</i> ^e	0.003	0.048	0.38	0.52

95% CI, 95% confidence intervals.

^a Anchor₂₅, Anchor₅₀, Anchor₇₅, and Anchor₉₀ represent the age at which 25%, 50%, 75% or 90% of the 19 mammary carcinoma cases were diagnosed among 242 females in this study population.

^b Age-anchored life expectancy, calculated as the median number of years of life remaining after a specified age (i.e., median time to death) for mammary carcinoma cases and for non-cases who never developed mammary carcinoma. CI = 95% confidence intervals for life expectancy (median time to death) in mammary carcinoma cases and non-cases calculated using Kaplan–Meier estimator.

^c *n* = number of females alive at anchor point.

^d Difference and 95% CI between the life expectancy of cases and non-cases for each anchor was calculated using Hodges–Lehmann estimator of median differences. Estimates of the difference and confidence interval obtained using this method provide a more robust estimate than is provided by the subtraction of two medians. A difference in age-anchored life expectancy with positive value indicates that cases have longer life expectancy than non-cases, i.e. no survival disadvantage is observed among mammary carcinoma cases. A 95% confidence interval that includes zero indicates a non-significant difference in age-anchored life expectancy between cases and non-cases ($P>0.05$).

^e For each anchor, *P* values calculated by comparison of life expectancy of cases versus non-cases using Mann Whitney *U* test.

Table 4
Relationship between age and pyometra risk in 242 female Rottweilers.

Age at diagnosis (years)	Dogs with pyometra (n)	Dogs without pyometra (n)	Total uterus-years ^a	Rate of pyometra per 100 dog-years (95% CI)	Rate ratio (95% CI)
Total cohort	16	226	1078	1.48 (0.91–2.42)	
Category					
<3.9	4	238	706	0.57 (0.21–1.51)	1.0 (ref)
4.0–7.9	10	121	321	3.12 (1.68–5.79)	5.5 (1.7–17.5)
≥8.0	2	29	51	3.92 (0.98–15.65)	6.9 (1.3–37.7)

95% CI, 95% confidence intervals.

^a Represents sum of years at pyometra risk contributed by females with intact uterus in cases and non-cases.

Table 5
Life course analysis of age-anchored life expectancy in pyometra cases versus non-cases.

Age at anchor (years)	Time points (anchors) that reflect the distribution of age at diagnosis of pyometra in this study population ^a			
	Anchor ₂₅	Anchor ₅₀	Anchor ₇₅	Anchor ₉₀
Life expectancy in years ^b (CI)				
Case (n) ^c	6.8 (5.2–7.7) (16)	5.5 (3.9–6.4) (16)	4.3 (3.3–5.4) (14)	3.7 (2.7–4.8) (14)
Non-case (n)	6.0 (5.7–6.4) (226)	4.7 (4.4–5.1) (223)	3.7 (3.4–4.2) (205)	3.2 (2.8–3.7) (198)
Difference between cases and non-cases in years (CI) ^d	+0.6 (–0.4 to 1.5)	+0.5 (–0.4 to 1.5)	+0.7 (–0.2 to 1.5)	+0.6 (–0.3 to 1.4)
P ^e	0.28	0.32	0.14	0.19

CI, confidence intervals.

^a Anchor₂₅, Anchor₅₀, Anchor₇₅, and Anchor₉₀ represent the age at which 25%, 50%, 75% or 90% of the 16 pyometra cases were diagnosed among 242 females in the study population.

^b Age-anchored life expectancy, calculated as the median number of years of life remaining after a specified age (i.e., median time to death) for pyometra cases and for non-cases who never developed pyometra. CI = 95% confidence intervals for life expectancy (median time to death) in pyometra cases and non-cases calculated using Kaplan–Meier estimator.

^c n = number of females alive at anchor point.

^d Difference and 95% CI between the life expectancy of cases and non-cases for each anchor was calculated using Hodges–Lehmann estimator of median differences. Estimates of the difference and confidence interval obtained using this method provide a more robust estimate than is provided by subtraction of two medians. A difference in age-anchored life expectancy with positive value indicates that cases have longer life expectancy than non-cases, i.e. no survival disadvantage is observed among pyometra cases. A 95% confidence interval that includes zero indicates a non-significant difference in age-anchored life expectancy between cases and non-cases ($P > 0.05$).

^e For each anchor, P values calculated by comparison of life expectancy of cases versus non-cases using Mann Whitney U test.

is a result that would be expected for a disease condition with low (<10%) case fatality.

Life course analysis of four disease conditions: Comparison of mammary carcinoma and pyometra with the impact of appendicular bone sarcoma and lymphoma on age-anchored life expectancy and years of life lost (YLL) per diagnosis

Because age-anchored life expectancy for females with mammary carcinoma and pyometra failed to show shortened survival versus non-cases over the life course, we expanded our analysis to include two other age-related disease conditions. We selected two conditions that commonly affect Rottweilers and are typically associated with high (≥90%) case fatality. Comparison of age-anchored life expectancy in 61 females with appendicular bone sarcoma and 20 females with lymphoma versus non-cases indicated that both diseases were associated with shortened life expectancy (Table 6). Life expectancy was shortened by 9 months (25%) in females with appendicular bone sarcoma at Anchor₂₅ ($P = 0.001$) and shortened by 24 months (44%) in females with lymphoma at Anchor₂₅ ($P = 0.002$). A clear difference could be seen in the direction of the impact of the four disease conditions on age-anchored life expectancy (Fig. 2). The figure displays the difference in age-anchored life expectancy between cases and non-cases for the four disease conditions at 4, 6, 8, and 10 years of age, providing a more direct comparison of the directionality and magnitude of the impact that each disease had on life expectancy over the life-course. Statistically significant reductions in life expectancy were found for appendicular bone sarcoma at age 6 and 8 years

(reduction of 8 months and 9 months, respectively), and for lymphoma at age 4 and 6 years (reduction of 22 months and 15 months, respectively). Females with mammary cancer and pyometra showed no such longevity disadvantage (Fig. 2).

To illustrate further the relative longevity-shortening force of these four diseases, we estimated the years of life lost (YLL) associated with premature death (prior to 10 years of age) attributable to each disease (Table 7). The longevity-shortening impact of appendicular bone sarcoma and lymphoma was strong, accounting for 27% and 18% of the total YLL in the study sample, respectively. Years of life lost per diagnosis—a measure independent of incidence rate or the timing of deaths due to other causes—was calculated using two different cut points to define premature mortality: death prior to 9 years; or death prior to 10 years. Comparing the results from these two assumptions, the relative impact of the four diseases on premature mortality per case remained consistent (Figs. 3A and B). For deaths prior to 10 years (Fig. 3B), YLL per diagnosis of appendicular bone sarcoma and lymphoma were 1.10 years and 2.22 years, respectively. Years of life lost per diagnosis for pyometra was only 0.25 years. Although 84% of the mammary carcinoma cases had been diagnosed prior to 10 years of age, no deaths attributable to mammary carcinoma were observed during the first 10 years of life.

Discussion

A growing imperative to find and implement effective strategies to promote longevity prompted this line of inquiry. While innovative efforts will continue to focus on finding new agents

Table 6

Life course analysis of age-anchored life expectancy in cases of appendicular bone sarcoma and lymphoma versus non-cases.

	Time points (anchors) that reflect the distribution of age at diagnosis of cases within this study population ^a			
	Anchor ₂₅	Anchor ₅₀	Anchor ₇₅	Anchor ₉₀
Appendicular Bone Sarcoma				
Age at anchor (years)	7.6	9.0	10.0	11.2
Life expectancy in years ^b (CI)				
Case (n) ^c	2.1 (1.5–2.8) (52)	1.6 (0.8–1.9) (35)	1.2 (0.7–1.5) (22)	0.4 (0.1–0.6) (11)
Non-case (n)	3.2 (2.6–3.4) (147)	2.0 (1.8–2.2) (125)	1.2 (1.0–1.5) (94)	0.8 (0.7–1.1) (48)
Difference between cases and non-cases in years (CI) ^d	–0.8 (–1.3 to –0.3)	–0.4 (–0.8 to 0.01)	–0.3 (–0.6 to 0.2)	–0.4 (–0.7 to –0.04)
<i>P</i> ^e	0.001	0.06	0.24	0.03
Lymphoma				
Age at anchor (years)	5.5	7.0	10.0	10.6
Life expectancy in years (CI)				
Case (n)	1.8 (0.6–5.3) (20)	1.8 (0.3–4.2) (13)	1.2 (0.8–2.6) (6)	0.6 (0.2–2.0) (6)
Non-case (n)	4.5 (4.2–5.0) (215)	3.3 (2.9–3.7) (199)	1.2 (1.0–1.5) (110)	0.9 (0.6–1.1) (90)
Difference between cases and non-cases in years (CI)	–2.0 (–3.1 to –0.7)	–0.8 (–2.1 to 0.3)	0.1 (–0.6 to 0.7)	–0.2 (–0.8 to 0.4)
<i>P</i>	0.002	0.12	0.86	0.50

CI, confidence intervals.

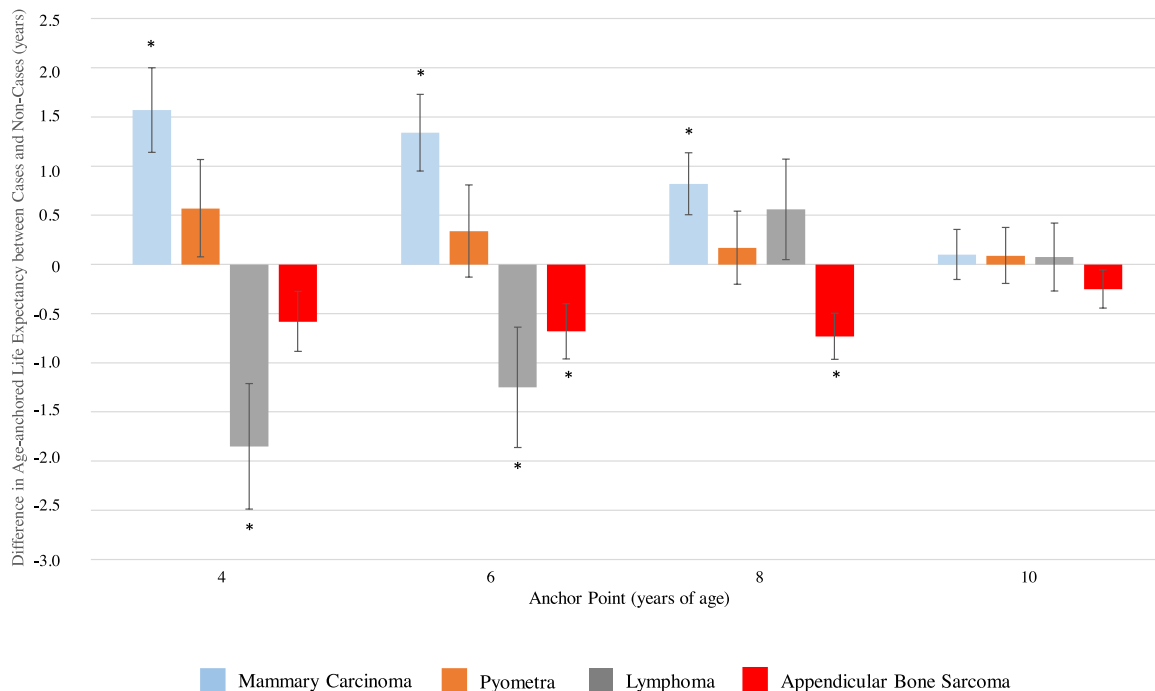
^a Anchor₂₅, Anchor₅₀, Anchor₇₅, and Anchor₉₀ represent the age at which 25%, 50%, 75% or 90% of the 61 appendicular bone sarcoma and 20 lymphoma cases were diagnosed among 242 females in the study population.^b Age-anchored life expectancy, calculated as the median number of years of life remaining after a specified age (i.e., median time to death) for cases and for non-cases who never developed the disease condition. CI = 95% confidence intervals for life expectancy (median time to death) in cases and non-cases calculated using Kaplan–Meier estimator.^c *n* = number of females alive at anchor point.^d Difference and 95% CI between the life expectancy of cases and non-cases for each anchor was calculated using Hodges–Lehmann estimator of median differences. Estimates of the difference and confidence interval obtained using this method provide a more robust estimate than is provided by subtraction of two medians. A difference in age-anchored life expectancy with negative value indicates that cases have shorter life expectancy than non-cases, i.e. a survival disadvantage is observed among cases of appendicular bone sarcoma and lymphoma. A 95% confidence interval that includes zero indicates a non-significant difference in age-anchored life expectancy between cases and non-cases ($P > 0.05$).^e For each anchor, *P* values calculated by comparison of life expectancy of cases versus non-cases using Mann Whitney *U* test.**Fig. 2.** Difference in age-anchored life expectancy of cases of mammary carcinoma, pyometra, lymphoma, and appendicular bone sarcoma versus non-cases. Age-anchored life expectancy was calculated for specified time anchors: 4 years, 6 years, 8 years, and 10 years of age. The height of each bar in the histogram indicates the difference in age-anchored life expectancy between cases and non-cases expressed in years. Each bar in the histogram that extends below the horizontal line (labelled 0) depicts a negative point estimate for the difference in age-anchored life expectancy between cases and non-cases indicating that cases have shorter life expectancy, i.e., cases have a survival disadvantage. Note that at no time during the life course is there a negative point estimate for mammary carcinoma or pyometra (no histogram bars appear below the horizontal line). An asterisk (*) denotes a significant difference in age-anchored life expectancy between cases and non-cases, indicating the disease and age at which Hodges–Lehmann estimator of difference yields a 95% confidence interval that does not include zero ($P < 0.05$). Error bars represent \pm standard error of the point estimate.

Table 7
Years of life lost (YLL) associated with premature mortality (death prior to 10 years of age) attributable to mammary carcinoma, pyometra, lymphoma, and appendicular bone sarcoma in 242 female Rottweilers.

	(1) Number of diagnoses	(2) Proportion of deaths prior to 10 years	(3) Proportion of premature deaths with death attributable to case fatality	(4) Years of life lost (YLL) attributable to case fatality ^a	(5) YLL per diagnosis ^b	(6) Proportion of total YLL in study sample attributable to case fatality ^c (%)
Mammary carcinoma	19	2/19	0/2	0	0	0
Pyometra	16	7/16	1/7	4.0	0.25	2
Appendicular bone sarcoma	61	38/61	36/38	67.5	1.10	27
Lymphoma	20	14/20	14/14	44.4	2.22	18

^a For each disease, years of life lost (YLL) attributable to case fatality equals the sum of YLL (10 minus age at death) contributed by each case whose death prior to age 10 was attributed to that disease.

^b Years of life lost per diagnosis was calculated for each disease as the sum of years of life lost (YLL) attributable to case fatality (column 4) divided by number of cases diagnosed (column 1).

^c Calculated for each disease as the sum of years of life lost (YLL) to case fatality (column 4) divided by 251, which represents the total number of YLL contributed by 126 females that experienced premature mortality (death prior to 10 years of age).

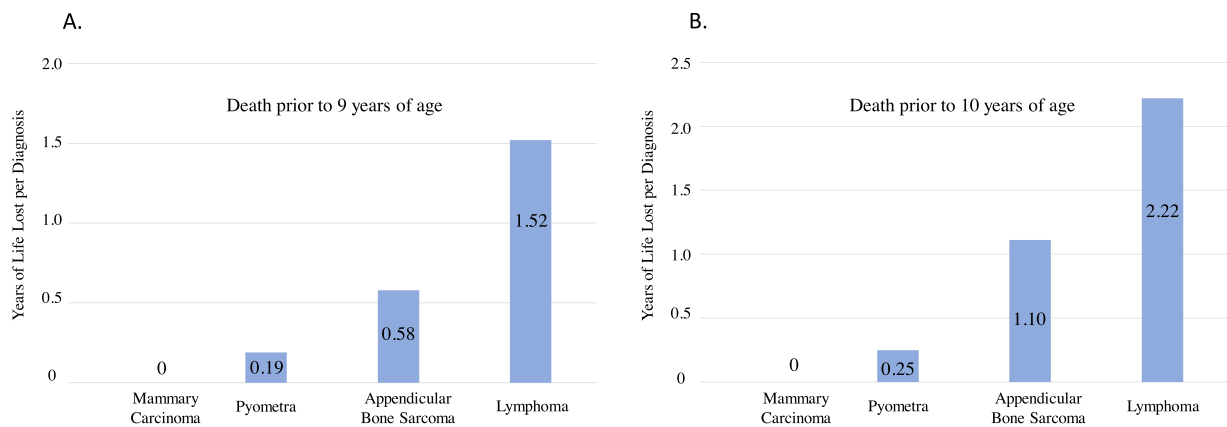


Fig. 3. Comparison of years of life lost (YLL) per diagnosis attributable to mammary carcinoma, pyometra, appendicular bone sarcoma, and lymphoma among 242 female Rottweilers. Years of life lost per diagnosis was calculated using two different cut points to define premature mortality: death at 9 years of age (Fig. 3A) or death at 10 years of age (Fig. 3B). For each disease, YLL per diagnosis was calculated as the sum of years of life lost attributable to case fatality divided by number of cases diagnosed.

and approaches that will combat aging, previous reports have not adequately documented the life-long health consequences of elective ovariohysterectomy, a current intervention widely practiced by veterinarians in North America. The statement ‘Ovariohysterectomy can lower the risk or even prevent two bad diseases’, may accurately sum up a prevailing belief among some clinicians, but offers no insight into the overall impact that removing or conserving ovaries has on longevity. Here, we penetrate further the longevity consequences of ovariohysterectomy by conducting the first life course analysis of the impact of mammary cancer and pyometra on age-anchored life expectancy. We show that at no time during the life course does the current or future diagnosis of these two health conditions shorten subsequent survival compared to females who never developed these diseases. These findings fail to support the notion that a strategy to avoid the development of mammary cancer or pyometra will beneficially impact overall longevity in pet dogs. Moreover, as we look to design strategies that will optimize longevity, this study points to the need for a new kind of thinking: *whole organism thinking*—a careful considering of the biological trade-offs that are likely induced by all interventions that impact health (Waters, 2014). This re-envisioning will redirect emphasis away from solely studying the incidence of a particular disease and toward understanding the influence that the age at onset and mortality attributable to each disease exerts on overall longevity.

The main purpose of this report was to evaluate the impact of mammary cancer and pyometra on longevity. For mammary cancer, we compared the longevity of females who developed mammary carcinoma versus those females who never developed the disease in a single-breed cohort, rather than studying a hodgepodge of different breeds, so that we might make surer interpretations of results that hinge upon breed-specific lifespan, disease occurrence, age at onset and case fatality risk, and other competing causes of mortality in dogs at different ages. We limited our mammary cancer cases to females diagnosed with mammary carcinoma—excluding benign neoplasms and malignant mesenchymal tumors—in an attempt to reduce the heterogeneity inherent in studying canine mammary neoplasms (Goldschmidt et al., 2011; Im et al., 2014). We evaluated life expectancy of females alive at different time points during the life course, selected at the age at which 25%, 50%, 75%, and 90% females were diagnosed with mammary cancer. This enabled us to evaluate whether the association between mammary cancer and life expectancy changed over the life course using non-arbitrary anchors that reflected the distribution of age at diagnosis of mammary carcinoma over the life course (Ricklefs and Cadena, 2007). This careful attention to life course perspective (Waters and Kariuki, 2013; Waters, 2014) was motivated by results from human studies suggesting that, when it comes to health questions as divergent as the influence of obesity on all-cause mortality (Lee et al., 2012) or

the association between high serum cholesterol and dementia (Kivipelto et al., 2001; Mielke et al., 2005; Kivipelto et al., 2006; Zuliani et al., 2010), the direction of impact of a given factor on health outcome may flip-flop during the life course, i.e., shifting from detrimental to beneficial with advancing age. Here, for mammary cancer, our analysis showed a consistent direction of impact—at no time during the life course was a diagnosis of mammary cancer associated with shortened life expectancy. Further analysis, evaluating age-anchored life expectancy of females alive at the anchors of 4, 6, 8, and 10 years of age, confirmed this result. In this cohort, females that developed mammary carcinoma had *superior survival*—age-anchored life expectancy that actually exceeded females who never developed this disease, with mammary carcinoma cases enjoying more than 12 months of life expectancy advantage at 4 and 6 years of age.

To our knowledge, this work provides the first life-long analysis of the impact of mammary carcinoma on longevity in female dogs. It may be instructive to situate these new results within the context of a much larger study of mammary tumors conducted in a population of more than 80,000 insured female dogs in Sweden published more than a decade ago (Egenvall et al., 2005). That study reported data on mammary tumor incidence, survival after mammary tumor diagnosis, and proportion of deaths attributable to mammary tumors (i.e., proportionate mortality) in a study sample of females from age 3 years up to 10 years. The vast majority of mammary tumor diagnoses occurred in older dogs. Swedish investigators reported that 93% of female Rottweilers were not affected by mammary tumors until after age eight; in our study sample, 96% of Rottweilers were free of a diagnosis of mammary carcinoma during the first 8 years of life. In the Swedish study, when all breeds were combined, time to death after mammary tumor diagnosis was shorter than in females that died by age 10 without a mammary tumor diagnosis. However, estimates of the impact of these mammary tumors on overall longevity were not obtainable because more than 50% of females with mammary tumors were alive at 10 years of age, therefore age at death and age-anchored life expectancy could not be ascertained. Interestingly, breed-specific analysis showed the Rottweiler breed had significantly higher mortality after mammary tumor diagnosis compared to other breeds. This finding may indicate that Rottweilers are more prone to developing biologically aggressive (i.e., malignant) mammary tumors than other breeds. At least one report (Itoh et al., 2005) supports the notion that large breeds that develop mammary tumors may have a poorer prognosis than small breeds. Unfortunately, in the Swedish study, mammary tumor diagnoses were not distinguished as benign or malignant tumors and no information was available on treatment. Although most bitches in Sweden have intact ovaries for most of their lifetime—approximately 7% of insured female dogs in that country undergo ovariectomy during the life course (Egenvall et al., 1999)—the proportion of deaths attributable to mammary tumors was still very low. Overall, the proportion of deaths attributable to mammary tumors was 2.5% in the Swedish study, which parallels the 2.9% proportionate mortality attributable to mammary carcinoma that we report here.

The generalizability of our results on the life expectancy consequences of mammary cancer to other populations rests on the reliability of our estimates of: (1) age at mammary carcinoma diagnosis; and (2) case fatality, i.e., the proportion of mammary cancer cases that experience death attributable to their mammary cancer. In our study sample of Rottweilers, median age at diagnosis was 8.5 years and case fatality was 37%. Data from the literature on breed-specific age at mammary cancer diagnosis and mortality attributable to mammary cancer are surprisingly limited (Im et al., 2014), but the figures we report here for these two critical cancer characteristics are consistent with previous reports across

different pure breeds. Median age reported for canine mammary cancer diagnosis usually ranges from 8 to 10 years (Hellmén et al., 1993; Salas et al., 2015; Vascellari et al., 2016). Case fatality for mammary cancer ranges from 18% to 63% in previous reports (Schneider et al., 1969; Misdorp and Hart, 1976; Allen and Mahaffey, 1989), which closely parallels the 95% CI of the 37% case fatality for the mammary cancers in our study (19–59%). With respect to the particular subset of mammary neoplasms diagnosed as mammary carcinoma, 42% case fatality was reported in 60 female dogs (28 different breeds) after surgical excision and 20 months follow-up in a recent randomized clinical trial (Kristiansen et al., 2016). Case fatality for mammary carcinoma was also 42% after surgical excision in 69 female dogs (27 different breeds) with mammary carcinoma in the Purdue Comparative Oncology Program's tumor registry (D.K. Allen, unpublished data). Taken together, the results from our study and from the literature support the notion that canine mammary cancer is a late-onset disease with moderate case fatality. For this reason, we believe our work provides a perspective on the impact of mammary carcinoma on longevity that may have important implications for other canine populations, including other breeds.

A full interpretation of the longevity-shortening force of any disease, including mammary cancer, also depends on the age structure and mortality of the population, i.e., the age at which individuals die of competing causes. It is conceivable that our failure to detect a longevity disadvantage among mammary cancer cases might indicate that the Rottweilers we studied had a lower than expected average age at death due to competing mortalities, which would not have provided the necessary time window to allow a sufficient number of mammary cancers to develop and shorten life expectancy. However, the lack of longevity disadvantage in mammary cancer cases that we report here was observed in female Rottweilers with a median age at death of 9.9 years, which equals or exceeds the average age at death for Rottweilers in other studies (9.8 years, $n=101$ dogs, [Michell, 1999](#); 9.0 years, $n=98$ dogs, [Proschowsky et al., 2003](#); and 8.0 years, $n=105$ dogs, [O'Neill et al., 2013](#)). Thus, this critical aspect of our study sample seems well-suited for our research aim—to critically evaluate the longevity-shortening impact of mammary cancer and pyometra.

Similar to our results with mammary cancer, we found no evidence in our age-anchored analysis that pyometra cuts short life expectancy at any age throughout the life course. This pattern is consistent with the behavior of a disease with low case fatality. The median age at diagnosis of pyometra in our study was 5.4 years, which is similar to the median age at pyometra diagnosis of 6.5 years reported in Rottweilers in a Swedish study (Egenvall et al., 2001), and the average age at diagnosis for Labrador retrievers and Golden retrievers reported by [Hart et al. \(2014\)](#), which was 5.5 years and 6.4 years, respectively. Among females diagnosed with pyometra, the low case fatality (7%) in our study sample is similar to the case fatality of pyometra reported across different pure breeds—1–5% of deaths in pyometra cases were attributable to perioperative complications ([Wheaton et al., 1989](#); [Gibson et al., 2013](#); [Jitpean et al., 2014](#)) and up to 10% of cases were euthanased without surgery due to comorbid conditions ([Jitpean et al., 2014](#)). Taken together, it suggests that canine pyometra is a disease with low case fatality, even in breeds such as the Rottweiler that appear more prone to developing the disease (Egenvall et al., 2001). It is plausible that a disease with low case fatality might still be associated with an overall survival disadvantage if the genetic and environmental factors that combine to trigger the disease also combine to trigger other adverse health issues that might cause premature death due to other causes ([Capocaccia et al., 2015](#)). But the results of this first life course analysis of life expectancy in canine pyometra do

not support the notion that such triggers translate into a disadvantage in overall longevity in females with pyometra compared to females that never develop the disease.

After failing to find evidence that two health hazards associated with ovary conservation—mammary cancer and pyometra—adversely influence life expectancy during the life course, we questioned whether these null results in some way reflected an inability of our method to detect disease-specific longevity disadvantages in this cohort. To test this possibility, we conducted an analysis of age-anchored life expectancy of females that developed two other disease conditions—appendicular bone sarcoma, lymphoma—selected because of their high ($\geq 90\%$) case fatality (Withrow et al., 2012). We showed that females with these disease conditions had an up to 40–50% shortening of age-anchored life expectancy, with differences from non-cases reaching statistical significance at Anchor₂₅ for both diseases. These results added confidence that our main result—the absence of an adverse effect of mammary cancer or pyometra on overall longevity—did not reflect an artifactual shortcoming in our method. Interestingly, with advancing age, the impact of lymphoma on life expectancy flip-flopped, switching from a significant life-shortening at age 4 and 6 years to a trend toward a longevity advantage at 8 years of age. Whether or not this change in directionality represents important differences in the etiology and biological behavior of early-onset versus late-onset lymphomas should be explored.

It is expected that the incidence of ovary-driven conditions may vary considerably between populations depending upon duration of lifetime ovary exposure. Thus, we made no attempt here to estimate a ‘true’ incidence rate for mammary carcinoma or pyometra. Instead, we focused our efforts on *estimating the relative lethality of these diseases*. Our research strategy—the focused interrogation of a sample containing complete data on age at death—enabled us to determine age-anchored life expectancy and years of life lost per diagnosis. These two measures provide an estimate of the force of a disease on life-expectancy and premature mortality *on a per case basis*, which is not affected by incidence rate. Thus, if the incidence of mammary cancer or pyometra were 50% lower or two-fold higher, it would not have altered our estimates of the impact that each diagnosed case exerts on life expectancy or average years of life lost. The information the two measures provide are complementary. Age-anchored life expectancy estimates the average remaining years of life for each case, regardless of cause of death. Years of life lost per diagnosis describes the premature mortality attributable to case fatality, not case mortality due to other causes (Brown et al., 2009; Carter and Nguyen, 2012; Thiam et al., 2016). Years of life lost may offer valuable opportunities to model assumptions about important diseases (e.g., the impact of lowering case fatality, delaying age of disease onset, or varying the proportion of cases euthanased at the time of diagnosis), which might inspire fresh insights into the possible longevity consequences of current health practices and future interventions designed to reduce premature mortality (Burnet et al., 2005; Carter and Nguyen, 2012). Because the aim of this study was to critically evaluate the impact of age-related diseases on longevity, an age at death of 4 years was considered to be a reasonable lower boundary to study the longevity consequences of mammary cancer and pyometra. Conditions that may significantly contribute to early-life mortality—poisonings, road traffic accidents, neonatal infectious diseases, behavioral issues—were not the focus of this analysis. Although conditions associated with early-life mortality would contribute to the *total* years of life lost (i.e., the total burden of disease within a population), the calculation of the two measures used here to describe the longevity-shortening impact of mammary cancer and pyometra were not distorted by their exclusion.

Our openness to re-thinking the relationship between two ovary-driven diseases and longevity was a logical outgrowth of our work on the biology of exceptional longevity. In a previous study of 83 female Rottweilers who reached exceptional longevity—living at least 13 years, which represents more than 30% longer than average for this breed (Michell, 1999; Proschowsky et al., 2003; O’Neill et al., 2013)—keeping ovaries longer was associated with a longevity advantage (Waters et al., 2009). To determine whether this clue obtained from a group of Rottweilers with highly successful aging might be a biological signal operational in members of this breed with more typical longevity, we re-tested the association between years of ovary exposure and longevity in 242 females that lived up to 12.9 years (i.e., none of the females in this study sample reached exceptional longevity). Females with longer ovary exposure (≥ 4.3 years) had a statistically significant 17 months longevity advantage over females with shorter ovary exposure. Reconciling the notion that keeping ovaries longer increases the development of both mammary cancer and pyometra in this study population, but also promotes longevity might seem counterintuitive, even problematic. It is not. The concept of whole organism thinking predicts that any intervention—including the decision to remove or conserve ovaries—would be associated with biological trade-offs (Waters, 2014). Seen through the lens of whole organism thinking, it may be concluded that the beneficial effects of ovary conservation on longevity in this study cohort outweighed any detrimental effects. It should be noted that there are other considerations that figure into the decision by pet owners to pursue elective ovariohysterectomy that are not addressed by our study, such as limiting overpopulation of unwanted dogs, and other behavioral and quality of life issues. For certain, broader dialogues concerning optimal timing and techniques of sterilization are warranted. But results from focused studies such as this one can provide essential starting points to launch such dialogue. Our findings here that the two diseases considered to be the major health hazards of ovary conservation—mammary cancer and pyometra—are not associated with shortened longevity, situates use of whole organism thinking as all the more prescient as we take further steps toward understanding the physiological trade-offs provoked by elective endocrine organ removal.

As part of a separate but related line of inquiry, we also evaluated the association between lifetime ovary exposure and the risk of developing mammary cancer. Our motivation for this was the systematic review by Beauvais et al. (2012), which called into question the strength of evidence supporting a significant relationship between age at ovariohysterectomy and the development of mammary cancer. In contrast to many other canine study populations in North America (for example the Veterinary Medical Databases), our study cohort was uniquely suited for this sort of investigation because data on the number of years of lifetime ovary exposure were available for each subject. In agreement with previous work by Schneider et al. (1969), our results suggest there may be a critical time window for the protective effect of ovariohysterectomy on mammary cancer risk. We found a strong protective effect of ovariohysterectomy in young females—no cases of mammary carcinoma developed in 79 females spayed within the first 2.5 years of life. Schneider et al. (1969) reported that spaying after 2.5 years of age had no significant impact on subsequent mammary cancer risk, a proposition which remains under considerable debate. In our study sample, we could not demonstrate that additional ovary exposure after 2.5 years was associated with a further increase in mammary cancer development. But, the heterogeneity of canine mammary tumors should inform us that few conclusions fit neatly into all-or-none compartments. Therefore, future work should determine whether later-life ovary exposure significantly affects the susceptibility of particular bitches to develop

mammary cancer, or impacts the growth or metastasis of a subset of resultant tumors (Kristiansen et al., 2016).

Study results must always be interpreted within the context of certain methodological considerations, such as selection of study sample, the manner in which data are collected, risk for case misclassification, and whether cases receive unusual medical surveillance or care. Dogs in this study were owned by individuals who were motivated to complete a health questionnaire, and so the possibility should be considered that the study sample reported here might suffer from selection bias that would limit the external validity (generalizability) of results (Sedgwick, 2013). For example, it is likely that participating Rottweiler owners were particularly concerned about cancer as a major life-threatening disease of their breed, which may explain the high proportion of deaths in this study cohort attributable to cancer. If this is true, then it seems more likely that the observed frequency of mammary cancers would *overestimate*, rather than underestimate, any adverse impact of mammary cancer on longevity in this breed. Moreover, with regard to the quality of information collected on three key variables that would be expected to strongly influence the main inferences drawn from this work (i.e., age at diagnosis, case fatality, and age at death), it is difficult to envision that owners who participated would either preferentially own or report females with late- rather than early-onset mammary cancers, or preferentially report cases of pyometra that did not result in death, or preferentially inflate the life expectancy of females with mammary cancer or pyometra when participants were not informed that the age at onset or lethality of these conditions were under study. That our estimates of age at diagnosis and case fatality of mammary cancer and pyometra reported here are in such close agreement with previous reports in the literature further supports this conclusion. Consequently, we have every reason to believe that the pet owners who participated in this study are likely to be representative of those who would be motivated to explore best practices to optimize the healthy longevity of their pets.

Although previous work has shown the feasibility of using questionnaires to generate reliable data on exposures and disease outcomes in pet dogs (Glickman et al., 1989, 2000), there is always potential for misclassification. One can envision this to be particularly problematic for certain disease conditions, such as hemangiosarcoma of the spleen, in which ascertaining a diagnosis of an intracavitary cancer process can be more challenging, leading to non-specific diagnoses (i.e., ‘abdominal mass’) and outcome underreporting. In contrast, we targeted for careful study mammary cancer and pyometra—two disease conditions that are at much lower risk for misclassification. Cases of mammary cancer and pyometra undergo treatment with surgical procedures requiring general anesthesia, which are noted in medical records and readily recalled by owners. Moreover, the effects of underreporting or exclusion of cases are buffered by our use of measures of life expectancy and premature mortality that estimate the longevity disadvantage of these conditions on a per case basis, independent of any imprecision in the estimating of true incidence rates. Further, in our study sample, all cases of mammary cancer and pyometra were diagnosed and treated by primary care veterinarians, not specialists—lowering the likelihood that a high proportion of cases received ‘elite’ care that might bias their longevity outcome.

Finally, the strengths and limitations of a single breed study should be considered. We conducted a study of a single breed because single-breed studies enable investigators to make observations relevant to exposures and outcomes within a more homogeneous set of variables relevant to health, such as body size, risk for particular diseases, rates of disease progression, and expected life span. We reasoned that navigating a less heterogeneous

physiological context could expand our understanding of the interplay between aging and disease, creating opportunity to generate new hypotheses based upon clearer signals. To gain clearer signals on potential factors that impact disease resistance and susceptibility in humans, researchers have capitalized on the study of homogeneous populations, rather than general populations. For example, the relationship between BRCA-2 gene mutations and breast cancer susceptibility in women was a hypothesis fueled by observations of Icelandic women, then later translated from this relatively isolated subset of women to include women of other ethnicities and geographical locations throughout the world (Tryggvadottir et al., 2006). Recognizing the tremendous morphological diversity that exists across breeds of the canine species, it is altogether reasonable to question the extent to which the results from a single breed are translatable to other breeds (Runge et al., 2010). But this questioning is really no different than questioning whether the results of any health study can yield accurate predictions for other individuals—even members of the same breed—who were not involved in that particular study. Each clinician will have to decide whether the results of this study of competing causes of mortality in Rottweilers are relevant to members of other breeds that share similar body size and overall disease spectrum, and thus share a similar set of mobility-threatening orthopedic diseases and a preponderance of mesenchymal and lymphoid (rather than epithelial) neoplasms. With regard to interpreting the data presented here, clinicians should consider that the Rottweiler breed is among the top 25% of breeds at highest risk for mammary cancer (Jitpean et al., 2012), and the top 5% highest risk for pyometra (Egenvall et al., 2001; Jitpean et al., 2012), suggesting that a minority of other breeds carry a stronger predisposition for developing these conditions. Today, single-breed studies such as the Golden Retriever Lifetime Study are being rightfully revered for their translational potential—not only to other dog breeds but to humans (Guy et al., 2015). The challenge ahead is to see such studies—all health-related studies—as sources of clues, not proof, providing each clinician an opportunity to re-shape their beliefs, and providing the veterinary profession with new hypotheses that will help to re-frame ideological starting points.

Conclusions

Our findings point to two important conclusions. First, our results fail to provide evidence that either mammary cancer or pyometra have an adverse impact on overall longevity. These findings call into question the argument that elective ovariectomy should be advocated as a longevity-promoting intervention on the basis of protecting against disease conditions that display late-onset, moderate case fatality risk (mammary carcinoma) or low case fatality risk (pyometra). It is hoped that these results, which challenge some prevailing beliefs, will stimulate new starting points for use by a freshly motivated field of investigators. Second, and perhaps more importantly, this study points to the need for redirecting our emphasis away from a disease incidence-only approach and toward evaluating the impact of particular diseases in the context of competing causes of mortality and overall longevity. If our intent is to design and implement strategies that will promote healthy longevity, we will need to embrace the idea of trade-offs. This maturation toward whole organism thinking will provide a necessary corrective against the natural preoccupation of specialized investigators who find their own investigations focused on a particular disease. The quest for longevity-promoting strategies belongs in the territory of the non-specialized. Focusing solely on the avoidance of a single disease, rather than engaging in a life course analysis of all-cause mortality that takes into account physiological trade-offs, will only hinder the sound selection of such strategies.

Conflict of interest statement

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

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