Changes in Behavioural and Leukocyte Parameters Associated with Canine Ovariohysterectomy

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Lynn P. Robblee

Charlottetown, P. E. I.

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Abstract

Ovariohysterectomy is one of the most routine surgical procedures performed by veterinary clinicians. Very little, however, is known about the non-reproductive behavioural changes associated with this procedure. The objectives of this study were to determine the non-reproductive behavioural changes associated with ovariohysterectomizing, evaluate if these behaviours were associated with differences in white blood cell counts (as a measure of long-term stress), and determine if these behavioural changes were associated with changes in testosterone concentrations in ovariohysterectomized versus intact bitches.

One hundred and three bitch owners were recruited from veterinary clientele in Atlantic Canadian provinces of New Brunswick, Nova Scotia, Prince Edward Island and Newfoundland and Labrador. Two telephone questionnaires were completed by one hundred and three of the bitch owners of both ovariohysterectomized (test subjects, n=84) and intact bitches (controls, n=19). The first questionnaire was completed before the animal was ovariohysterectomized and the second approximately 8 months after ovariohysterectomy. Aggression was determined from the questionnaire results by the incidence of growling. Appetite and excitability scores were also recorded between the ovariohysterectomized and intact groups of bitches . Two blood samples were also taken, one at the time of ovariohysterectomizing, and one 8 months after ovariohysterectomizing. Neutrophil to lymphocyte ratios and absolute lymphocyte values were determined from the blood samples as a potential measure of long-term stress. Testosterone results were evaluated using an enzyme linked immunosorbent assay, but were inconclusive and only indicated as an appendix of this document.

Results indicated that ovariohysterectomized bitches were more likely to develop growling behaviour than intact bitches (OR=21.54, P= 0.015). Ovariohysterectomized bitches were also more likely to be excitable (OR=6.83, P=0.019). There were no significant relationships between the reported appetite of the bitches and ovariohysterectomy. Intact bitches were more likely to have a neutrophil to lymphocyte ratio higher than 3.5 which may be indicative of long-term stress, however, absolute lymphocyte values (lymphopenia) may be a more accurate reflection of long-term stress in the bitches. There were too few bitches with absolute lymphocyte values indicative of a lymphopenia to determine if differences existed between the groups.

These results have begun to elucidate clinically important behavioural changes associated with ovariohysterectomizing bitches. Understanding of these effects can help caretakers of bitches to diminish undesirable behaviours that may result from ovariohysterectomy and in turn improve animal welfare and human-animal interactions.

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1. Literature Review

Abstract

Ovariohysterectomy is routinely performed by veterinarians throughout North America. Very little is known, however, about the non-reproductive behavioural consequences of this procedure. Based on a handful of studies which have investigated behaviours associated with ovariohysterectomizing bitches, it appears that there may be an association between ovariohysterectomy and an increase in aggression. Other behaviours, such as increases in activity levels and appetite, have also been reported to be associated with ovariohysterectomy. It is unclear whether the behavioural changes thought to be associated with ovariohysterectomy are due to the different environments of ovariohysterectomized versus intact bitches, or whether they are a result of an underlying physiological change. It is possible that factors such as chronic stress, training methods, exercise levels and socialization may also influence the behaviour of bitches. An examination of the behavioural, physiological and physical characteristics of ovariohysterectomized versus intact bitches, and the environments within which they live, is necessary to fully understand which factors contribute to any changes in behaviour associated with ovariohysterectomy.

1. 1 Introduction

One of the strongest heterospecific relationships existing between mammalian species is the relationship between man and dog. The dog has truly been a grand and faithful companion to humans for many years. The origin of the dog is a topic of great debate. It is uncertain whether the dog has originated from multiple canine species, however DNA sequencing strongly

indicates that the dog appears to be most closely related to its wild ancestor *Canis lupis*, commonly known as the wolf (Savolainen et al., 1997; Vila et al.,1997). The time of domestication is thought to date back as early as the late Pleistocene period (Coppinger and Coppinger, 2001). At this time, dogs may have introduced themselves into human societies as camp scavengers, but were adopted by humans for hunting purposes. The hunting behaviour itself, however, may not have been as important as the companionship that the dog provided to humans during this activity (Coppinger and Coppinger, 2001). The close cooperation and social interaction involved in hunting is what is believed to have facilitated the origin of the attachment that exists between man and dog.

Over the years, humans began to enjoy the comfort and companionship that dogs provided, and today, dogs are generally regarded as companion animals. Although some dogs are used for working purposes, 73% of dog owners cite companionship as the main reason for obtaining their dog (American Pet Products Manufacturers Association, 1988).

In recent years much effort has been devoted to understanding and strengthening the human-animal bond. Research has been done in this area to determine how the relationship between humans and animals can be optimized (Knol, 1987; Beaver, 1994; Endenburg and Knol, 1994; Reisner et al., 1994; Patronek et al., 1996). Understanding the behaviour of dogs is essential if we are going to fully enjoy their company in a household setting, as well as train them for working purposes. Many dogs are relinquished for undesirable behaviour in both the household setting and working environment; 50-70% of dogs in the United States that are euthanized at humane societies are relinquished due to behaviour problems (Sigler, 1991). Aggression is the

most frequently reported behaviour problem to referral practices (Houpt, 1985; Wright and Nesselrote, 1987; Guy, personal communication; Beaver, 1994). Current research has indicated that the reproductive status of an animal may be associated with aggression and that aggression may be exacerbated in female dogs that are ovariohysterectomized (Voith and Borchelt 1982; O'Farrell and Peachey, 1990; Guy et al., 2001). Ovariohysterectomy is one of the most frequently performed surgeries; an estimated 79% of female bitches are ovariohysterectomized in the United States (Patroneck and Glickman, 1994). It is therefore important to understand the behavioural implications associated with this common surgery.

This review will summarize current research on aggressive behaviour by bitches towards people living in the same household, and the relationship between aggression and whether bitches are ovariohysterectomized or intact. It will also outline other behavioural, physical and physiological characteristics of bitches and other species that may be associated with ovariohysterectomy.

1.2. The Anatomy and Physiology of the Canine Female Reproductive Tract and the Procedure of Ovariohysterectomy

1.2.1. Anatomy of the canine reproductive tract

The canine uterus is classified as bicornuate (Nickel et al., 1973). It is located caudal to the kidneys in the sublumbar region, and has 2 long uterine horns or pedicles and a short body (Pasquini et al., 1995). The ovaries are attached to the uterine horns by the proper ligament of the ovary, and to the body wall by the suspensory ligament (Dyce et al., 1996). The mesosalpinx and the mesovarium are peritoneal folds that form a connective tissue bursa which

entraps the ovary. The mesometrium is a fold of peritoneum that attaches the uterine horns and body to the body wall. The mesometrium, mesosalpinx, and mesovarium make up the broad ligament (Dyce et al., 1996). The uterine tube courses from the uterine horn to the ovary and is comprised of three areas, the ampulla, the isthmus and the infundibulum, and facilitates transport of the fertilized ovum from the ovary to the uterus (Bearden and Fuquay, 2000). The placenta of the bitch is zonary, having bands of chorionic villi which form a ring around the placenta (Hafez, 1993).

1.2.2. The estrous cycle of the bitch

The reproductive cycle of the bitch is described as in Feldman and Nelson (1987). The estrous cycle of small-breed bitches generally starts between 6-10 months but may not start until 18-24 months in larger breeds. Bitches commonly cycle continuously throughout the year. The cycle consists of 4 phases: proestrus, estrus, diestrus and anestrus. Proestrus is the time of hightened follicular growth and is 6-11 days in duration. During this time plasma estrogen concentrations progressively rise from around 25 pg/ml to 60-70 pg/ml. Estrus is the period of standing heat when the bitch is receptive to breeding by male dogs and shows behaviour characteristics related to estrus, producing a vaginal discharge which is straw colored or pink. Behaviour characteristics related to estrus include crouching and elevating the perineum toward a male. Pressure on the lower back will cause the tail to move to one side and the back legs to be held tense. At this time plasma estrogen is at its peak (60-70 pg/ml). Estrus duration is variable between breeds and may last from 1-20 days. During this period the female will attract males over long distances due to the production of pheromones.

Diestrus is the period when the corpus luteum is fully functional and is secreting progesterone. The corpus luteum is formed by the leutenization of the ovarian follicle. During this time the hormone production switches from predominantly estrogen to progesterone by the thecal and granulosa cells of the follicle. The corpus luteum will persist if the bitch becomes pregnant or, in the absence of pregnancy, will regress at approximately 65 -80 days after the onset of diestrus. After the regression of the corpus luteum a corpus albicans remains and the bitch will enter anestrus.

Anestrus is the final phase and is a period of quiescence when the uterus prepares for another cycle. During anestrus small bursts of leutenizing hormone, follicle stimulating hormone and estrogen occur and are believed to contribute to the growth of follicles during this period. Progesterone is also produced in very small quantities during this time and remains relatively stable. The length of anestrus is variable but usually lasts approximately 4 ½ months. When anestrus is complete the cycle repeats.

1.2.3. The procedure of ovariohysterectomy and related concerns

Preparation for the procedure of ovariohysterectomy typically involves a thorough physical exam to determine if there are any unusual surgical risks. If there are none, the animal may be premedicated with glycopyrrolate or atropine to help reduce the bradycardia that may be induced by visceral manipulation (Hedlund et al., 2000). Opioids (ie:morphine, butorphanol, fentanyl) are often given to provide peri and postoperative analgesia. Once general anaesthesia has been induced using gas inhalation, the skin at the site of the incision is clipped, cleaned, and sterilized (Hedlund et al., 2000). One technique for the procedure of ovariohysterectomy is

described as in Slatter (1985). The area between the umbilicus and the pubis is divided into thirds and an incision is made in the cranial one third of this area. The size of the incision is dependent on the size of the bitch. The right uterine horn is located with the index finger and a clamp is placed on the proper ligament which is used to retract the ovary. A window is then made in the mesovarian and the ovarian pedicle is triple clamped. The pedicle is then incised between the middle clamp and the clamp closest to the ovary. The clamp most distal to the ovary is removed and a ligature is put in its groove. The remaining clamp is removed and the pedicle is checked for any indications of bleeding. The same procedure is then repeated on the left uterine horn. Clamps are then placed on the uterine body cranial to the cervix and the body of the uterus is severed between the proximal and middle clamps. A ligature is then placed around each uterine artery just beneath the most caudal clamp, and the caudal clamp is then removed and the uterus is ligated in the groove that remains. The uterine pedicle is then inspected for bleeding and placed back into the abdomen.

The commonly recommended age for ovariohysterectomizing bitches and cats is approximately 6 months, or just prior to their first estrus cycle (Slatter, 1985). Ovariohysterectomy before the first estrous cycle may decrease the risk of mammary cancer. In a study by Schneider et al. (1969) it was found that if ovariohysterectomy is performed after the first estrous cycle, the risk of mammary gland cancer increases to 8 percent, if it is done after two ovarian cycles then the risk increases to 26%, and after two years ovariohysterectomy has no preventative effect on mammary gland cancer. Ovariohysterectomizing of bitches eliminates the possibility of uterine diseases such as neoplasia, or more commonly, pyometra (Sorenmo et al., 2000). Although six months remains the typical age, some humane societies

and animal shelters are neutering at an earlier age in an attempt to increase the amount of animals adopted from the shelter, and to prevent unwanted litters due to owners failing to take the initiative to have their pet neutered (Olson et al., 1986; Lieberman, 1988; Salmeri et al., 1991; Theran, 1993; Kustritz, 1999).

Although early age neutering has been a topic of concern for veterinarians and animal shelter managers, very few controlled studies have been conducted on this method. Salmeri et al. (1991) evaluated three groups of mixed-bred dogs: dogs neutered at 7 weeks, dogs neutered at 7 months, and dogs that were sexually intact. Growth plate closure was delayed in dogs neutered at 7 weeks of age compared to those neutered at 7 months resulting in greater final radial/ulnar length. There were no changes in the food intake, back fat depth or weight gain between any of the groups. With regards to behavioural changes the only significant difference was that dogs neutered at 7 weeks of age were judged to be more active than the sexually intact dogs, however this was a subjective assessment of the behaviour of the dogs over an observation period of 15 months. The observers were not blinded to the reproductive status of the three groups of dogs. Also, the authors did not indicate if they controlled for age in the statistical analysis when comparing the behaviour of the three groups of dogs. It would therefore be difficult to determine if the behavioural changes seen were a result of the early neutering, or actually differences in maturity. In general, it appears that there are no behavioural changes that occur as a result of early ovariohysterectomy.

Another concern regarding prepubertal gonadectomy is the possibility of complications that may occur during and shortly after the surgery in young animals. Dogs and cats less than 12

weeks of age had more minor complications as a result of gonadectomy when compared to animals that were over 24 weeks of age, and females had more overall and minor complications than males amongst the animals in the 12 week old group (Howe, 1997). The minor complications included such things as swelling, bruising and inflammation at the incision site, torn vascular pedicles, cardiac rate abnormalities, presumed hypoglycemia, gastric dilation during anesthesia, and mild vomiting, diarrhea, or anorexia. Protocols for early age neutering have been developed which take into consideration physiological differences between immature and mature animals (Howe, 1997). The lower hematocrit and blood volume of younger animals make control of blood loss essential, and thermoregulation should also be considered due to the decreased ability to shiver, less body fat, and a smaller volume to surface area ratio in younger animals (Theran, 1993). Liver enzymes for metabolism of drugs are less developed and must be acknowledged when administering anesthesia (Theran, 1993). There has been some concern from veterinarians that prepubertal gonadectomy may result in a persistent infantile vulva that may lead to perivulvar dermitis (Joshua, 1965), however there have been no controlled studies done to determine the validity of this concern.

The estimated average cost for ovariohysterectomy in bitches in the Maritime provinces is $165.00 (based on one cost estimate from one veterinary clinic in each of Nova Scotia, New Brunswick, Prince Edward Island, and Newfoundland). Most veterinary clinics suffer an economic loss because the price charged does not cover even half of the real costs involved with the procedure. These include such things as the cost for premedication, anesthesia, and other materials required to complete the surgery, the cost of running preoperative blood tests, hospital charges for housing the animal overnight, employment fees for surgeons and

administrative personnel, and general overhead (W. Grasse, Personal Communication). Even though veterinarians do not yield a profit from ovariohysterectomy many pet owners may still feel that the procedure is too expensive. Today's veterinarian is obligated to perform surgery to the best of his or her ability and in a humane and sterile way, and all of the facilities required to meet these demands cost money (Miller, 1977). Until veterinary clientele realize the costs and the importance of the procedure, the price for ovariohysterectomy will remain low and veterinarians will suffer an economic loss with this procedure. This economic loss, however, is typically recouped in the purchase of other services and products throughout the life of the pet (Miller, 1977).

1.3. Perceptions of the Public and Veterinary Professionals Regarding Ovariohysterectomy

1.3.1. Attitudes towards ovariohysterectomy in North America

Ovariohysterectomy is one of the most common surgical procedures performed by veterinarians. Previous studies consistently indicate that the majority of the female dog population is ovariohysterectomized. Patronek and Glickman (1994) have estimated that 79% of all pet female bitches are ovariohysterectomized in the United States. Remarkably similar results were found in Canada where Guy et al. (2001) found that 78.3% of bitches were ovariohysterectomized when evaluating the caseload of 20 clinics in the Maritime provinces of New Brunswick, Nova Scotia and Prince Edward Island.

The main reason for public interest in the neutering of companion animals is to control the dog and cat population. An estimated 3-4 million dogs are euthanized by animal shelters

annually in the United States (HSUS, 2003). Animals that are euthanized in shelters represent one third of all canine deaths (Patronek et al.,1996). Dog owners also prefer not to have to live with the inconvenience of estrous cycles and clinical pseudopregnancies. Clinical pseudopregnancy, which is an abnormal occurrence in bitches, is an exaggeration of the normal physiological events that happen in a non-pregnant bitch and can result in lactation and mothering behaviours which may be bothersome to owners (Feldman and Nelson, 1987). Bitches may also attract unwanted dogs to the house when they are in estrus due to the release of pheromones, they may become restless and be more vocal (Feldman and Nelson, 1987). These factors may have led to an increasing number of pet owners having their bitches ovariohysterectomized.

Other alternatives to ovariohysterectomy are available but are not widely used in North America. These methods involve chemical sterilants, cytotoxins and vaccines (Bloomberg, 1996). Various hormones administered orally or injected can be used to interfere with the ovarian cycle of the bitch, such as natural progesterone and testosterone, and a variety of synthetic steroids derived from testosterone and progesterone which probably function by suppressing gonadotropic hormone and in turn ovarian cyclicity (Concannon and Meyers-Wallen, 1991). Androgens, however, may lead to masculinization, and progesterone treatments may lead to cystic endometrial hyperplasia and subsequent uterine infection, mammary development, and post-therapy lactation (Concannon and Meyers-Wallen, 1991). Therefore, if hormonal therapies are used correctly they may contribute to a decreased number of unwanted litters, but would not be beneficial in controlling other unwanted characteristics related to an intact bitch. Chemical sterilants are only available to control fertility in male cats and dogs.

They are injected into the testes, ductus deferens or epididymis and result in permanent azoospermia (Bloomberg, 1996). These are used for permanent sterility and decrease androgen production which may help diminish undesirable behaviours related to circulating androgens (Bloomberg, 1996). Zona pellucida vaccines are also being evaluated for their efficacy and would render the ovaries permanently nonfunctional (Bloomberg, 1996). This would also be a useful method to ensure permanent sterilization, but such vaccines have not yet been completely developed (Bloomberg, 1996).

1.3.2. Attitudes toward ovariohysterectomy and neutering in other societies

Caution must be used when comparing information on views and policies regarding neutering in different societal areas. Each society may have its own distinct culture and ethics regarding pet neutering. As indicated above, North American views on neutering are skewed toward the majority of the population believing that bitches should be ovariohysterectomized. The reverse, however, is true for Europe, where Jochle (1991) has estimated that only 10% of companion animals are neutered and the other 90% of female bitches are treated with progestin injections to control the pet population. Europe is advanced in the study of pharmacological intervention as a method of controlling the pet population. The United States veered away from this option when the initial pharmacological dose of progestin to bitches was too high, resulting in a high incidence of pyometra (Jochle, 1991).

Great success has been achieved in Europe by the development of a safe and effective progestin therapy, in contrast to the therapies that failed in the United States. Many companion animal owners in Europe consider neutering inhumane and degrading (Jochle,

1991). Europeans want their pets to enjoy the benefits of contraceptives while avoiding the risks associated with surgery (Jochle, 1991). The veterinary medical profession also believe that contraceptive hormonal therapies may strengthen the bond between owners and their pets without hurting the economics of their profession (Jochle, 1991). The progestin therapy must be purchased from and administered by the veterinarian every 4-5 months for 5-10 years (Jochle, 1991). Progestin therapies therefore require frequent visits to the veterinarian.

Effort is also being made in Europe to enact laws that prohibit neutering of healthy animals. These laws are being pushed by animal welfare advocates with the intention of fostering animal welfare and improving humane treatment of companion animals (Jochle, 1991). As indicated in the review by Jochle, 1991, it appears that in Europe animal rights attitudes may be more prevalent regarding neutering, alternative methods to neutering, and the health consequences of this procedure. The information provided by Jochle (1991), however, is not a controlled study, but a veterinarian's perception of what he saw in his practice.

In Australia 47.3% of women who own dogs and 61.1% of men who own dogs would not have their dog neutered if it had not already been done, based on the results of a questionnaire administered to dog owners (Blackshaw and Day, 1994). Neutering in Australia does not appear to be as prevalent as in the United States, but seems to be more prevalent than in Europe (Jochle, 1991, Blackshaw and Day, 1994, Patronek and Glickman, 1994). Some of the reasons stated by owners in Australia for not neutering dogs and bitches were: the owners planned on breeding the dog, they did not have enough money for the procedure, it was generally

unnecessary, or they believed that the dog might become overweight (Blackshaw and Day, 1994).

1.4. Behavioural, Physical and Physiological Consequences of Ovariohysterectomy

1.4.1. Normal reproductive behaviour of the bitch during the estrous cycle

During proestrus plasma concentrations of estrogen rise gradually from 25 pg/ml to 60-70 pg/ml until the onset of estrus in the bitch (Concannon et al., 1977; Wildt et al., 1979; Feldman and Nelson, 1987). This rise in estrogen may cause the bitch to become more restless and nervous, her appetite may increase or decrease, and she may exhibit polydipsia and polyuria, which may be related to the facilitation of the dispersal of pheromones to attract males (Feldman and Nelson, 1987). Estrogen at this time causes bitches to be attracted to males, but full receptivity to males does not occur until the progesterone concentrations rise prior to ovulation (Concannon et al., 1977). Full receptivity will occur when there is a decrease in estrogen:progesterone ratio (Concannon et al., 1979). Receptivity of the bitch for the male gradually declines during estrus and she becomes nonreceptive from early diestrus to late anestrus (Feldman and Nelson, 1987).

1.4.2. Behavioural consequences of ovariohysterectomy

Even though ovariohysterectomy is one of the most common surgical procedures performed by veterinarian practitioners, very little is known about the non-reproductive behaviours associated with this procedure. There have been few objective and controlled studies which examine non-reproductive behaviours related to ovariohysterectomy. Voith and Borchelt (1982), indicated that the majority of the dogs referred to their behaviour clinic for dominance aggression were ovariohysterectomized females. This was a descriptive study since no control

subjects (intact bitches) were used for comparison with the ovariohysterectomized females. Explanatory statements indicating an association between aggression and ovariohysterectomizing can therefore not be stated, because there was neither a control group or any information provided on the number of ovariohysterectomized bitches in the general or referral population in that area. It is possible that ovariohysterectomized female bitches may have been more common in the population of bitches belonging to owners who were using this referral service, resulting in what appears to be a higher risk of dominance aggression. This might give a false perception that ovariohysterectomized female bitches are at a higher risk for dominance aggression.

In a study conducted by Podberscek and Serpell (1996), aggressive behaviours were examined in 1008 English Cocker Spaniels using randomly distributed questionnaires to United Kingdom Kennel Club members. They found that neutered bitches were more likely to exhibit numerous forms of aggressive behaviours when compared to intact females. Further analysis of their data revealed that all forms of aggressive behaviours disappeared when bitches that were neutered because they were aggressive were excluded from the analysis, with the notable exception of aggression towards children. This would suggest that aggression does persist after ovariohysterctomy, and that aggression towards children in the household is actually associated with ovariohysterectomy. Ovariohysterectomy may therefore not be a good preventative measure or treatment for aggression.

The most pertinent work examining the association between non-reproductive behaviours and ovariohysterectomy was by O'Farrell and Peachey (1990). This study is the only one to date

that specifically examined the behavioural changes that occurred as a result of ovariohysterectomy. It involved two questionnaires completed by 150 owners of ovariohysterectomized dogs and 150 owners of intact female dogs regarding the general behaviour of their bitches, with the bitches being matched for breed and age. The owners completed the first questionnaire at the time of ovariohysterectomy and the second approximately six months later. The results from these questionnaires indicated that ovariohysterectomized bitches had a higher dominance aggression score[1] towards family members when compared to intact controls. The ovariohysterectomized bitches that were most likely to elicit dominant aggressive behaviours were under one year of age and had already shown some form of aggression. This suggests that aggression in these bitches may become exacerbated after ovariohysterectomy and that aggression does not appear to occur spontaneously as a result of the procedure. Podberscek and Serpell's (1996) results are similar to those of O'Farrell and Peachey's (1990) , in that both results indicate that aggression persists after bitches are ovariohysterectomized, but does not persist in intact bitches. It must be considered, however, that in the study by O'Farrell and Peachey (1990) neither the experimenters nor the owners were blinded to the reproductive status of the bitches.

1.4.3. Effects of ovariohysterectomizing on behavioural, physiological and physical parameters in other species

With the exception of ferrets, dogs and cats are the only species that are routinely ovariohysterectomized. There are, therefore, few studies examining non-reproductive

[1] Dominance aggression was not defined in this study, therefore it is difficult to determine if the aggression that occurred was truly dominance aggression and not another type of aggression.

behaviours associated with ovariohysterectomy in other mammalian species. Those studies that have been conducted in other species typically involve the animals being housed in research settings, not homes, and examine behaviour directed towards their conspecifics (Debold and Miczek, 1984; Albert et al., 1989; Stavisky et al., 1999). In bitches, aggressive behaviour towards humans tends to be the focus of interest. Regardless of the environment in which the animals are housed, similar results have been found in other species. In rats, aggression persists after ovariohysterectomy (Debold and Miczek, 1984). Aggression that occurred due to changes in housing did not appear to become worse or better after ovariohysterectomy. In contrast, the male rats showed a complete loss of aggressive behaviour after castration (Debold and Miczek, 1984). In female Macaques, aggression also persists after ovariohysterectomy. A two to threefold increase in aggression after ovariohysterectomy was seen in comparison to those Macaques that were left intact (Stavisky et al., 1999).

1.4.4. Physiological and physical consequences of ovariohysterectomy

The most obvious physiological consequence of ovariohysterectomy is the removal of the sex hormones released from the ovaries. Estrogen, progesterone and androgens the main steroid hormones produced by the ovaries, while relaxin, inhibin, activin, activin-binding protein and prostaglandins are also produced in smaller quantities (Greenspan and Gardner, 2001). Very little research has been conducted in bitches on the role steroid ovarian hormones play in modulating non-reproductive behaviours, although a great deal of information is available from other species (Suchowsky et al., 1971; Gandleman and vom Saal, 1975; Van Oortmerseen et al., 1987; Sharma and Rissman, 1994; Romeo et al., 2000). When considering the behavioural effects of the steroid hormones, the effects of the steroids released from the adrenal gland must

also be considered, since progesterone, androgens and small amounts of estrogens are secreted by the adrenal cortex (Greenspan and Gardner, 2001).

In adult female mice that were administered a combination of four anabolic steroids (testosterone, testosterone cypionate, methyltestosterone and norethandrolone) there was increased pair fighting between mice, but a decrease in overall activity (Bronson, 1996). When female mice were administered the normal maintenance level ("capsule size of hormone administered required to maintain the seminal vesicles of a castrate male mouse at a weight typical of intact males") for males, they showed no difference in behaviour when compared to female mice that received five times this level. This suggests that there is a threshold effect for testosterone in female mice and that testosterone administered above this level has no additional effect. Gandleman and vom Saal (1975) found that administration of testosterone to female mice induced killing of their pups and intraspecific aggression.

Suchowsky et al. (1971) found that testosterone proprionate causes the manifestation of aggressive behaviour when given to intact or ovariohysterectomized female mice. Studies in male mice indicate that attack latency is not only related to the baseline plasma testosterone level, but is also dependent on responsiveness to testosterone induced before adulthood (Van Oortmerseen et al., 1987). Steroid receptors have been proven to start developing during puberty (Romeo et al., 2000). These steroid sensitive areas may be responsible for the behaviours elicited as a result of increased circulating testosterone during puberty. Testosterone must be aromatized to estrogen in order to initiate sexual behaviour in most female mammals (Sharma and Rissman,1994). It has also been demonstrated that only aromatizable androgens

are associated with aggressive behaviours in male mice (Christie and Barfield, 1979; Compaan et al., 1994; Toda et al., 2001). In female mice, testosterone has been shown to facilitate aggressive behaviours, but estrogens have been shown to have no effect on mediating aggressive behaviours (Rines and vom Saal, 1984; Simon et al., 1985; Simon and Masters, 1987).

Estrogen therapy in elderly humans has been shown to decrease aggression (Kyomen et al., 1999). Estrogen has also been shown to decrease aggression in humans with a genetic disorder called Sanfilippo syndrome, a rare degenerative disorder with severe intellectual and behavioural consequences such as aggression and disruptive behaviours (Hier et al., 1999). In this case, it is difficult to know if estrogen has a direct effect on neural activities in the brain related to aggression, or if estrogen has a therapeutic role in the treatment of the disease which in turn may cause a decrease in aggression. Estrogen appears to have a dose-dependent effect in women; low estradiol (<50pg/ml) is depressive, medium (150pg/ml) has a pleasant mood effect and excessive amounts (>150pg/ml) may cause irritability and aggression (De Lignieres and Vincens, 1982). Care must be taken to note the amount of estrogen used in experimental studies with women before concluding an association with decreased aggression. Reportings in humans may also be difficult to compare with dogs due to the nature of assessment of aggression. In human studies, aggression in subjects is determined by self-assessments (Soler et al., 2000). This may subjectively bias the studies. Studies in rats and mice may be more comparable to canines since in most cases the incidence of aggressive behaviours are objectively determined by a researcher who is blinded to the categorization of test and control subjects.

It is difficult to determine if behaviour changes associated with ovariohysterectomy are associated with the changes in circulating sex hormones when the gonads are removed. Sex steroids are produced from the adrenal gland (Norman and Litwack, 1987) and estrogen, testosterone and progesterone are produced in various regions in the brain (Lombardi et al., 2001; Bixo et al., 1995). Aromatase (responsible for steroid conversion) , is also found in adipose tissue, and in bone in humans (Simpson et al., 2002). In bitches, testosterone concentrations are still detectable in small quantities during anestrus. Olson et al. (1984) found testosterone concentrations of 63 pg/ml during late anestrus in the bitch. It may be possible that small amounts of circulating estrogens and androgens from extragonadal sources may be contributing to non-reproductive behaviours associated with ovariohysterectomy. No information is available on other sources of androgens in ovariohysterectomized bitches. In ovariectomized women, only androgen concentrations are lower in ovariectomized women when compared to concentrations in postmenopausal women, estrogen concentrations are similar in ovariectomized and postmenopausal women (Vermeulan, 1976). The same may be true for ovariohysterectomized bitches when compared to anestrus bitches.

Decreased concentrations of testosterone may be associated with anxiety and/or depression (Bancroft and Cawood, 1996) and alterations in normal circulating androgens may be associated with cognition. Human subjects with congenital adrenal hyperplasia, which results in abnormally high testosterone concentrations, have a significantly lower general intelligence level than control subjects that did not have the disease (Helleday et al., 1994). Difference in cognition between animals with differing androgen concentrations may in turn affect other behaviours. Cognition will determine if an animal can predict and control its environment by

adjusting its behaviour (Weiss, 1971). The inability of an animal to predict or control its environment may lead to stress, which in turn may lead to aggression (Weiss, 1971).

Some veterinary clients are concerned that their bitches will become obese after ovariohysterectomy due to an increase in appetite or a decrease in activity. O'Farrell and Peachey (1990) found that indiscriminate appetite (eats any commercial dog food, eats very quickly, eats rubbish) was higher in ovariohysterectomized bitches when compared to intact female bitches that were kept in a household environment. Intact bitches showed a decrease in indiscriminate appetite with maturity whereas ovariohysterectomized bitches showed no change in appetite. Salmeri et al. (1991), however, found that gonadectomy had no effect on food intake, weight gain, or back-fat depth among dogs housed in a laboratory setting that were fed ad lib initially then switched to specified meal times. Many studies have examined the population of dogs presented to veterinary professionals for obesity (Mason, 1970; Edney and Smith, 1986). Obesity is slightly higher in female bitches in general when compared to males (Mason, 1970) and ovariohysterectomized females were twice as likely to be obese as intact females (Edney and Smith, 1986). Without knowing the difference in food intake and quality, and the activity level of the dogs in question, it is impossible to determine whether the difference in body weight is truly the result of ovariohysterectomy.

1.5. Stress in Bitches and its Association with aggression

1.5.1. Evaluating stress in animals

Assessing the stress response expressed by animals is one way to evaluate animal welfare (Nelson, 2000). Nelson (2000) described the stress response as a combination of physiological

and behavioural responses that occur when an animal's homeostasis is disrupted in response to stressors in the environment. The stress response involves the release of epinephrine (within seconds) from the adrenal medulla and norepinephrine from the sympathetic nervous system under the immediate conditions of stress (Nelson, 2000). Norepinephrine and epinephrine are under the control of the central nervous system (Norman and Litwack, 1987). At the same time that this response is occurring, the hypothalamus is stimulated to release corticotropin-releasing hormone, which stimulates the adenohypophysis to release adrenocorticotrophic hormone (ACTH) and β-endorphin (Norman and Litwack, 1987). ACTH then stimulates the release of glucocorticoids from the adrenal cortex. Glucocorticoids cause gluconeogenesis, while epinepherine causes rapid conversion of stored glycogen (glycolosis) to be utilized to help combat stress (Norman and Litwack, 1987). Vasopressin is also released from the posterior pituitary and acts to increase blood volume and pressure and make energy transport to muscles more efficient (Nelson, 2000). The adenohypophysis will also release prolactin which temporarily suppresses reproduction while the animal is dealing with the stressor over a long period of time. Prolactin inhibits the responsiveness of the Leydig cells of the testes and ovaries to leutenizing hormone, resulting in less production of testosterone (Nelson, 2000). There is, therefore, a close relationship between the hormones of the stress response and sex steroid hormones. Through the mechanisms mentioned above the animal should be able to adapt to the environment. The general adaptation syndrome was described by Hans Selye in 1936 when he exposed mice to cold temperatures causing a classic stress response involving adrenal hypertrophy, gastrointestinal ulcers, and thymolymphatic atrophy . After two weeks, the animals adapted, and did not have any indication of this stress response. If, however, a stressor persists for a long period of time, pathological events such as protein breakdown in tissues,

decreased glucose utilization and decreased protein and nucleic acid synthesis may occur which can be harmful to the animal (Norman and Litwack, 1987).

Numerous attempts have been made to physiologically and behaviourally quantify an animal's stress response and determine what is an appropriate measure of stress in animals (Weiss, 1971; Tuber et al., 1996; Beerda et al., 2000). Animal welfare at the present time remains poorly defined. Good animal welfare can be described as an animal being in a satisfactory physical or physiological state of well being, or the ability of the animal to express its natural behaviour (Mason and Mendl, 1993).

Many problems arise when trying to quantitatively or qualitatively analyze the welfare of animals. The following factors must generally be considered: the species, the animal's age and gender, types of aversive stimuli, the duration of the aversive stimuli, and the time taken to measure the stress response (Mason and Mendl, 1993).

1.5.1.1. Age and stress

Variation may occur in the physiological mechanisms relating to stress as an animal ages. Immune system development in animals is not complete until one year of age and declines with age in geriatric animals, for example, sheep less than 12 months of age have poorer resistance to parasitic invaders and viruses than mature sheep (Tizard, 1996). Aged rats have an exaggerated corticosterone response, but a decreased level of corticosterone receptors in the hippocampal region when compared to younger rats (Lorens et al., 1990). Behaviours of animals related to stress would also change as a result of learned behaviours and experience (Lindsay, 2000). An

animal is more likely to respond to a stimulus that it has previously associated with a negative or positive experience. With repeated exposure to the stimulus, the animal may show a stress response just upon encountering objects associated with the stimulus. This is called a two stage theory of association (Barker, 1997). With age comes varied experiences and associations with objects and stimuli; this may cause behavioural differences in relation to stress as well as differences in mental well being.

1.5.1.2. Gender and stress

Gender of the animal may also be related to physiological stress responses in dogs (Garnier et al., 1990). Garnier et al. (1990) proposed that bitches are more susceptible to environmental stress because they have a significantly higher basal serum cortisol level than male dogs. When males and females were housed in unfamiliar settings, however, the difference in serum cortisol disappeared. Males in this study did appear to acclimatize to the new environment more quickly than females, based on serum cortisol concentrations. In contrast, Hennessy et al. (1997) found no gender associated differences when comparing plasma cortisol concentrations of dogs. It is interesting to note that neither of these studies considered the reproductive status of the animals, rendering interpretation difficult given the close relationship between sex steroids and gluccocorticoids.

1.5.1.3. Type of stimuli and stress, and the latency of stress measurement

The type of aversive stimuli may also determine the stress response that may occur. Behaviourally, animals may respond differently depending on the type of aversive stimulus. It is also important to consider duration of the stress response when evaluating the physiology and behaviour of the animal in relation to the stressor. Acute stressors may cause an up-regulation of the immune response while the animal produces β-endorphins to try to cope with a stressful event (Norman and Litwack, 1987). Long-term stress can cause immunosuppression due to cell damage as a result of chronic cortisol release (Norman and Litwack, 1987). Stereotypies may also develop if an animal experiences chronic stress. Stereotypies are defined by Houpt (1998) as behaviours that are repetitive, serve no purpose and occupy more than 10% of the animals time. They may occur in an attempt to behaviourally cope with the stress, which may be indicated by the release of endorphins.

The time taken to measure a stress reaction must be considered when choosing a method to quantify stress. For example, cortisol may be misleading as an indicator of chronic stress because it varies at the time of analysis due to changes in the environment which may cause the animal to become stressed. Plasma cortisol can rise in a matter of minutes (Norman and Litwack, 1987), and shows pronounced circadian rhythms (Nelson, 2000). A better approach to evaluate long-term stress may be to evaluate the pathological effects of the chronic release of cortisol on the immune system. Continuous exposure to corticosteroids results in lymphopenia, neutrophilia, and eosinopenia and a neutrophil to lymphocyte ratio greater than 3.5 (Aiello and Mays, 1998). Eosinopenia, neutrophilia and lymphopenia have all been reported following corticosteroid administration to dogs (Latimer and Rakich, 1989). This

effect occured 4-6 hours after administration of the corticosterone (Latimer and Rakich, 1989). Ratios of neutrophils to lymphocytes have been used to evaluate stress in horses that are cross-tied during 24 hours of road transport (Stull and Rodiek, 2002). In this study, these ratios were significantly elevated in horses that were cross-tied as opposed to those that were loose in the trailer. Neutrophil:lymphocyte ratios were also evaluated by Morrow-Tesch et al. (1993) who found that there were no diurnal variations over a 24 hour period in this ratio but there were diurnal variations during this time period in neutrophil, monocyte and eosinophil percentages. Absolute counts of white blood cells may be a more accurate predictor of long-term stress as a result of cortisol than neutrophil to lymphocyte ratios counts (Latimer and Rakich, 1989). Stress in laboratory beagles during transportation caused elevated cortisol, corticosterone and leukocytes when compared to values before transport (Kuhn et al., 1991). In pigs subjected to transport stress, total white blood cell counts increased significantly and the absolute lymphocyte values decreased significantly (Dalin et al., 1993). Stress associated with surgery may also affect white blood cell values (Kreeger et al., 1990). Foxes that had radio transmitters surgically implanted into their abdomen had an increase in total white blood cell counts 8 hours post surgery (Kreeger et al., 1990). Exercise may also affect white blood cell counts (Wigernaes et al., 2001). Strenuous exercise in fasted endurance athletes caused a decrease in total white blood cell counts (Wigernaes et al., 2001). Stress may therefore be affected by both the type of stimuli and the latency of the stress measurement, and these factors should be considered before measuring stress responses.

1.5.2. Stress in Canines

One of the most common stress-related disorders in the dog is separation anxiety/frustration (Knol, 1987; Wright and Nesselrote, 1987; Takeuchi et al., 2000; Flannigan and Dodman, 2001; Overall et al., 2001). The goal of the dog experiencing separation frustration is to regain contact with its attachment object, which in most circumstances is its owner (Lindsay, 2001). When this goal can not be attained, the dog becomes anxious and performs many different behaviours to try to cope with the change in its environment. The dog may demonstrate stress-related behaviours such as barking, or excessive chewing and the destruction of objects in the home. These stress related behaviours are also a frequent reason why dog owners seek advice from animal behaviour professionals and may lead to relinquishment of the animal (Houpt, 1985).

Another cause of stress that may occur in dogs is frustration in training. In training, and in general interactions with people, it may not always be clear to a dog what is expected of it. The dog may not understand what behaviours to perform to control and cope with its environment. An unpredictable and/or uncontrollable environment has been shown in rats to result in gastric lesions, possibly associated with a glucocorticoid response due to stress (Weiss, 1971). Predictable and controllable environments may be obtained for dogs through appropriate training and interactions. It allows them to be aware of what behaviours to perform in order to access resources in the environment, such as food. Control over resources enhances sense of control over the environment in general (Weiss, 1971).

Weiss (1971) also found that relevant feedback to rats after they performed the desired behaviour also reduced stress. Rats who received a safety signal (light signal) after performing the desired behaviour had reduced gastric lesions as a result of chronic cortisol release. Conversely, punishment for performing a behaviour (negative feedback) increased gastric lesions. Rats that could perform a behaviour to avoid a shock had more control over their environment and had fewer gastric lesions (Weiss, 1971), demonstrating the stress-reducing capacity of a predictable environment.

Tuber et al. (1996) measured stress-related glucocorticoid responses in dogs in the following five different environments:

1) alone in a novel environment
2) together with conspecific in novel environment
3) together with human in novel environment
4) alone in familiar environment; and
5) together with conspecific in familiar environment.

Dogs released the least amount of glucocorticoids when they were together with a human in a novel environment, even in comparison to being in a familiar environment with their own species (Tuber et al., 1996). It is therefore important to consider these factors when measuring stress responses in dogs housed in different environments. It also reinforces the significance of human interactions in the production or control of stress in dogs.

Exercise may also elicit a stress response in dogs. Depending on the intensity of the exercise, stress responses may vary. Radosevich et al. (1989) found that ACTH, beta-

endorphins and cortisol increase during exercise, and that the release of these hormones depends on the amount and type of exercise the animal performs. Campbell et al. (1988), however, they found that moderate exercise programs in purpose-bred beagles had no effect on serum cortisol or immune function. In humans, chronic intense exercise in Olympic athletes has been shown to impair immunity (Nieman, 2000). The stress during Olympic training may not be exclusively related to exercise, however, but may also be associated with severe mental stress, inadequate sleep, malnutrition, and weight loss in these athletes.

1.5.3. Aggression in Canines

One of the most commonly reported problems to veterinary behavioural services is aggression (Houpt, 1985; Wright and Nesselrote, 1987; Beaver, 1994; Guy, personal communication). The term aggression is applied to a wide variety of different behaviours, some of which are dangerous to people and other animals and some of which are not (Reisner, 2002). For example, a dog chasing a car may be considered a form of predatory aggression, but will not commonly cause harm to a person or animal, and does not pose a threat to any person or animal. Aggression may still be defined by some as an obvious behaviour expressed by an animal in attempt to injure another animal or person, or as an attack or threat of attack (Moyer, 1987; Francis, 1988). These definitions do not encompass aggression which does not injure other organisms and may therefore be incomplete. It may be better to classify aggression instead of defining it. It can be classified based on the target to which the behaviour is directed, by function (which considers the motivations for the behaviour), or as passive or active defensive reflexes based on Pavlovian reflexology (Voith and Borchelt, 1996). Classification methods, on their own, are not adequate because they all have specific shortcomings. For example,

classification by reflexology does not consider aggression which may be learned. Classification by function does not consider the reason for the behaviour, but may be most useful since it considers motivations and can, therefore, help formulate treatments for the aggressive behaviour (Voith and Borchelt, 1996). Incorporating all classification schemes together when describing aggression would be most useful.

There are many forms of aggression that have been classified in dogs. Dominance aggression has frequently been reported as the most common form of aggression in veterinary behaviour specialists' caseloads (Wright and Nesselrote, 1987). Unfortunately, dominance aggression is commonly misdiagnosed and there is sometimes confusion and misuse of the term (Mertens, 2000). Dominance aggression usually involves mature, male dogs between the ages of 2 and 3 years that are self confident and assertive and exhibit the body language of an offensively aggressive dog. Dominance aggression involves situations where the social position of the dog is challenged (Luescher, 2001). When evaluating aggression based on biting behaviour in a household environment, Guy et al. (2001) found that bitches and dogs that were fearful of men, children and strangers were most likely to bite. These characteristics do not fit the characteristics for a dominantly aggressive dog. Dogs that are aggressive towards household members are often expressing conflict aggression, due to some frustration resulting from conflict within the environment, including conflicts due to social interactions. They will respond aggressively due to the resulting anxiety (Luescher, 2001). Fear induced aggression may be closely associated with conflict aggression and involves a dog feeling threatened by a person or animal and may be more common in improperly socialized dogs (Mertens, 2000). Conflict aggression may involve fear aggression when a dog is in conflict with its environment,

because it is unsure of what behaviours to perform to please the owner and receive reinforcement. The dog may then become fearful if punished for not performing the correct behaviour (Leuscher, 2001).

Other forms of aggression that can be shown by dogs are territorial or possessive aggression where a dog is fundamentally protective of its resources and aggressive while trying to guard them (Mertens, 2000). Wright and Nesselrote (1987) indicated that 32% of the 72 dogs evaluated for aggression by behaviour specialists were diagnosed as being territorial or possessively aggressive. Maternal aggression occurs when a bitch is attempting to protect or guard her puppies and may be associated with hormonal changes related to pregnancy and lactation (Voith and Borchelt, 1996; Nelson, 2000). Predatory aggression in dogs is a natural behaviour that stems from hunting and involves activities such as chasing fast moving objects like cars, other animals, joggers, etc. These behaviours have a high heritability in certain breeds of dogs such as hunting and herding dogs (Mertens, 2000) Animals may also become aggressive when they experience pain (Mertens, 2000). This is termed pain-induced aggression and is frequently encountered in the veterinary clinic. It involves a response to a painful stimulus or the fear of a painful stimulus (Houpt, 1998). Redirected aggression involves aggression being directed toward another person or object which did not evoke the initial aggression and may be an expression of frustration (Mertens, 2000). Other forms of aggression are inter-male and inter-female aggression, irritable aggression, play aggression and learned aggression (Mertens, 2000).

Growling, lunging, snapping and biting are behaviours that are typically considered aggressive. Annual dog bites to humans in the United States range from 1-2 million (Marder, 1991). In Canada epidemiological investigations in Guelph Ontario by Szpakowski et al. (1989) suggest a dog bite rate of 160/100,000 people per year in that area. The Canadian Hospitals Injury Reporting and Preventing Program indicated in 1996 that as many as 1042 bite-related injuries were reported per 100,000 injuries seen in the emergency room of 10 children's hospitals and six general hospitals. As prevalent as the occurrence of dog bites appears from published reports, many dog bites go unreported and the numbers reflected in most studies are those obtained from health care officials and municipal authorities. Guy et al. (2001) found that as few as 9.3% of dog bites that occur within the family home are reported to health care officials. Therefore, the estimates from studies which gather information from these sources may greatly misrepresent the number of dog bites that actually do occur, especially those of a minor nature.

1.5.4. Stress and Aggression

Previous research has identified an association between stress or anxiety and aggression (Paroli et al., 1972; Liptrap and Raeside, 1978; Wright and Nesselrote, 1987; Rugaas, 1997; Beerda et al., 1998). Paroli et al. (1972) found that administration of hormones associated with a stress response, cortisone and hydrocortisone, also increased aggression in isolated mice. The same study also found that hydrocortisone administration increased killing behaviour in rats. The removal of hydrocortisone, however, did not completely impair fighting in these mice. This may indicate that some of the fighting behaviours are learned. Other triggers in the mouse's environment may generate the fighting response, but not to the same extent as the administration of cortisone or hydrocortisone. Studies in boars also indicate that an increase in testosterone in

plasma during aggressive behaviour is highly correlated with an increase in the plasma corticosteroids (Liptrap and Raeside, 1978). Although research in other species indicates that there may be an association between stress and aggression, very little research with dogs has been conducted in this area to date.

General behaviours associated with stress in dogs are low postures, enduring autogrooming, paw lifting, vocalizing, copraphagy, repetitive or displacement behaviour, circling, trembling, body shaking and yawning (Beerda et al., 1999; Beerda et al., 2000). More severe avoidance or escape behaviours and anxiety-related behaviours can be seen in severe acute stress responses in dogs in situations that have been known to trigger this response, such as with separation anxiety, or thunder storm phobias. Such behaviours would include destruction of inanimate objects in the environment, inappropriate elimination, and even jumping through windows (Takeuchi et al., 2000; Flannigan and Dodman, 2001; Overall et al., 2001).

Dogs that are stressed may be more likely to be aggressive (Beerda et al., 1998). Beerda et al. (1998) found that male dogs and bitches subjected to spatial and social restrictions were more likely to exhibit a heightened state of aggression, excitement and uncertainty. In this study, aggressive behaviours believed to be associated with stress were piloerection along the dorsum, growling, placing a paw over and standing over other dogs. This study examined intraspecific aggression but did not evaluate aggression towards humans. The study by Beerda et al. (1998) was also conducted in an kennel environment. Studies in a household setting would be useful to determine if stress and/or aggression is associated with humans in that environment.

Avoidance-related aggression may also occur as a result of stress. Some behaviours associated with avoidance aggression are barking, shaking and moving away from the target stimulus to a safer location such as a corner of the room or under a bed or couch (Wright and Nesselrote, 1987). Avoidance behaviours and aggression towards unfamiliar people and aggression in a veterinary clinic have been associated with the dog having been raised in a non-domestic maternal environment and a lack of socialization between the ages of 3 and 6 months (Appleby et al., 2002). A dog may show a particular behaviour when it is in conflict with its environment or is stressed, in an attempt to avoid aggression and calm or stop the undesired behaviour in the person or animal which is the source of the conflict (Rugaas, 1997). The dog will show conflict-related behaviours to prevent aggression from occurring. This would include turning its head away from the source of the conflict, yawning, nose licking, freezing or walking away slowly from the conflict area, sitting or laying down, and even tail wagging (Rugaas, 1997). These are ways in which a dog tries to signal to other animals or people that it does not want to engage in an aggressive encounter, but is uncomfortable with its surrounding environment.

1.6. Conclusions

Due to the prevalence of bitches that are ovariohysterectomized it is imperative that we have better information regarding the behavioural, physical and physiological consequences of this procedure. Information must be derived to determine which behaviours may be associated with ovariohysterectomy and if the behaviours are related to a physiological change in the body as a result of ovariohysterectomy, or if the behaviour changes are simply a result of the environment in which the dog is kept. There are many factors in a dog's environment which must be considered

when evaluating behaviour such as exercise and training schedules, physical confinement, socialization, and housing with other dogs.

The **hypothesis of this study** is that bitches that are ovariohysterectomized will show behavioural changes that are associated with the procedure, and that these behaviour changes may be associated with physiological stress responses (as measured by neutrophil:lymphocyte ratios), environmental differences and characteristics of ovariohysterectomized versus intact bitches

The **objectives of this study** were to determine what behavioural changes occurred as a result of ovariohysterectomy. In particular, growling, excitability and appetite levels were analyzed as specific behavioural outcomes of interest and risk factors for these three outcomes were determined. Two physiological outcomes of interest were also evaluated, neutrophil:lymphocyte ratios and testosterone levels, and risk factors were developed for these variables. The main objective was to determine if spaying was a risk factor for the particular outcomes of interest listed above.

2. Reported Behavioural Changes Associated with Canine Ovariohysterectomy

Abstract

One hundred and three owners of bitches were recruited from the clientele of 24 veterinary clinics in Atlantic Canada to participate in a survey of canine behaviour. The bitches were between 4 and 14 months of age and lived in household settings. A 13 page telephone questionnaire was completed with the dog owners to quantify their dog's behaviour before spaying, and again approximately 8 months post-spaying. Owners of intact animals were also surveyed twice during similar time frames, serving as controls. Outcomes of interest evaluated from the questionnaires were the incidence of growling and snapping, the reported appetite level of the dog, and reported excitability levels. The incidence of growling and snapping was used as a measure of aggressive behaviour. Risk factors for the three outcomes of interest were determined by comparing other behaviours, environmental and demographic conditions, and training methods used by owners as determined from the questionnaire responses. Questionnaire responses indicated that ovariohysterectomized bitches were 21.54 times more likely to have growled or snapped by the time of the second questionnaire when compared to intact bitches (P=0.015). Ovariohysterectomized bitches were also reported to be more excitable than intact bitches (OR=6.83, P=0.019). There were no significant differences between the reported appetite levels of the ovariohysterectomized and intact bitches at the time of the second questionnaire.

2.1. Introduction

In North America approximately 79% of the pet dogs are ovariohysterectomized or castrated (Patronek and Glickman, 1984; Guy et al., 2001). More literature is available on the non-reproductive behavioural changes associated with castrating male dogs (Hart and Barrett, 1973; Beach, 1974; Hopkins et al., 1976; Neilson et al., 1997) than is available on non-reproductive behavioural changes associated with ovariohysterectomizing bitches.

There have been a small number of studies that have indicated that ovariohysterectomy may be associated with increased aggression in dogs. Voith and Borchelt (1982) found that the majority of the dogs seen in their referral behavioural clinic were ovariohysterectomized females. No controlled study was conducted by Voith and Borchelt (1982) to validate their observation, therefore, the bitches are only representative of bitches seen in a behavioural referral practice and not the general household population. There were also no indications of the number of spayed dogs in the reference population in this study. This is necessary to determine if the results of this study are only due to differences in numbers of spayed versus intact dogs and not a true association between ovariohysterectomized dogs and aggression. Podberscek and Serpell (1996) found that in a household environment owners reported that ovariohysterectomized cocker spaniels were more likely to show numerous aggressive behaviours when compared to intact bitches. O'Farrell and Peachey (1990) also determined that ovariohysterectomized bitches had a higher dominance aggression score toward family members when compared to intact bitches. Ovariohysterectomized bitches were also found to be more likely to have bitten when compared to intact females (Guy et al., 2001).

Other behaviours that may be associated with ovariohysterectomy are the general activity level of the bitches, and their appetites and obesity status (Edney and Smith, 1986; O'Farrell and Peachey, 1990; Salmeri et al., 1991) . Ovariohysterectomized bitches may have a higher general activity level than intact bitches (Salmeri et al., 1991), however, ovariohysterectomized bitches have been found to be twice as likely to be obese when compared to intact bitches (Edney and Smith, 1986). It is not clear whether this is related to an actual change in the appetite or to a change in the dog's metabolism after the ovariohysterectomy procedure. Previous literature reveals conflicting results concerning appetite levels of bitches after ovariohysterectomy (O'Farrell and Peachey, 1990; Salmeri et al., 1991). O'Farrell and Peachey, (1990) found that indiscriminate appetite was higher in ovariohysterectomized females when compared to intact bitches, however, Salmeri et al. (1991) found that there was no difference in food intake, weight gain, or back-fat depth between ovariohysterectomized and intact bitches.

The objectives of this study were to determine if ovariohysterectomy was associated with changes in aggressive behaviours, and to determine whether the appetite and excitability levels of ovariohysterectomized bitches differed significantly from intact bitches while controlling for confounding factors.

2.2 Materials and Methods

2.2.1. Subjects

In November of 2000, a total of 10 veterinary clinics were initially recruited for participation in the survey to recruit bitches for this study. The clinics were chosen from the Atlantic provinces of Nova Scotia, Prince Edward Island, New Brunswick and Newfoundland and Labrador. They

were selected on a convenience basis based on the size of their small animal clientele. The 10 clinics were contacted by phone, introduced to the project, and asked if they would participate. Nine of ten clinics agreed to participate in the study based on this initial contact. To obtain 10 clinics for participation, another clinic was contacted and agreed to participate. In January of 2001 materials for the survey were delivered in person by the principal investigator (author) to all participating veterinary clinics in Nova Scotia, New Brunswick and Prince Edward Island. The materials for Newfoundland were sent by mail. Upon delivery of the material to the clinics, the project guidelines were discussed in detail with both the veterinarians and staff members. Difficulty was encountered in obtaining sufficient control animals (bitches which the owners were electing not to ovariohysterectomize). For this reason, additional measures were undertaken to recruit more control subjects. This included the addition of a further 7 clinics, and the utilization of a breeders list for the Atlantic Provinces. Each breeder was called and asked if they had a dog between the ages of 4 and 14 months that had not yet had an estrus, that lived in a household setting, and was not going to be ovariohysterectomized in the next year. If they answered yes to all of these questions they were asked to participate in the survey. Due to some breeders being clients of veterinary clinics that were not already part of the survey, 7 more clinics were recruited. The materials were sent to these clinics by mail and instructions and guidelines were discussed with veterinarians over the phone. In all, 24 clinics participated in the study.

The materials sent to each clinic included a 2 page information sheet (appendix 1) and 1 page laminated instruction sheet (appendix 2) for the clinic, 20 log sheets (appendix 3) to record client information, 20 client consent forms (appendix 4), and 20 information sheets (appendix 5) for the clients. The first 10 clinics also received a display sign about the project to exhibit at the

admitting desk. The first 17 clinics were also supplied with 7ml heparinized and non-heparinized vacuum-sealed tubes (Vacutainers©) for blood sample collection (to collect samples for a separate phase of the study).

The veterinarians and veterinary staff were responsible for introducing the survey to clients that could participate in the study: clients that had bitches that were between 4 and 14months of age, that had a minimum body weight of 4kg, had not yet had an estrus, and lived in a household setting. If the client agreed to participate they were given a client consent form to read and sign, as well as an information sheet regarding the survey. The veterinarian filled out a log sheet regarding information on the client and the patient. The veterinarians and staff of the clinics participating in this study received ten dollars for every animal recruited, and the owners of the bitches received a thirty dollar credit to use towards services at the veterinary clinic.

2.2.2. Telephone questionnaire design

The telephone questionnaire, based on a questionnaire developed by Guy et al. (2001) (see appendix 6), was 13 pages in length, including an introductory page, and was comprised of 96 questions. The introductory page provided a description of the research project and its purpose, what types of questions would be asked, and approximately how long it would take to complete. The introduction did not mention aggression as an area of research in the study, but only indicated that the relationship between ovariohysterectomy and dog behaviour would be evaluated by recording general behaviours of the bitches before and after ovariohysterectomy.

There were a total of 79 closed-ended questions and 17 open-ended or descriptive questions in the questionnaire. The questionnaire was divided into several categories:

1) household information and general demographic information about the home

2) demographic information about the dog

3) behaviour of the dog in the first two months of ownership

4) general behaviour of the dog

5) owner attachment levels

6) aggressive behaviours of the dog.

The respondents were given the opportunity ask questions regarding the questionnaire at the end of the interview.

2.2.3. Telephone survey implementation

All interviews were conducted by the author. Pre-testing of the questionnaire was also conducted by the author with 3 convenience selected respondents with variable backgrounds. These included a veterinary student, a graduate student and a dog owner from the general public. Pre-testing took between 15 and 20 minutes with each respondent. No changes in questionnaire design were necessary after pretesting. Two sets of telephone questionnaires were completed. The first set was conducted from February 2001 to December 2001, as bitches were recruited into the study. The second set was conducted from January 2002- May 2002. Owners of controls (intact bitches) were also re-interviewed during this time period. Respondents were required to be a member of the household who was an adult and was responsible for helping take care of the dog. Extensive effort was made to contact clients who had moved or who had changed their telephone numbers.

2.2.4. Data management

Variables were developed for each individual question using the software program EpiData (EpiData Association, Odense, Denmark, Europe). The variables were then electronically transferred from EpiData into the statistical software program, STATA version 7 (STATA Corporation, College Station, Texas, U.S.A.), for analysis. All closed-ended questions were coded as dichotomous variables (1=yes, 0=no), or continuous variables where the values were numerical (ex: age, weight). Open-ended questions were compiled into lists of responses and then broken down into categories. The categories were then analyzed as dichotomous variables, being either yes, belonging to a category, or no, not belonging to a category. Any response of "don't know" was coded as missing data. Data was scanned for outliers, and unusual values were checked against questionnaire responses. All data were entered by the author.

The outcomes of interest for the analyses were growling at the time of the second questionnaire, growling at the time of the first questionnaire, bitches that growled during the second questionnaire, but not the first, excitability levels, and appetite levels of the bitches. Unconditional analyses were conducted on the outcomes of interest using all variables from both the first and second questionnaire responses. Risk ratios were then evaluated to measure the association between the outcomes of interest and the variables. All variables that were significantly associated with the outcomes of interest for this study were included in a multivariable logistic regression model. Only variables that had associations with a *P*-value less than 0.05 were retained in the final models, however, age, weight and reproductive status were forced into the model due to the probability that they may act as confounders. Proportional

differences were also analyzed between ovariohysterectomized and intact bitches for various behaviours.

2.3. Results

2.3.1. Questionnaire completion rates

A total of 137 respondents completed the first telephone questionnaire, and 103 respondents completed the second telephone questionnaire. Thirty-four respondents who completed the first questionnaire could not complete the second for various reasons. No individual refused to complete the questionnaire. The main reason that respondents could not complete the second questionnaire was because their dog went into estrus before the ovariohysterectomy procedure. Animals in estrus represented 41.0% of the non-respondents. Other reasons for inability of the respondents to complete the second telephone questionnaire are listed in Table 1. Eight of the initial owners of the 27 intact animals that completed the first questionnaire did not complete the second, and 26 of the initial 110 animals that were ovariohysterectomized did not complete the second telephone questionnaire for a total of 34 non-respondents. Completion rate for the questionnaire was therefore 73% (see Table 1 for reasons for non-response). The mean time required to complete the first telephone questionnaire was 17.27 minutes, and 15.39 minutes to complete the second.

2.3.2. Demographic characteristics of ovariohysterectomized and intact bitches

A total of 136 respondents (99.3%) reported age. Mean ages of the ovariohysterectomized and intact bitches can be seen in Table 2. The age distribution for the total population of bitches for the first and second questionnaires can be seen in Figures 1 and 2. The mean age of the

ovariohysterectomized and intact bitches were not significantly different at the time of the first questionnaire, but were significantly different at the time of the second questionnaire ($P<0.001$).

Weight was reported by 134 (97.8%) respondents in the first questionnaire and by 102 (99.0%) respondents in the second questionnaire. The mean weights for the intact and ovariohysterectomized bitches were not significantly different in both the first and second questionnaire responses (see Table 2).

Family size, number of children and adults, and number of caretakers of the dog are also presented in Table 2. The distribution of family size for intact bitches, ovariohysterectomized bitches and the total population can be seen in figure 3. The largest proportion of respondents (33.8%) reported that there were 2 people in the family. There were no significant differences between family size or number of adults in the home between the intact and ovariohysterectomized bitches. More than half of the respondents (60.2%) in the total population of bitches reported that they had no children. Owners of intact bitches were more likely to not have children, however this difference was not significant. Approximately seventy-four percent of intact dog owners said that they had no children whereas 57.1% of ovariohysterectomized dog owners reported that they had no children in their home. In both groups, the majority of caretakers were female (79.0% for intact bitches, 84% for ovariohysterectomized bitches).

Breed description and origin of the bitches are reported in Table 2 for intact and ovariohysterectomized bitches. All of the intact bitches were purebred with 58% from the owner's own litter, and 42% from another breeder. Table 3 is a list of breeds in the study. Of

the intact animals who originated from breeders, more than half of the bitches were to be used for breeding purposes. In the ovariohysterectomized bitches, the majority were purebred. Nearly half of the ovariohysterectomized bitches came from breeders (45.23%), however, only one ovariohysterectomized dog owner reported that the dog was obtained for breeding purposes, but that they had later decided not to breed her. A summary of the reasons dog owners listed for obtaining the dog can be found in Table 4.

2.3.3. A summary of reasons given by owners for ovariohysterectomizing their bitches

Reasons for ovariohysterectomizing bitches can be seen in Table 5. Owners could give more than one reason for ovariohysterectomizing their dog. More than half of the total population of dog owners (53.57%) listed that the main reason they ovariohysterectomized their dog was so that the dog could not reproduce. The next most commonly reported reason listed for ovariohysterectomizing was that it was a requirement of a Humane Society or breeder's contract (25%). The third most frequent reason listed for ovariohysterectomizing was to prevent estrus (22.6%). Owners who answered that they ovariohysterectomized their dog to prevent estrus stated that they did not want the complications or behaviours associated with estrus. Some owners answered one or both of these reasons for wanting to ovariohysterectomize. A small proportion of dog owners reported that they wanted to ovariohysterectomize their pets to prevent health problems (7.1%). An additional 5.9% of owners stated that the veterinarian recommended they ovariohysterectomize their pets. The smallest proportion of dog owners indicated that they ovariohysterectomized their dog in hopes that it would calm her down and make her easier to deal with (4.8%). This was the only response that indicated that owners would like to change the behaviour of the dog by ovariohysterectomy.

2.3.4. Destructive and fear-related behaviours in ovariohysterectomized versus intact bitches

Univariable analysis identified significant differences between ovariohysterectomized and intact bitches for destructive behaviours and fear-associated behaviours. A summary of these results can be found in Table 6. Ovariohysterectomized animals were reported in the second questionnaire to be significantly more likely to destroy objects while left home alone (P=0.001). Fourteen of the 50 bitches that were reported to have destroyed things were not solely alone at home, they were housed with another household dog. Thirteen of the fourteen bitches that destroyed things that were housed with another dog were ovariohysterectomized, and only one was intact. There were no significant differences between ovariohysterectomized and intact bitches for other behaviours that may be associated with distress while left home alone, such as whining, vocalizing, or inappropriate elimination.

A number of behaviours that may be associated with fear in bitches were more likely to be reported in ovariohysterectomized than in intact bitches. Ovariohysterectomized bitches appeared to be more fearful of inanimate objects such as vacuum cleaners (P=0.006), and unusual noises (P<0.001), compared to intact bitches. There was also a trend that spayed bitches were more likely to hide underneath furniture than intact bitches (P=0.071). This may be an avoidance behaviour related to fear. There were no significant difference between the weight of the dog and hiding behaviours (P>0.05). It is therefore unlikely that the difference in hiding behaviour between the ovariohysterectomized and intact groups is due to size of the animal (ie: that smaller bitches are more likely to hide underneath objects). There were also no differences between ovariohysterectomized and intact bitches for fear of thunder.

2.3.5. Methods and devices used for training in ovariohysterectomized and intact bitches

A list of the main difference between ovariohysterectomized and intact bitches for training devices and methods can be found in Table 7. Owners of intact bitches were more likely to use crate training (P=0.002) than owners of ovariohysterectomized bitches. Intact dog owners were less likely to use head halters (P=0.046) than owners of ovariohysterectomized dogs, and to obtain information about training from their veterinarian (P=0.035).

In the second questionnaire 38.8% of the total population of dog owners reported that they had purchased books or videos on dog training in the last year. Owners of intact bitches showed a trend of being more likely to purchase books or videos on dog training (P=0.059) than ovariohysterectomized dog owners, and they were significantly more likely to attend training classes (P<0.001). Sixty-two percent of the intact dog owners who said there was a reason for attending training classes stated that socialization was the main reason for attending the classes. Other reasons listed by intact dog owners for their dog attending training classes were obedience training and training for conformation shows. The majority (25.0%) of ovariohysterectomized dog owners stated that the reason for their dog attending obedience classes was to prevent or change a certain behaviour, such as jumping up on people or pulling on the leash while walking.

In the total population of both intact and ovariohysterectomized bitches, 92.2% of owners reported in the second questionnaire that they gave their bitches treats in the form of food for good behaviour. However only 2.9% of the population reported that they made their bitches work for their meals. There were no significant differences between ovariohysterectomized and intact bitches for either of these variables. Only 10.7% of owners said they used some form of

physical punishment to reprimand their bitches when they misbehaved. There were no significant differences between the ovariohysterectomized or intact dogs in the number owners reporting using physical punishment to train their bitches. Owners of intact bitches, however, were more likely to ignore their bitches or withhold a treat for misbehaving (P=0.046). Only 9.5% of ovariohysterectomized dog owners withheld treats or ignored their bitches for misbehaving while 26.3 % of intact dog owners responded that they used this form of negative punishment to correct their bitches for misbehaving.

Incomplete housetraining was defined as a dog having more than 2 training accidents (urinating or defecating in the house) in the previous month before the second telephone questionnaire was conducted. Only 7 bitches (6.8%) in the total population were incompletely housetrained by the time of the second questionnaire. There were no differences between the ovariohysterectomized and intact animals for incomplete housetraining.

2.3.6. Differences between feeding and exercise levels between ovariohysterectomized and intact bitches

Results for feeding and exercising can be found in Table 8. Intact dog owners were more likely to report in the second questionnaire that they did not take their bitches for walks at all when compared to ovariohysterectomized dog owners (P=0.001). There were no differences between the number of ovariohysterectomized and intact dog owners who stated that they let their bitches off leash while on walks, or in the amount time spent free roaming outdoors. Intact bitches were more likely to have been reported to have specialized meal times in the second questionnaire than ovariohysterectomized bitches (P=0.014). Ovariohysterectomized bitches were much more likely to have free choice meals than intact bitches (P=0.002). A special treat is defined as any

food besides regular dog food. There was a trend in the amount of treats that were received by intact versus spayed bitches, but this trend was not statistically significant. Intact bitches were more likely to receive more than five special treats a week when compared to ovariohysterectomized bitches at the time of the second questionnaire (P=0.059).

2.3.7. Difference between socialization with other bitches between ovariohysterectomized and intact animals

Results summarizing socialization with other bitches can be found in Table 9. Numerous questions were asked regarding how much time the dog spent with other bitches, and where the dog spent this time. Spending time with another dog everyday was much more prevalent in intact bitches (P<0.001), as well as spending time with another household dog (P<0.001). The majority of intact bitches spent time with another dog everyday, and in all cases it was with another household dog. Ovariohysterectomized bitches spent more time visiting dogs at a neighbour or friend's house (P=0.002). Thirty-four percent of ovariohysterectomized bitches (compared to 0% of intact bitches) spent time at a neighbour or friends home. Ovariohysterectomized dog owners were also less likely to deliberately socialize their bitches. Owners of ovariohysterectomized bitches showed a trend of being more likely to report that their bitches rarely spent time with other bitches (P=0.055). None of the intact dog's owners reported that their bitches rarely spent time with another dog compared to 16.67% of ovariohysterectomized dog owners.

2.3.8. Aggression and ovariohysterectomy

Growling was defined as "growling at a human even if the caretaker thought it was in play". Growling was a dichotomous variable in which bitches reported to have growled were considered a case and bitches that had not growled were considered a control. New cases of growling were defined as bitches that were reported to have growled during the time of the second questionnaire (after ovariohysterectomy), but had not growled at the time of the first questionnaire (before ovariohysterectomy). New cases of growling was also a dichotomous variable.

Table 10 depicts the proportion of aggressive behaviours in ovariohysterectomized and intact bitches reported in both questionnaires, before and after ovariohysterectomy. Of the ovariohysterectomized animals, 39.28% of them had been reported to have growled at the time of the second questionnaire which was significantly higher than the incidence in the intact group (P=0.004). Table 10 shows that growling is the only aggressive behaviour that is significantly different between the ovariohysterectomized and intact bitches at both the time of the first questionnaire and the time of the second (P=0.006 and P=0.004 respectively). Biting was significantly different between the two groups of bitches before the ovariohysterectomy at the time of the first questionnaire (P=0.040), but was not significant at the time of the second questionnaire. Other aggressive behaviours listed in Table 10 were not different between the ovariohysterectomized and the intact groups, however, only very small numbers of bitches in the total population actually showed these behaviours, so a significant difference between the two groups would be difficult to detect.

The number of ovariohysterectomized and intact bitches that had growled exclusively in the first questionnaire or the second is reported in table 11. Table 11 indicates how many bitches growled in the first questionnaire, second questionnaire, and both questionnaires. Not all bitches that growled during the first questionnaire growled during the second, there were only 17 bitches altogether that growled at both the first and second questionnaire. Some of the bitches that growled during the first questionnaire were not reported to have growled during the second questionnaire, and some of the bitches that did not growl during the first questionnaire, started to growl by the time the second questionnaire was conducted. At the time of the first and second questionnaires roughly the same proportion of bitches from each population growled (38.09% of the bitches that were going to be ovariohysterectomized and 5.26% of the bitches that were going to remain intact (P=0.006)). However, the bitches that growled exclusively during the first questionnaire were different than the bitches that growled during the second questionnaire. Table 12 is a logistic regression model which identifies variables that were potential predictors of growling at the time of the first questionnaire. The sensitivity of the model was 47.06%, and the specificity was 86.57%. This means that bitches that did not growl would be more correctly detected by the model than would bitches that did growl. The positive predictive value was 64.00% and the negative predictive value was 76.32%. This indicates that the proportion of bitches predicted to be growlers, that actually were growlers, was slightly less than the proportion of bitches predicted to be non-growlers that actually were non-growlers, respectively. Age and weight of the bitches were forced into this model due to the possibility of these variables acting as confounding variables. Both these variables were continuous. Age appeared to be associated with incidence of growling at the time of the first questionnaire, but removing age from the model did not appear to change the magnitude of the values in the model. It was therefore probably not

acting as a confounding or interacting variable. Weight was not significantly associated with growling, but was forced into the model, because when it was removed from the model it changes the magnitude of the variables in the model. It was therefore probably acting as a confounding or interacting variable.

Reproductive status (whether the dog was going to be ovariohysterectomized or left intact) was found to be associated with growling at the time of the first questionnaire. Bitches that were to be ovariohysterectomized were 15.11 times more likely to growl at the time of the first questionnaire than bitches that were to remain intact (P=0.014).

Bitches that were obtained from a breeder showed a trend of being less likely to growl at the time of the first questionnaire (OR=0.42, P=0.075). This, however, is not significant, and is above the cutoff of P=0.05, but is included in the model due to its importance in evaluating the environments between the ovariohysterectomized and intact bitches. Also, if the dog's caretaker had previously owned a dog, then the current dog was less likely to growl at the time of the first questionnaire (OR=0.25, P=0.043)

Table 13 represents a logistic model which identifies variables that were potential predictors of new cases of growling that occurred after the ovariohysterectomy procedure. This method of definition for cases gives a better idea of whether the growling was related to the ovariohysterectomy procedure itself instead of other factors in the environment that may have contributed to the growling. The sensitivity of the model was 43.75% and the specificity was 94.23%. This indicates that bitches that were not new growlers would be more correctly detected

by the model than would new cases of growling, and there would be a low number of bitches that would be identified as false positives. The positive and negative predictive values were 70.00% and 84.48%, respectively. This indicates that the proportion of bitches predicted to be new growlers, that actually were new growlers, was slightly less than the proportion of bitches predicted to be non-growlers that actually were non-growlers, respectively. Weight was significant in the model, however, removal of weight in the model had no large effects on the associations between the other variables in the model, therefore it can be assumed that weight is not acting as a confounder. Age also did not appear to be acting as a confounder.

Ovariohysterectomy was found to be positively associated with new incidences of growling. Ovariohysterectomized bitches were much more likely to show new incidences of growling than intact bitches according to this logistic regression model (P=0.015).

Table 14 indicates which aggressive behaviours were associated with the general incidence of growling at the time of the second questionnaire. If a dog growled or snapped when a toy was taken away, while being moved, or while guarding food or toys, the dog was more likely to be reported by owners to growl in general. This indicates that growling was an effective measurement of aggression, it was associated with other forms of aggressive behaviours, and appeared to be repeatable in the questionnaire.

Table 15 represents a logistic regression model which identifies variables that were potential predictors for growling in general at the time of the second questionnaire regardless of their growling status at the time of the first questionnaire. The sensitivity of the model was 62.50%

and the specificity was 88.41%. The positive and negative predictive values of the model were 71.43% and 83.56% respectively. Age and weight were also controlled for in this model, but did not appear to be acting as confounders. In this model, ovariohysterectomized bitches were again more likely to growl when compared to intact bitches (P=0.008).

2.3.9. Other potential predictors for growling in ovariohysterectomized and intact bitches at time of second questionnaire

Other potential predictors for growling at the time of the second questionnaire can be seen in the logistic model for growling at the time of the second questionnaire in Table 15. There was a 3.8 kg increase in the mean weight over the 8.2 month period between the two questionnaires. Bitches that were older than the average age at the time of the first questionnaire were 1.5 times more likely to growl at the time of the second questionnaire (P=0.023). Animals that were heavier than the average weight were more likely to growl. Heavier animals were 1.07 times (per kilogram of body weight) more likely to growl at the time of the second questionnaire (P=0.036).

Owners that stated that there was a reason for obtaining the dog other than just wanting a pet were 7.73 times more likely to have a dog that growled (P=0.002). The reasons given for obtaining bitches that growled varied and no specific reason was more prevalent in bitches that growled when compared to bitches that didn't growl. Only one respondent of the bitches that did growl stated that they wanted a dog for protective purposes (see Table 4).

Owners that stated that they obtained their dog from a family member or friend were 4.99 times more likely to have a dog that growled than owners who obtained their dog from other sources

(P=0.002). Some of the reasons for obtaining bitches that were reported to have growled were that it was given as a gift or that a child had moved out and left the dog behind. There was no way to determine why people obtained their dog from a family member or friend if they responded that there was no reason for obtaining their dog besides just wanting a pet.

Bitches that spent time in the day sectioned off to part of the home prior to the time of the first questionnaire were 4.93 times more likely to growl prior to the time of the second questionnaire (P=0.009). The bitches that were reported as growlers prior to the time of the second questionnaire spent nearly half of their time (48.12%) sectioned off to part of the home in the day time hours. It was not determined just where this time was spent in the home.

Bitches that were walked more than once a day were less likely to growl than bitches who were walked less than this amount (OR=0.25, P=0.018). Univariable analysis also indicates that bitches that were walked more than once a day were also more likely to be allowed off leash while on walks (RR=2.06, P=0.018). There were no significant relationships between the weight of the dog and if it was walked more than once a day, therefore it is unlikely that weight is acting as a confounder in this association (P>0.05). There were also no significant differences in the types of collars or training devices such as head halters, choke chains, etc., used by dog owners that walked their bitches more than once a day (P>0.05). It is interesting to note, however, that the bitches that stopped growling between the first and second questionnaire were reported to be more likely to be walked more than once a day (OR=3.62, P=0.025)

2.3.10. Excitability and ovariohysterectomy

Excitability was a continuous variable and bitches were ranked on a scale of one to ten by the owners, one being very calm and ten being very excitable. The average excitability level reported by the owners in the first questionnaire was 7.11 with the minimum value reported being one and the maximum being 10. The average value reported in the second questionnaire was 7.15 with the minimum value was 1 and the maximum was 10. An increase in excitability was determined by comparing how the owner ranked their bitches for excitability in the first questionnaire to how they ranked their dog in the second. Any bitches that showed an increase in the excitability rank from the first to the second questionnaire were included in this model (n=102). As was done for growling, a logistic regression model was developed that evaluated an increase in excitability between the time of the two questionnaires as the outcome of interest. This model is depicted in Table 16. The sensitivity and specificity were 42.86% and 77.61% respectively, and the positive and negative predictive values were 50% and 72.22%. Ovariohysterectomized bitches were found to be 6.83 times more likely to show an increase in excitability than intact bitches (P=0.019), as seen in Table 16. Age and weight were locked into the model and did not appear to be acting as confounding or interaction terms.

Table 17 represents a logistic regression model that evaluated excitability rank in general for the second questionnaire. The first questionnaire results for excitability were not included in this model. The model looked at the bitches' excitability levels at the time of the second questionnaire and classified bitches that were above the average excitability ranking (5 out of 10) as being very excitable.

Excitability was a dichotomous variable with a value greater than the average excitability value coded as one and a value lower than the average coded as zero. The sensitivity and specificity of these models were 75.00% and 64.44% respectively, and the positive and negative predictive values were 67.35% and 72.50%. In this second model ovariohysterectomized and intact bitches did not differ. Age and weight were also locked into this model. The two models evaluating excitability indicate that ovariohysterectomized animals were more likely to develop an increase in excitability levels when compared to intact bitches but no significant predictors of overall excitability were found that would distinguish the two groups at the time of the second questionnaire.

2.3.11.Other potential predictors of high excitability in bitches at the time of the second questionnaire

Animals that were classified as being very outgoing during the first two months of living with their owners at the time of the second questionnaire were 5.95 times more likely to be highly excitable at the time of the second questionnaire (P=0.004). For this variable bitches were ranked on a scale of one to ten, one being shy and ten being outgoing. The average rank for this variable was 7.00, which was the same as the 50th percentile value for this variable. Bitches that had a ranking that was higher than 7 were classified as very outgoing. There were no associations found by univariable analysis between the ranking for being outgoing in adult bitches at the time of the second questionnaire and a dog being classified as very excitable.

Dog owners that reported that they used a choke chain to walk their bitches were 10.29 times more likely to have a dog that was classified as being highly excitable (P=0.012). Other training

devices were not found to be associated with a dog being highly excitable. There were no significant associations between ranking scores for the dog being outgoing and the use of a choke chain. No significant differences were found in the frequency that the dog was walked with if the dog was let off the leash on these walks, or if the owner used choke chains while walking these dogs.

2.3.12. Reported appetite levels of ovariohysterectomized versus intact bitches

The appetites of ovariohysterectomized and intact bitches were also evaluated. Appetite was a continuous variable and was ranked by the owners using a scale of one to ten, one being a dog that was a very picky eater and ten being a dog that was ravenous. Table 18 represents a logistic regression model with the outcome of interest being animals that had an increase in appetite between the first and second questionnaire. Sensitivity and specificity of the model were 53.85% and 82.54% respectively, and the positive and negative predictive values were 65.63% and 74.29%. There were no differences in appetite between ovariohysterectomized and intact bitches. Ovariohysterectomized bitches therefore did not appear to have an increase in appetite as a result of the ovariohysterectomy procedure.

Table 19 represents a logistic regression model with the main outcome of interest being the general appetite of the bitches at the time of the second questionnaire. The sensitivity and specificity of this model were 61.36% and 77.27% respectively and the positive and negative predictive values were 72.97% and 66.67%. There were also no differences found between ovariohysterectomized and intact bitches for their general appetite at the time of the second questionnaire.

2.3.13. Other potential predictors of above average appetites in bitches at the time of the second questionnaire

Bitches that were obtained directly from a breeder were 5.48 times more likely to have been reported to have an above average appetite (P=0.001), but this did not appear to be related to the purebred status of the dog. Bitches that had attended training classes were also classified as having an appetite that was above average (OR=4.70, P=0.015). Of the training classes attended by bitches that had an above average appetite, 77.27% of these classes used food as a training reward. Bitches that spent time in the day free roaming outside were less likely to be reported as having an above average appetite (OR=0.264, P=0.019).

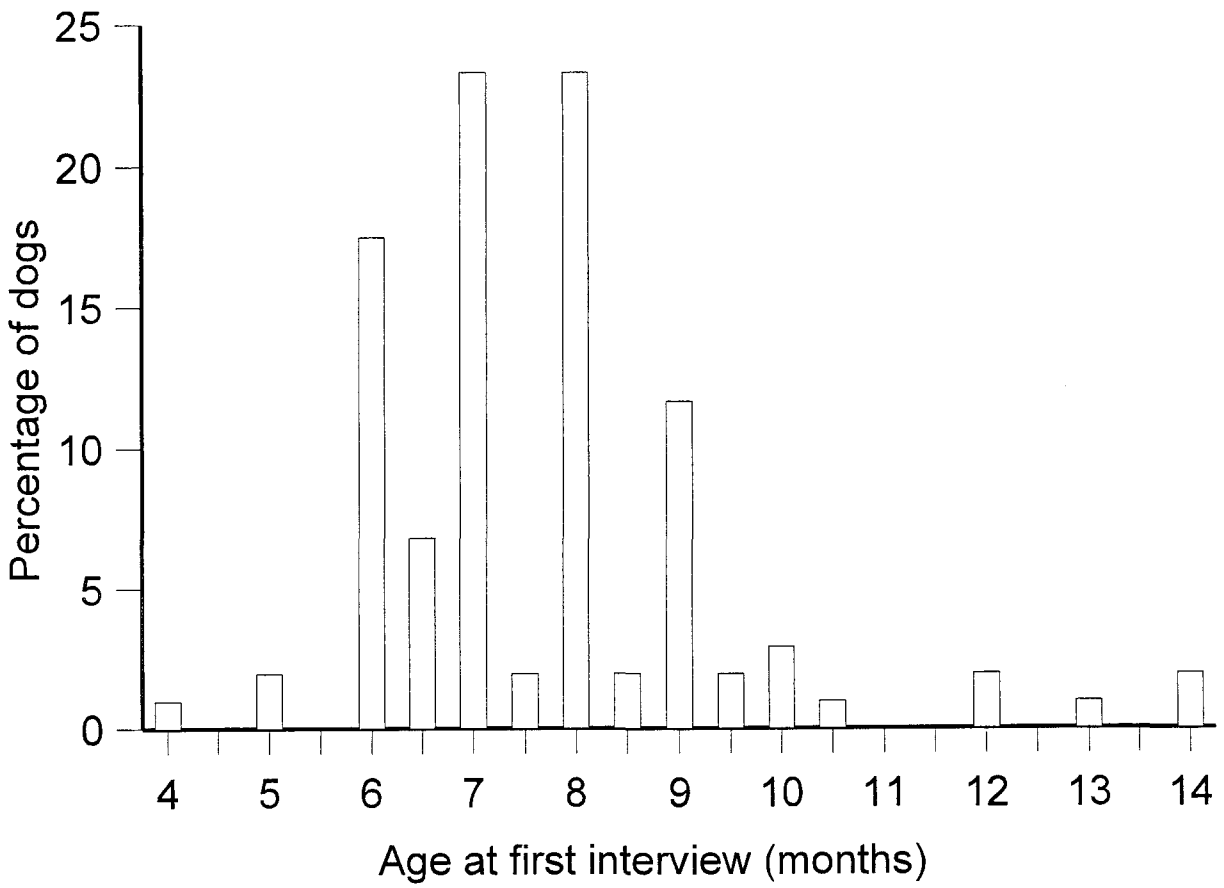

Figure 1. Age of the total population of bitches at the time of first interview (7.7 ± 1.7)

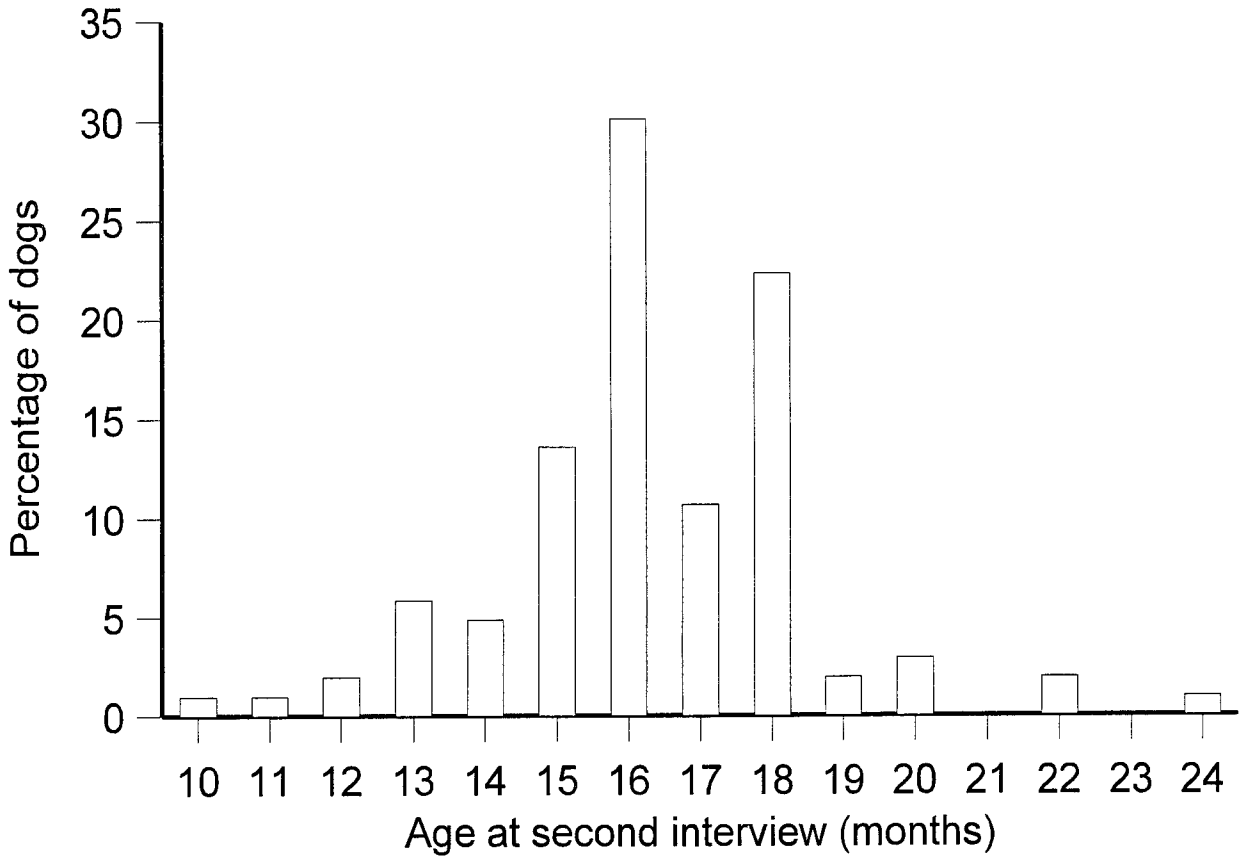

Figure 2. Age of the total population of bitches at time of second interview (16.4 ± 2.3)

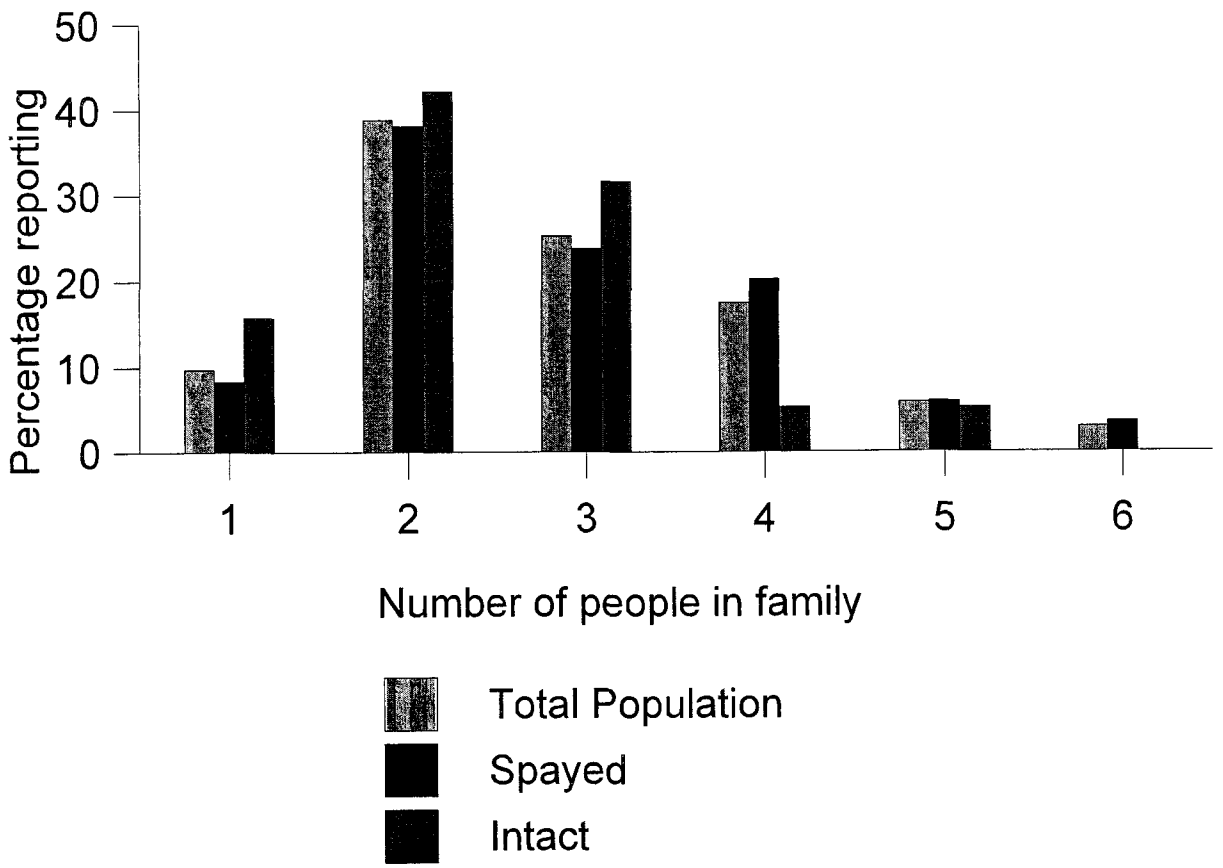

Figure 3. Number of people in family of ovariohysterectomized bitches, intact bitches, and total population

Table1.
List of some reasons for respondents' inability to participate in second questionnaire

Reasons for non-response	Intact	Ovariohysterectomized
Dog given away to another family due to behaviour problems	0	1
Dog euthanized or died	0	2
Dog given to humane society	0	1
Dog sold	2	4
Reason unknown	3	7
Dog was in Estrus	3	11

Table 2.
Demographic characteristics of ovariohysterectomized and intact bitches and their homes

	Intact (n=19)		Spayed (n=84)	
	Mean (SD)	Range	Mean (SD)	Range
Age at first questionnaire (months)	8 ± 2.31	37723	7.63±1.54	5.75-14
Age at second questionnaire (months)*	14.31 ± 2.03	37911	16.91±2.17	38346
Weight (kg) first questionnaire	17.03 ± 11.27	1.81-38.63	16.68±7.53	4.54-30.45
Weight (kg) second questionnaire	19.83 ± 14.82	1.36-54.54	20.87±10.04	3.64-45.45
Family Size	2.42 ± 1.02	37260	2.88±1.22	37261
# of Children in home	0.368 ± 0.76	37439	0.76±0.99	37440
# of adults in home	2.052 ± 0.62	37258	2.15±0.74	37261
# of caretakers for dog	1.053 ± 0.23	37257	1.31±0.58	37258

	Intact			Ovariohysterectomized	
	Description	% Bitches		Description	% Bitches
Breed	Purebred	100	Breed	Purebred	60.7
	Mixbred	0		Mixbred	35.71
	Owner Unsure	0		Owner Unsure	3.59
Origin	Own dog's litter	58	Origin	Own dog's litter	1.19
	Breeder	42		Breeder	45.23
				Humane Society	10.71
				Obtained from family member or friend	23.8
				Pet shop	7.14
				Other	15.47
Primary Caretaker	Female	78.95	Primary Caretaker	Female	84
	Male	21.05		Male	16

* Statistically significant

Table 3.
Breeds of bitches in study

Ovariohysterectomized	n	Intact	n
Beagle	1	Belgian Tervuren	1
Belgian Tervuren	1	Bichon Frise	1
Bernese Mountain Dog	1	Brittany Spaniel	1
Border Collie	1	Cairn Terrier	1
Boxer	3	Nova Scotia Duck Tolling Retriever	1
Dachshund	1	German Shepherd	1
Nova Scotia Duck Tolling Retriever	1	Golden Retriever	1
German Shepherd	2	Great Dane	2
German Wire Hair Pointer	1	Jack Russel Terrier	1
Golden Retriever	6	Labrador Retriever	3
Havanese	1	Shetland Sheep Dog	2
Jack Russel Terrier	1	Springer Spaniel	1
Labrador Retriever	11	Toy Manchester Terrier	1
Poodle (Miniature)	1	Welsh Terrier	1
Poodle (Standard)	1	Yorkshire Terrier	1
Poodle (Unspecified)	1		
Pug	2		
Rottweiler	1		
Samoyed	1		
Schnauzer (Unspecified size)	1		
Scottish Terrier	1		
Shetland Sheep Dog	2		
Shih Tzu	3		
Springer Spaniel	1		
Whippet	1		

Table 4.
Summary of some reasons* for obtaining dog at time of first interview

Reasons for obtaining dog	Ovariohysterectomized (n)	Intact (n)
Liked temperament of breed	8	1
Breeding, showing, genetics and conformation	1	19
Field trials, agility and hunting	3	2
Lost previous dog	15	0
Lonely because lost family member	2	0
Given as gift	1	0
Companion	3	0
To be active with	2	0
Given to owner because previous owner could not keep	4	0
Companion for other dog in house	1	0
Therapy for elderly or children	2	0
Protection	1	0

* Owners could list more than one reason

Table 5.
Summary of main reasons* owners listed for ovariohysterectomizing the dog

Reason	n
Calm her down/easier to deal with	4
Breeder or Humane Society contract	21
No estrus	19
No pups	45
Health	6
Keep other dogs away from owner's house	7
Veterinarian recommended	5

* Owners could list more than one reason

Table 6.
Differences in destructive and fear-related behaviours between ovariohysterectomized and intact animals

	Ovariohysterectomized		Intact		*P*-value
	%	n	%	n	
Destroying objects while left home alone	57.32	47	15.79	3	0.001
Hiding under furniture	29.23	24	5.6	1	0.071
Fearful of vacuum	44	40	10.5	2	0.006
Fearful of unusual noises	61.54	52	10.5	2	<0.001

Table 7.
Differences in training methods and devices between ovariohysterectomized and intact bitches

	Ovariohysterectomized		Intact		*P*-value
	%	n	%	n	
Used crate training	64.28	54	100	19	0.002
Attended training classes	25.3	21	68.42	12	<0.001
Purchased training materials	34.52	29	57.89	11	0.059
Obtained advice from veterinarian on training	47.56	40	21.05	4	0.035
Used head halters to walk their bitches	17.86	15	0	0	0.046

Table 8.
Differences in feeding schedules and exercise between ovariohysterectomized and intact animals

	Ovariohysterectomized		Intact		*P*-value
	%	n	%	n	
Not walked at all	5.95	5	31.58	6	0.001
Scheduled meal times	53.57	45	84.21	16	0.014
Free choice meal times (ad-lib)	42.85	36	5.26	1	0.002

Table 9.
Differences in socialization with other bitches between ovariohysterectomized and intact animals

	Ovariohysterectomized		Intact		*P*-value
	%	n	%	n	
Spent time with another household dog	23.81	20	84.21	16	<0.001
Spent time with another dog at a neighbour or friend's home	34.52	29	0	0	0.002
Spent time with another dog everyday	35.71	30	84.21	16	<0.001
Rarely spent time with other dogs	16.67	14	0	0	0.055

Table 10.
Differences in aggressive behaviours between ovariohysterectomized and intact animals

	Ovariohysterectomized		Intact		*P*-value
	%	n	%	n	
Reports of growling in first questionnaire	38.09	32	5.26	1	0.006
Reports of growling in second questionnaire	39.28	33	5.26	1	0.004
Reports of biting first questionnaire	27.38	23	10.5	2	0.039
Reports of biting in second questionnaire	17.86	15	5.26	1	0.171
Reports of snapping in response to discipline by first questionnaire	4.76	4	0	0	0.332
Reports of snapping in response to discipline by second questionnaire	4.76	4	5.26	1	0.972
Reports of increase in growling between questionnaires	17.86	15	5.26	1	0.171
Growling when toy taken away in first questionnaire	9.53	8	0	0	0.161
Growling when toy taken away in second questionnaire	17.86	15	5.26	1	0.171
Growling when stolen object taken away in first questionnaire	5.95	5	0	0	0.276
Growling when stolen object taken away in second questionnaire	7.14	6	0	0	0.23

Table 11.
Multivariable logistic regression model to explain risk factors for growling in ovariohysterectomized and intact bitches at the time of the first questionnaire (n=103)

Variable	Coefficient	*P*-value	Odds Ratio	95% CI*
Reproductive status (to be ovariohysterectomized =1)	2.44	0.014	15.11	1.71-133.23
Age (months)	2.08	0.038	1.37	1.01-1.84
Weight (kg)	1.29	0.197	1.04	0.98-1.11
Dog was obtained from a breeder	-1.78	0.075	0.42	0.16-1.09
Caretaker owned a dog before	-2.03	0.043	0.25	0.07-0.95

Table 12.
Comparison of the number of ovariohysterectomized versus intact bitches that growled in the first questionnaire and second questionnaire

	Ovariohysterectomized	Intact	TOTAL
Growled 1st questionnaire only	16	1	17
Growled 2nd questionnaire only	32	1	33
Growled at neither 1st or 2nd questionnaire	19	17	36
Growled at both 1st and 2nd questionnaire	17	0	17
TOTAL	84	19	

Table 13.
Multivariable logistic regression model to explain risk factors for new cases of growling in ovariohysterectomized and intact bitches (n=68)

Variable	Coefficient	*P*-value	Odds Ratio	95% CI*
Reproductive status (ovariohysterectomized =1)	2.43	0.015	21.54	1.08-257.24
Age (months)	1.8	0.071	1.55	0.96-2.52
Weight (kg)	1.97	0.048	1.07	1.00-1.15
Owner had reason for obtaining dog other than just wanting a pet	1.94	0.053	4.33	0.98-19.07

* 95 % Confidence Interval

Table 14.

Aggressive behaviours associated with growling incidence at time of 2nd questionnaire

Variable	Growling Bitches			Non-growling Bitches			
	n	Result	%	n	Result	%	*P*-value
Biting	33	no	79	70	no	87	0.852
		yes	21		yes	13	
Dog growled or snapped when toy was taken away	33	no	79	70	no	97	0
		yes	21		yes	3	
Dog growled or snapped while being moved	33	no	85	70	no	100	0
		yes	15		yes	0	
Growling or snapping while guarding food or toys	33	no	70	70	no	90	0.01
		yes	30		yes	10	

Table 15.
Multivariable logistic regression model to explain risk factors for growling at the time of the second questionnaire in ovariohysterectomized and intact bitches (n=101)

Variable	Coefficient	*P*-value	Odds Ratio	95% CI*
Reproductive status (ovariohysterectomized =1)	2.64	0.008	23.5	2.26-224.37
Age (months)	2.27	0.023	1.52	1.05-2.18
Weight (kg)	2.1	0.036	1.06	1.00-1.13
Owner had reason for obtaining dog other than just wanting a pet	3.07	0.002	7.73	2.09-28.56
Dog was obtained from a family member or friend	2.38	0.017	4.99	1.33-18.78
Sectioned off to part of the home in the daytime at the time of the first questionnaire	2.62	0.009	4.93	1.49-16.29
Walked more than once a day	-2.37	0.018	0.25	0.08-0.78

* 95 % Confidence Interval

Table 16.
Multivariable logistic regression model to explain risk factors for increase in excitability in ovariohysterectomized and intact bitches (n=102)

Variable	Coefficient	*P*-value	Odds Ratio	95% CI*
Reproductive status (ovariohysterectomized =1)	2.35	0.019	6.83	1.37-34.00
Age (months)	-1.15	0.251	0.84	0.62-1.12
Weight (kg)	-0.47	0.642	0.99	0.95-1.03
Dog wore traditional buckle collar while on walks	-2.7	0.007	0.29	0.12-0.71

* 95 % Confidence Interval

Table 17.
Multivariable logistic regression model to explain risk factors for highly excitable bitches at time of second questionnaire (n=89)

Variable	Coefficient	*P*-value	Odds Ratio	95% CI*
Reproductive status (ovariohysterectomized =1)	1.25	0.21	3.23	0.51-20.26
Age (months)	-0.16	0.872	0.97	0.72-1.31
Weight (kg)	1.04	0.33	1.02	0.98-1.08
Dog was reported as very outgoing at time of first questionnaire	2.9	0.004	5.95	1.78-19.89
Dog wore choke chain while on walks	2.51	0.012	10.29	1.67-63.55

* 95 % Confidence Interval

Table 18.
Multivariable logistic regression model to explain risk factors associated with increase in appetite levels in ovariohysterectomized and intact bitches (n=102)

Variable	Coefficient	*P*-value	Odds Ratio	95% CI*
Reproductive status (ovariohysterectomized =1)	0.61	0.898	1.07	0.35-3.26
Age (months)	0.12	0.666	0.94	0.73-1.22
Weight (kg)	0.01	0.769	1	0.97-1.04
Dog was fed free choice at the time of first questionnaire	1.37	0.019	2.97	1.20-7.35

* 95 % Confidence Interval

Table 19.
Multivariable logistic regression model to explain risk factors associated with an above average appetite in ovariohysterectomized and intact bitches at time of second questionnaire (n=88)

Variable	Coefficient	*P*-value	Odds Ratio	95% CI*
Reproductive status (ovariohysterectomized =1)	0.56	0.574	1.63	0.29-8.97
Age (months)	0.228	0.228	0.82	0.60-.1.3
Weight (kg)	0.745	0.745	1.01	0.96-1.06
Dog was obtained from a breeder	0.001	0.001	5.48	1.94-15.49
Dog attended training classes	0.015	0.015	4.7	1.35-16.37
Dog spent time free roaming outside in the daytime at time of second questionnaire	0.019	0.019	0.26	0.09-0.80

* 95 % Confidence Interval

2.4. Discussion

2.4.1. Telephone questionnaire response rates and effectiveness of statistical methods

The response rates for telephone questionnaires are typically the highest next to in-person interviews (Dillman, 1978). The telephone questionnaire used in this study had an excellent response rate, facilitated by the fact that owners were asked to participate by their veterinarian prior to being contacted by the interviewer. Not one person refused to complete the telephone questionnaire solely because they did not care to do it. Failure to respond to the questionnaire were due to circumstances such as the dog being relinquished to the humane society for behaviour reasons, the dog having died, or the breeder having to sell the dog.

Information was acquired from this telephone questionnaire regarding the environment of the bitches, and the bitches' health, exercise levels, feeding schedules and owner attachment levels. Some of these variables were associated with the behavioural outcomes of interest in our study and were analyzed to ensure that a complete picture of the association is seen. This study used multivariable statistical models that evaluate the combined effect of many variables on the outcome of interest. This may help identify results that would not be seen if only univariable analyses were conducted.

2.4.2. Comparison with previous surveys

Very few studies have examined the behavioural consequences of ovariohysterectomy in particular. Most studies evaluate the reproductive status of the dog in relation to another behavioural outcome being examined (O'Farrell and Peachey, 1990; Podberscek and Serpell, 1996; Guy et al., 2001). Podberscek and Serpell (1996) determined that neutered cocker spaniel

bitches were more likely to be aggressive when compared to intact females. They were not focusing particularly on the behaviours associated with ovariohysterectomy, but were evaluating general aggressive behaviours in purebred English Cocker Spaniels. Their results may not be applicable to all breeds of bitches. In contrast, our study looked at multiple breeds of bitches, and the results should be more widely applicable.

Guy et al. (2001) conducted a detailed telephone questionnaire which determined the risk factors for dog bites to family members in 227 bitches. In the study by Guy et al. (2001) ovariohysterectomized bitches were 2.13 times more likely to have bitten than intact bitches. This study involved multivariable logistic regression models which determined associations between behaviour, environment and reproductive status. O'Farrell and Peachey (1990) evaluated behaviours associated with ovariohysterectomy in particular, and conducted a detailed in person interview with dog owners regarding the behaviour of their ovariohysterectomized or intact dog. The data analysis of the study by O'Farrell and Peachey (1990) only evaluated correlations between individual behaviours. No multivariable analysis was conducted. Valuable information can be lost by not incorporating multiple factors that may be associated with the behaviour in one statistical model.

2.4.3. Consideration for bias

Selection bias may have occurred when choosing breeders for participation in this study. Breeders were contacted because of the need to recruit more intact bitches (controls) for the study. The breeders chosen were a convenience sample and were not chosen randomly. Control bitches needed to be recruited immediately to adhere to the timeline for the questionnaires, and control

bitches were difficult to obtain due to the small number of people in the general population who don't ovariohysterectomize their bitches. Breeders were most likely to have bitches with the characteristics needed for controls. It was very difficult to obtain a large number of bitches that were intact that lived in a normal household environment. Studies could be conducted with bitches raised in a laboratory setting with equal numbers of intact and ovariohysterectomized animals, however, the behaviour of bitches in this environment would not be representative of bitches raised in a household setting. This would severely limit the relevance of the results.

Due to the fact that all of the intact bitches were either raised by, or obtained from a breeder, it is possible that knowledge regarding dog behaviour among owners of intact bitches may have been different than that of the ovariohysterectomized dog owners. This may have influenced both the responses given by the owners on the questionnaire and the behaviours shown by the bitches. Differences in knowledge may effect training methods used by the owners which would effect the behaviours of the dog. The differences between groups was controlled for by looking at characteristics of the different households, such as the number of dogs in the home, exercise levels, feeding schedules, training methods and the general environment in which the bitches were housed. These variables were incorporated into the logistic regression model. Incorporation of these variables helps to ensure that the difference seen between the two groups was most likely due to the ovariohysterectomy procedure and not due to any other variables that may be influencing the outcomes of interest. All of the bitches in this study were raised in a household environment. Dog owners that were breeders must have raised their dog in a household environment and not in a kennel to be included in this study. Bitches that are housed in a kennel may have different training methods and social interactions with both humans and other dogs than

bitches that are raised within a household. Only selecting bitches that came from a household environment would ensure a more homogenous group of animals.

Clinical trials could also be conducted where the owners of bitches could be asked to refrain from ovariohysterectomizing their dog until the study was completed. This, however, would not be in the best interest of the animal or the owner due to the risk of pregnancy or diseases associated with keeping a bitch intact.

Consequently, an observational study was the only ethical way to evaluate the association between ovariohysterectomy and behaviour. The bitches in this study are ovariohysterectomized at the recommended age, and are raised in a typical household environment. This would most accurately reflect the behaviour of a dog that is kept as a companion animal. With this design it is obviously important to control for as many potential differences between the groups as possible.

2.4.4. Demographic characteristics of ovariohysterectomized and intact bitches

Bitches between the ages of 4 and 14 months were recruited to participate in this study, but the mean age was 7.63 months, reflecting the typical time to neuter a dog of 6-7months of age. It was mandatory that the bitches did not have a estrus before the time of the first questionnaire to remove this potential source of variability among the bitches. The age of the bitches was fairly consistent at the time of the first questionnaire, but was more variable by the time of the second questionnaire. The discrepancy in age between the two groups may have been because a larger number of participants in the ovariohysterectomized group did not comply with the time that they

were recommended to return to their veterinarians for their second visit and telephone questionnaire.

The average family size (2.42) in our data is still fairly low when compared to the family size reported by Statistics Canada in 2000 (3.0). The difference in our results may be due to the smaller sample size compared to the census information, or because dog owners may have smaller families than the average population. Guy et al. (2001), however, conducted a survey with 227 dogs in the same geographic region and found a family size of 3.13 which was consistent with the report of the 1996 Canadian census.

Due to the large diversity of breeds in this study, the bitches were given the basic classification of either mixedbred or purebred for the analysis. All of the intact bitches were purebred and most were from a breeders' own litter or were purchased for the purpose of breeding or showing. Only one ovariohysterectomized dog owner that obtained a dog from a breeder indicated that the dog was originally obtained for breeding purposes, and owners of intact bitches were more likely to report that there was a specific reason for obtaining their dog. Owners of ovariohysterectomized bitches appeared to be adopting bitches primarily for reasons of companionship. This difference in attitude between the two groups may reflect different attitudes toward training and behaviour.

The percentage of bitches that were mixbred or of unspecified breeding is comparable to results reported by Guy et al. (2001) which indicated that 39.9% of bitches seen in veterinary clinics in this region were mixbred. It is possible that purebred bitches are seen more frequently by veterinarians. This may be because owners that have more economic interest in a purebred dog

are also more likely to spend money on veterinary services for their bitches. It is also possible that purebred bitches are more predisposed to health problems than mixbred (Proschowsky et al., 2003).

2.4.5. Public perception of ovariohysterectomy

Control of pet population was the most frequently reported reason for ovariohysterectomizing bitches in this study. More than half of the dog owners (53.57%) reported that they ovariohysterectomized their dog to prevent unwanted puppies. It is evident that pet owners in this region may believe that ovariohysterectomizing is the most effective way to prevent unwanted litters of puppies. European dog owners are using alternative approaches to control pet population such as progestin therapies (Jochle, 1991). Only 4.76% of dog owners in this study stated that they ovariohysterectomized their dog in attempt to calm her down or make her easier to deal with. The small proportion of owners that were concerned with changes in behaviour believed the opposite of what previous literature has reported which is that ovariohysterectomizing may make a dog more active (Salmeri et al., 1991).

Despite the decreased risk of mammary carcinoma in ovariohysterectomized bitches (Sorenmo et al., 2000), decreased risk of pyometra (Feldman and Nelson, 1987), and obesity (Edney and Smith, 1986), very few owners (7.14%) reported that they ovariohysterectomized their dog to prevent health problems. Thirty-one percent of the population reported that a breeder, humane society or veterinarian recommended that they ovariohysterectomize their dog. The intent of these recommendations may have involved ovariohysterectomizing to prevent health problems.

2.4.6. Risk factors for new cases of growling in ovariohysterectomized and intact bitches

Ovariohysterectomized bitches were more likely to develop a new case of growling when compared to intact bitches (OR=21.54, C.I.=1.08-257.34). The confidence interval for this association is very wide, suggesting that there is considerable uncertainty about the strength of the association between ovariohysterectomy status and incidence of new cases of growling. The exact magnitude of the association is not clear, but the direction of the association is strongly positive. This association indicates that the ovariohysterectomy itself may be related to growling in bitches, and that the difference may not be due to other differences between the ovariohysterectomized and intact groups of bitches.

Another risk factor for a new case of growling was if the owner stated that there was a specific reason for obtaining the dog other than just companionship. Owners who said there was a reason showed a trend of being more likely to have a dog that developed a new case of growling after the time of the ovariohysterectomy procedure (P=0.053). It is difficult to say why owners who had a reason for obtaining their pets would be more likely to have a dog that developed growling behaviours. It is possible that the owners with different expectations for their dog had different ways of interacting with it. The majority of intact dog owners stated that the reason for obtaining their dog was for breeding, showing, genetics or conformation, which are mainly working purposes. Most ovariohysterectomized dog owners, however, stated that they obtained their dog because their previous dog had died or was given away. This suggests that they obtained a new dog to replace the companionship that the old dog provided. Owners who had reason for obtaining their new dog may been more frustrated when the dog did not meet their expectations. This in turn may affect the bond between the owner and the dog. Owners who stated that they had a reason

for obtaining their dog other than just companionship were less likely to seek out their dog for companionship when they were feeling lonely, frustrated or sad (RR=0.634, P=0.018,). This suggests that owners that stated there was a reason for obtaining their dog, and had particular expectations may be less attached to them. Serpell (1996) found that moderately attached dog owners appeared to be less satisfied with their dog's behaviour when compared to highly attached owners. Serpell (1996) recorded the actual and ideal rating reported by dog owners for the behaviour of their dogs. Dogs in the moderately attached group had larger differences between the actual and ideal ratings, suggesting that these dogs did not meet the moderately attached owners' expectation of an ideal dog. Caution must be used when interpreting the results of the study by Serpell (1996) because it involved self-assessments of the owners' attachment levels, and was based on a convenience sample of only 37 dog owners. Other questions which may indicate attachment levels, such as purchasing gifts for their dog, giving her treats for good behaviour or allowing her to rest on furniture, were not significantly different between owners who had a reason for obtaining their bitches and those who didn't. Voith et al. (1992) did not find a relationship between permissive behaviour of dog owners and reports of aggression.

Age and weight were locked into this model because of their potential to act as confounding variables. Adding or removing age or weight did not, however, have a significant impact on the association between ovariohysterectomy and new cases of growling. Weight appeared to be only marginally associated with new cases of growling. Animals that were larger were more likely to develop a new case of growling. Body weight has generally been regarded as being associated with the onset of puberty in domestic animals; larger animals take a longer time to reach their mature body weight and therefore will reach puberty at a later age (Arthur et al., 1983). This

physiological difference may be contributing to the difference in aggression, although it is important to remember that no bitches in the study were reported to have had an estrous cycle.

2.4.7. Risk factors for growling at the time of the second and first questionnaire in ovariohysterectomized and intact bitches

At the time of the second questionnaire ovariohysterectomized bitches were 23.50 times more likely to growl when compared to intact bitches (P=0.008, C.I.=2.26-244.37). It is difficult to determine from this information whether the growling was due to the demographic differences between the two groups, or whether it was due to the ovariohysterectomy procedure itself. In the first questionnaire, the bitches that were to be ovariohysterectomized were also more likely to growl than bitches that were to be left intact (P=0.014, C.I.=1.71-133.23). This indicates that the group of ovariohysterectomized bitches may have been more likely to growl even before the ovariohysterectomy procedure. It is possible that there may have been a fundamental differences between the two groups of bitches and the environments in which they were raised, an argument which is further supported by the trend that bitches that are obtained from a breeder were less likely to growl (P=0.075, C.I.=0.16-1.09). This is only marginally significant, but nonetheless is important to take into consideration. The magnitude of the odds ratio for growling at the time of the second questionnaire, however, is much higher than that of the first questionnaire, suggesting that even though differences between the groups of ovariohysterectomized and intact animals were most likely present, the ovariohysterectomy procedure itself may also be playing a part in contributing to the increase in growling in the ovariohysterectomized group of bitches. Also, as indicated above, not all bitches that growled in the first questionnaire continued to growl at the time of the second questionnaire, and there were bitches that did not growl at the time of the first

questionnaire that started to growl by the time of the second questionnaire. A model was developed that accounted for this by evaluating only new cases of growling, with the model indicating that ovariohysterectomized bitches were more likely to growl than intact. It is therefore possible that the ovariohysterectomy procedure itself could be contributing to new cases of growling. Finally, every effort was made to control for environmental factors in the logistic regression models for growling, which should act to minimize the effects of these factors. Next to a laboratory study or clinical trial, which are unlikely to be reflective of a bitches' natural environment, this is the best that can be done to obtain accurate information regarding incidence of growling.

Having a reason for obtaining the dog was also associated with general growling in the second questionnaire which would be expected since it was associated with new cases of growling. Owners that obtained their dog from a family member or friend were also more likely to have a dog that growled at the time of the second questionnaire (P=0.017). Guy et al. (2001) also found that a dog was more likely to have bitten if it was obtained from a relative or friend. It is possible that a dog may be given away from its original home due to behaviour problems, or that people may be talked into taking a puppy from a family member or friend when they are not truly prepared to do so.

Bitches that were restricted to part of the home in the day at the time of the first questionnaire were more likely to growl at the time of the second questionnaire (P=0.009). The dog may have been restricted to part of the home at an early age in attempt to deal with problem behaviours at that time. It is also possible that spatial or social restriction may cause bitches to become more

stressed and in turn aggressive. Beerda et al. (1998) found that dogs that were switched from a spacious group housing setting to a 1.7m^2 isolated kennel showed aggressiveness towards conspecifics, a heightened state of excitablity and significantly more behaviours associated with chronic stress such as autogrooming and placing paws on conspecifics. Conversely, Leadon and Mullins (1991) found increasing cage size appeared to have no effect on plasma cortisol or ACTH, when evaluating greyhounds that were accustomed to cages that were 38cm in width who were subsequently switched to wider cages that were 80 cm in width.

Bitches that were walked more than once a day were less likely to growl than bitches that were walked at lower frequencies (OR=0.251, P=0.018). Bitches that were walked more than once a day were also more likely to have stopped growling by the time of the second questionnaire if they had growled at the time of the first questionnaire (OR=3.62, P=0.025). Guy et al. (2001) also found that in a telephone questionnaire involving 227 dog owners, 6 bitches had been reported to never go outside, and all 6 of these bitches had been reported to have bitten. Well-behaved dogs, in general, may be more likely to be taken for walks. Owners may find it easier to take a dog that does not behave aggressively on walks. Frequent walks may also be protective in preventing aggression in bitches. The physical exercise may be producing physiological effects such as moderate glucocorticoid and endorphin release, which can have a morphine-like effect (Kaneko, 1989; Norman and Litwack, 1987). It is also possible that bitches that were walked more than once a day had more of an opportunity to be socialized with people and other animals. Beerda et al. (1998) found in a variety of challenges that socially and spatially restricted dogs exhibited a heightened state of aggression towards conspecifics. Socialization may stabilize the communication between people and animals. Clear communication will generally make a dog less

likely to respond to a situation with aggression (Rugaas, 1997). Dogs that are walked more than once a day may also be more likely to experience novel situations more regularly than dogs that are walked at lower frequencies. This may make the animal more adaptable to different environments and possibly less likely to be aggressive.

2.4.8. Demographic characteristics of bitches reported to have growled in second questionnaire

There were no significant differences between purebred and mixbred bitches regarding incidence of growling at the time of the second questionnaire. This is in contrast with results found by Guy et al. (2001) who reported that mixed bred dogs were more likely to growl, but not to bite. This may be due to the larger sample size in the study by Guy et al. (2001).

The family size of growling bitches was slightly larger than the average reported family size of 3.0 reported by Statistics Canada in 2000, and was larger than family size of the total population in this study. This suggests that bitches that growl may be more likely to live in homes with more people. Owners of growling bitches were found to have more children (<18 years of age) in the home (P=0.013). This result is consistent with Guy et al. (2001) who found that the presence of one or more teenagers in the home was a risk factor for biting in dogs.

2.4.9. Excitability levels in ovariohysterectomized versus intact bitches

Ovariohysterectomized bitches were found to be more likely to have an increase in excitability after the time of the ovariohysterectomy procedure (P=0.019). Previous literature has indicated that ovariohysterectomized bitches are more likely to have a higher general activity level when compared to intact bitches (Salmeri et al.,1991). It is possible that the owners would describe a high activity level as being excitable.

2.4.10. Other risk factors for high excitability levels in ovariohysterectomized and intact bitches at the time of the second questionnaire

Excitability was not found, however, to be associated with growling or biting in our study. Guy et al. (2001) found that dogs that had bitten were ranked by their owners as being more excitable as puppies than dogs that had not bitten. It is possible that the behaviour of the dog as a puppy had influenced the owner's perception of its excitability as an adult. The owner may have remembered a particular instance where the puppy was excitable, or base their ranking on a few events. Age and weight were locked into the model but neither were associated with an increase in excitability suggesting that they were not acting as confounders.

Using a standard buckle collar as opposed to another type of collar for the purpose of walking the dog appears to be associated with less excitability. Dog owners that used a standard buckle collar on their dog, as opposed to any other type, had a dog that was less likely to develop an increase in excitability (P=0.007, OR=0.288). The difference in excitability may be associated with the fact that owners who have a dog that is less excitable may be more likely to use a standard collar. If a dog is easier to walk then there is no need to use another type of collar.

Previous studies have indicate that there are no differences in cortisol levels between dogs who wear regular buckle collar and those who wear a head halter (Ogburn et al., 1998). Forceful restraint with choke or prong collars, however, may cause anxiety and frustration, and in turn, trigger aggression (Lindsay, 2001).

Owners who have reported that they used a choke chain to walk their bitches were more likely to have a dog that was excitable (P=0.012). The choke chain may have been used on bitches that were excitable in the first place in an attempt to control this behaviour. It is also possible that the choke chain itself is causing the behaviour. If used appropriately, the dog will learn to avoid the correction which is a negative reinforcement of heeling behaviour. If used inappropriately, the aversive stimulus is unpredictable and may cause stress in the dog

Bitches that were reported as being outgoing as a puppy were more likely to be highly excitable (OR=5.95, *P*=0.004). This indicates that the behaviour of the dog when she was a puppy may be a predictor of how excitable she will be as an adult. Owners should be aware that puppies that are highly outgoing may be excitable as adults, and should be prepared to use preventative strategies to avoid problem behaviour. Alternatively, Wilsson and Sundgren (1997) found that the willingness of 8 wk old puppies to approach and make contact with people had no significant associations with "nerve stability" at maturity (15-20 months of age). "Nerve stability" in this study was not defined. Nerve stability as reported by Wilsson and Sundgren (1997) and the owners' perception of excitability in our study may be quite different.

2.4.11. Reported appetite levels in ovariohysterectomized versus intact bitches

Obesity is one of the most common concerns regarding malnutrition in bitches (Edney and Smith, 1986; Sibley, 1984). A common question that is asked by dog owners when having their dog neutered is if their pet will gain weight as a result of the procedure. Although this study does not examine obesity, appetite (or perceived attitude toward food) and body weight were recorded. The appetite evaluated was as reported by the dog owners. This was examined to determine if an increase in appetite occurred after ovariohysterectomy.

Ovariohysterectomized bitches were no more likely to develop an increase in appetite than the intact bitches. The results for appetite in ovariohysterectomized versus intact bitches are varied in previous literature. Salmeri et al. (1991) reported that there were no changes in appetite, food intake or weight gain after gonadectomy in male and female dogs. Other studies have found the opposite, that there is an increase in appetite after the ovariohysterectomy procedure (O'Farrell and Peachey, 1990) and that neutered bitches were twice as likely to become obese when compared to intact bitches (Edney and Smith, 1986). The appetite levels reported by Salmeri et al. (1991) were reported by the researcher, and the bitches were raised in an atypical laboratory environment, whereas in this study and the study conducted by O'Farrell and Peachey (1990) appetite levels were reported by the owners of the bitches. This difference in both environment and reporter may cause the appetite scores between studies to appear different and give varying results. The study conducted by Edney and Smith (1986) which reported obesity levels in ovariohysterectomized bitches used veterinarians to rank the bitches with a condition score on a five point scale. Again, different reporters may reveal different results, and condition score was used as opposed to actual weight gain. Houpt et al. (1979) found that the time of the estrous cycle

was associated with mean daily caloric intake in bitches. Mean caloric intake was lowest during estrus, and that ovariohysterectomized bitches gained significantly more weight and consumed more calories in the first ten days after the surgery, when compared to sham operated controls. The study by Houpt et al. (1979) used purebred beagles that were individually housed in fiberglass cages in a laboratory environment. This environment may not be comparable to a household setting. Bitches in a laboratory setting may not eat the same amount as bitches in a household environment because there are many factors, such as the presence of other people and animals, which may influence how much food a dog consumes.

Although the study that most closely reflects our design for indication of appetite levels is that reported by O'Farrell and Peachey (1990), which allowed the owners to rank their bitches appetite, it yielded opposite results to ours. O'Farrell and Peachey (1990) found that there was an increase in indiscriminate appetite in ovariohysterectomized bitches. There were no differences between whether or not a dog was purebred and it's appetite score, however there were quite a wide variety of purebred bitches in our study, which could in turn cause great variability in appetite scores. O'Farrell and Peachey (1990) matched for breed, which may account for some of the difference. Although there is no specific information on appetite levels of different breeds of dogs, incidence of obesity may vary in different breeds (Mason, 1970). This may or may not be related to appetite.

Neither the age nor the weight of the dog appeared to have an association with an increase in appetite. Animals that were fed free choice at the time of the first questionnaire were more likely to develop an increase in appetite. It may be that the animals fed free choice are always expecting

to find food . When there is no food in the animals' bowl it may seek out the owner to replenish it. This may give the appearance that the dog has a large appetite.

2.4.12. Risk factors for large appetites in ovariohysterectomized and intact bitches

An "above average appetite" was defined as an appetite that was above the average value for the total population of bitches. Ovariohysterectomy did not appear to influence whether or not a dog had a large appetite. Age and weight were locked into the model, but did not appear to be significant and were not acting as confounders. One risk factor for an above average appetite was being obtained from a breeder. Bitches obtained from a breeder were more likely to have an above average appetite when compared to bitches obtained from other sources (P=0.001). This may be due to the fact that animals obtained from breeders are purebred. Univariable analysis indicated that purebred bitches were more likely to have an above average appetite when compared to mixbred bitches (RR=1.326, P=0.039). Other studies have indicated that various purebred bitches were more likely to be obese when compared to mixbred bitches (Edney and Smith, 1986). No reports have been made on the appetite of purebred versus mixbred bitches.

Bitches that attended training classes were 4.70 times more likely to have an above average appetite (P=0.015). If the dog received treats as a reward for good behaviour in class, it may show more interest in treats than a dog that was not receiving food rewards for training. There was no association, however, between the owner reporting that he/she gave the bitches treats and the dog having an above average appetite. It is also possible that more purebred than mixbred bitches may attend training classes. There was no significant association between attending training classes

and being a purebred dog when univariable analysis was performed, however, bitches that were purebred showed a trend of being more likely to attend training classes (RR=1.29, P=0.065).

Bitches that were allowed to roam freely outdoors in the daytime were less likely to have a large appetite. The dog may be eating other sources of food outdoors, bitches that are outside may not be concentrating on food as much as a dog that is housed indoors and has little stimulation. Outdoor bitches may have more environmental enrichment and may be less likely to eat as a displacement behaviour.

2.5. Conclusions

Response rates for this telephone questionnaire were excellent, with no respondent refusing to complete the questionnaire. Level of participation was higher in the first telephone questionnaire than the second, mainly due to a small population of owners that had relinquished or sold their bitches. Ovariohysterectomized dogs were more likely to have become aggressive, when measured by incidence of growling, when compared to intact bitches. Dogs in the ovariohysterectomized group were more likely to growl even at the time of the first questionnaire, but the risk of growling at the time of the second questionnaire was much larger. Ovariohysterectomized dogs were also more likely to develop new cases of growling than intact bitches. Growling in general appeared to be a good indicator of aggression since it was associated with other possible forms of aggression such as growling or snapping when a toy was taken away, or when the dog was moved, and growling or snapping while guarding food and toys. Ovariohysterectomized bitches were also more likely than intact bitches to become be reported to have become more excitable. Appetite did not appear to differ between the ovariohysterectomized

and intact bitches. In conclusion, there were significant differences in incidence of growling, and excitability, but not appetite levels when comparing ovariohysterectomized and intact bitches. It is still not certain whether these differences are due to the ovariohysterectomy procedure, or are partially a result of the fundamental undetected differences between the two groups.

3. Changes in Leukocyte Parameters Associated with Canine Ovariohysterectomy

Abstract

A sample of 103 pet bitches from the Canadian provinces of Nova Scotia, New Brunswick, Prince Edward Island and Newfoundland were selected in order to determine risk factors associated with a pet bitch developing a neutrophil to lymphocyte ratio greater than 3.5, or a lymphopenia, both of which were considered potential indicators of enduring stress in bitches. A total of 84 bitches that were ovariohysterectomized and 19 that were intact were included in the population. All bitches were between the ages of four and fourteen months when they were recruited, and all prior to their first estrus. The bitches were derived from household settings to ensure that there were no extreme variations in environmental conditions, such as living in a kennel environment. Two blood samples were taken from the ovariohysterectomized and intact bitches to determine if the bitches had either of these white blood cell measures . The first blood sample was taken at the time of ovariohysterectomy (or similar age in intact bitches) and the second was taken approximately 8 months after the time of ovariohysterectomy. A 13 page telephone questionnaire was conducted with the owners of the bitches at the approximate time of ovariohysterectomy, and again eight months later. The telephone questionnaire provided detailed information about aggressive behaviours in the bitches, demographics of the household, demographic information about the dog, general behaviour of the dog, and owner attachment levels. Risk factors for a neutrophil to lymphocyte ratio higher than 3.5 were determined from the information provided by the questionnaire. Significant risk factors for a dog having a ratio higher

than 3.5 at the time of the second questionnaire were if the dog was intact (P=0.028, OR=12.19), and if the dog had worn a head halter on walks (P=0.011, OR=26.27). Variables that were protective were the owner having purchased books or videos on dog training (P=0.021, OR=0.06) and the dog having been walked more than once a day (P=0.037, OR=0.06). Few animals had lymphopenia, therefore difference between ovariohysterectomized and intact bitches for lymphopenia could not be determined. Aggression was also evaluated in detail to determine if a dog having a ratio higher than 3.5 was a risk factor for incidence of growling. A dog having a ratio higher than 3.5 did not appear to be a risk factor for incidence of growling.

3.1. Introduction

A stress response is a combination of physiological and behavioural responses that help an animal to cope with its environment and re-establish homeostasis (Nelson, 2000). The evaluation of stress in domestic animals has been a topic of controversy for many years. For accuracy in interpretation, behavioural and physiological responses to stress must be considered together, along with many other factors. The animal's age, sex, species, the type of and duration of aversive stimuli and time taken to measure the stress response must be considered when determining or evaluating a stress response in domestic animals (Mason and Mendl, 1993). In the domestic dog stress may arise from the animal trying to either reunite with the owner or cope with its social environment in a household setting (Lindsay, 2001). One of the most common stress-related behavioural problems in dogs is separation anxiety/frustration (Knol, 1987; Wright and Nesselrote, 1987). This in turn may lead to behavioural problems such as excessive barking, chewing and house-soiling in an attempt to cope with the stress of the owner not being present (Lindsay, 2001). Dogs may also become stressed if the household environment is not predictable

or controllable. In an examination of the effect of environmental control in rats Weiss (1971) found that the length of gastric lesions resulting from long-term stress was less when the rat had greater control over its environment and when the environment was predictable. In an analagous situation, dogs may be stressed in training if the owner is not predictable and the dog does not know what behaviours to perform to avoid punishment or earn rewards. Bitches that have attended training classes, for instance, may be less likely to show fearful behaviours such as shooting phobias related to hunting (Rugbjerg et al., 2003). Phobias in dogs may be indicative of other stress-related problems in dogs. For example, Overall et al. (2001) found that there was an 88% probability that dogs with noise phobias would also have separation anxiety. Stressors in dogs are usually not acute, such as extreme temperature variations that might occur in the wild, but are long-term (ie: separation anxiety) and will probably not result in death (Lindsay, 2001). They may, however, result in both physiological and behavioural responses that are evidence of compromised welfare.

For the purpose of this study, long-term stress was evaluated to ensure that the measurements reflected the household situation, and not acute stress as a result of the blood collection procedure. White blood cell counts were used to determine if the ratio of neutrophils to lymphocytes was elevated (indicative of long-term stress) or if a lymphopenia was present. Stress that is longer than 4-6 hours is considered long-term stress for the purpose of this study because this is the time it takes corticosteroids to cause lymphopenia (Norman and Litwack, 1987, Latimer and Rakich, 1989).

In dogs, the ratio of neutrophils to lymphocytes is normally 3.5:1 (Aiello and Mays, 1998). Inflamation and stress can cause changes in this ratio (Aiello and Mays, 1998). Acute stress due to excitement and fright will cause lymphocytosis and the neutrophil to lymphocyte ratio will decrease. Stress which causes cortisol release for periods longer than 4-6 hours will cause lymphopenia, and the neutrophil to lymphocyte ratio to go up (Latimer and Rakich, 1989). The normal absolute value for lymphocytes in dogs is 1.0-4.8 x 10^9/L (12-30%) (Atlantic Veterinary College, 2003). Although neutrophil to lymphocyte ratios and percentages have been used in the past and may still be used to measure stress (Song et al., 1995; Connor et al., 1997; Stull and Rodiek, 2002; Zahorec, 2001), absolute values of white blood cells are used more commonly at present because they are a more accurate method of evaluation (Latimer and Rakich, 1989; S. Burton, 2003, personal communication). The objective of this study was to determine if ovariohysterectomized bitches were more likely to have white blood changes indicative of stress compared to intact bitches, and evaluate which factors other than ovariohysterectomy were risk factors associated with these measures of stress in bitches.

3.2. Materials and Methods

3.2.1. Subjects and clinic recruitment

In November of 2000, a total of 10 veterinary clinics were initially recruited for participation in this survey. The clinics were chosen from the Atlantic provinces of Nova Scotia, Prince Edward Island, New Brunswick and Newfoundland and Labrador. These clinics were a convenience sample and were selected based on the volume of their small animal clientele. The 10 clinics were contacted by phone, introduced to the project, and asked if they would recruit subjects for this study. Nine of ten clinics agreed to participate in the study based on this initial contact, and

an additional 10th clinic was then recruited. In January of 2001 materials for the survey were delivered in person by the principal investigator to all participating veterinary clinics in Nova Scotia, New Brunswick and Prince Edward Island. The materials for Newfoundland were sent by mail. Upon delivery of the material to the clinics, the project guidelines were discussed in detail with both the veterinarians and staff members. Difficulty was encountered in obtaining sufficient control animals (bitches which the owners were electing not to ovariohysterectomize). A breeders list for the Atlantic Provinces was used to recruit more bitches that were intact. Each breeder was called and asked if they had a dog between the ages of 4 and 14 months that had not yet had a estrus, that lived in a household setting, and was not going to be ovariohysterectomized in the next year. If they answered yes to all of these questions they were asked to participate in the survey. Because some breeders were clients of veterinary clinics that were not already part of the survey, 7 more clinics were recruited. The materials were sent to these clinics by mail and instructions and guidelines were discussed with veterinarians over the phone. In all, 24 clinics participated in the study. These clinics and subjects were the same as those discussed in the first chapter of this thesis.

The materials sent to each clinic included a 2 page information sheet (appendix 1) and 1 page laminated instruction sheet (appendix 2) for the clinic, 20 log sheets (appendix 3) to record client information, 20 client consent forms (appendix 4), and 20 information sheets (appendix 5) for the clients. The first 10 clinics also received a display sign about the project to exhibit at the admitting desk. The first 17 clinics were also supplied with heparinized and non-heparinized vacuum-sealed tubes (Becton Dickinson Vacutainer ©) for blood sample collection.

The veterinarians and veterinary staff were responsible for introducing the survey to clients who owned bitches that were eligible for participation in the study, ie: bitches that were within 4-14 months of age, that had a minimum body weight of 4kg, had not yet had a estrus, and lived in a household setting. If the client agreed to participate they were given a client consent form to read and sign, as well as an information sheet regarding the survey. The veterinarian filled out a log sheet of information on the client and the patient, and collected 2 tubes of blood, one heparanized and one non-heparanized tube. The veterinarian then forwarded the blood, log sheet and consent form to the Atlantic Veterinary College by courier. A complete blood count was performed by the diagnostic services unit of the Atlantic Veterinary College. Serum samples were frozen and stored for future analysis.

3.2.2. Blood sample collection and analysis

Blood samples were collected by the veterinarian when the dog was presented to the clinic for the ovariohysterectomy procedure, or at the approximate age the dog would be ovariohysterectomized for intact animals, and again approximately 8 months later. One heparanized and one non-heparanized Vacutainer TM brand (Becton Dickinson, Franklin Lakes, NJ) tube was taken from all bitches at both sampling times. No blood samples were taken from bitches that were determined by the veterinarian to be ill or injured.

Complete blood counts were performed on whole blood samples using an Abbott cell dyne 3500 (Accelerated Technology Laboratories, West End, NC). Differential blood samples were evaluated and white blood cell ratios were calculated by hand. Calculations for neutrophil to lymphocyte ratios were done by adding the neutrophil, and eosinophil counts and dividing them by

the lymphocyte counts. Serum samples were centrifuged within 24 hours after collection and stored at -30° Celsius until testosterone assays were conducted (see Appendix 7 for testosterone analysis methods and results).

3.2.3. Telephone questionnaire design

The telephone questionnaire was the same as that discussed in the first chapter of this thesis. The telephone questionnaire was based on a questionnaire designed by Guy et al. (2001) (see appendix 6). It was 13 pages in length, including an introductory page, and was comprised of 96 questions. The questionnaire took 18 minutes on average to complete with each respondent. The introductory page provided a description of the research project and its purpose, the types of questions that would be asked, and approximately how long it would take to complete. The introduction did not mention aggression as an area of research in the study, but only indicated that the relationship between ovariohysterectomy and dog behaviour would be evaluated by assessing general behaviours of the bitches before and after ovariohysterectomy.

There were a total of 79 closed-ended questions and 17 open-ended or descriptive questions in the questionnaire. The questionnaire was divided into several categories: 1) household information/demographic information about the home, 2) demographic information about the dog, 3) behaviour of the dog in the first two months of ownership, 4) general behaviour of the dog, 5) owner attachment levels, 6) aggressive behaviours of the dog. The respondents were given the opportunity to respond to or ask any questions regarding the questionnaire at the end of the interview.

3.2.4. Telephone survey implementation

All questionnaires were conducted by the author. Pre-testing of the questionnaire was also conducted by the author with 3 convenience selected respondents with variable backgrounds. These included a veterinary student, a graduate student and a dog owner from the general public. Pre-testing took between 15 and 20 minutes with each respondent. No changes in questionnaire design were necessary after pre-testing. Two sets of telephone questionnaires were completed. The first set was conducted from February 2001 to December 2001, as bitches were recruited into the study. The second set was conducted from January 2002- May 2002. Owners of controls (intact bitches) were also re-interviewed during this time period. Respondents were required to be a member of the household who was an adult and who was responsible for helping to take care of the dog. Extensive effort was made to contact clients who had moved or who had changed their phone numbers.

3.2.5. Data management

Variables were developed for each individual question of the questionnaire using the software program EpiData (Epidata Association, Odense, Denmark). The variables were then electronically transferred from EpiData into a statistical software program, STATA version 7 (STATA Corporation, College Station, Texas, U.S.A.), where they were then analyzed. All closed-ended questions were coded as dichotomous variables (1=yes, 0=no), or continuous variables where the values were entered as a numerical value (ex: age, weight). Open-ended questions were compiled into lists of responses and then further broken down into categories. The categories were then analyzed as dichotomous variables, being either yes, belonging to a category, or no, not belonging to a category. Any response of "don't know" was coded as missing data. Whether or

not the bitches had a neutrophil to lymphocyte ratio greater than 3.5 was coded as a dichotomous variable. Absolute lymphocyte values were recorded and reported as a continuous variable. Data was scanned for outliers, and unusual values were checked against questionnaire responses.

Univariable analysis was conducted on all variables from both the first and second questionnaire responses. Risk ratios were then evaluated to measure the association between the variables. All variables that were significantly associated ($P \leq 0.05$) with the outcomes of interest for this study (neutrophil to lymphocyte ratios and growling at the time of the second questionnaire) were incorporated into a multivariable logistic regression model for each outcome of interest. Proportional differences were also analyzed between ovariohysterectomized and intact bitches for various behaviours.

3.3. Results

3.3.1. Neutrophil to lymphocyte ratios in ovariohysterectomized versus intact bitches and absolute lymphocyte counts

Whether or not a dog had a neutrophil to lymphocyte ratio of greater than 3.5 was a dichotomous variable potentially indicative of long-term stress in the bitches. Bitches with a ratio higher than 3.5 were classified as a case, and bitches that did not have this characteristic were classified as a control. A logistic model was developed with the ratio values recorded from the blood sample taken at the time of the second questionnaire as the outcome of interest and can be seen in Table 1. The sensitivity and specificity of the model were 41.67% and 95.24% respectively which indicates that the model is more likely to detect animals that do not have a ratio greater than 3.5 than it would be able to detect animals that did have a ratio greater than 3.5. The

positive and negative predictive values for the model were 62.50% and 89.55% respectively. In other words, the proportion of bitches predicted to have a ratio of greater than 3.5 which actually had a ratio greater than this was lower than the proportion of bitches predicted to not have a ratio greater than this that actually did not have a greater ratio.

Absolute values for lymphocytes were recorded, however there were only five animals from the first blood sampling and one animal in the second blood sampling that had a characteristic lymphopenia (lymphocyte value less than 1.0 x 10^9/L). Regression models were not developed for the absolute lymphocyte values due to the small number of animals that had a lymphopenia. The lymphocyte values ranged from 0.75 x 10^9/L - 4.97 x 10^9/L with an average value of 2.20 x 10^9/L ± 0.83 x 10^9/L in the first blood sampling, and from 0.99 x 10^9/L - 5.45 x 10^9/L with an average value of 2.48 x 10^9/L ± 0.90 x 10^9/L in the second blood sampling. Linear regression revealed that the absolute lymphocyte values were strongly associated with the neutrophil to lymphocyte ratio values (P<0.001).

Intact animals were 12.19 times more likely to have a ratio greater than 3.5 at the time of the second questionnaire when compared to ovariohysterectomized animals (P=0.028). A summary of the statistically significant univariable associations between questionnaire responses and a dog having a ratio above 3.5 in the first questionnaire can be seen in Table 2. There were no associations found between the ages of the dog and the ratios at the time of the first questionnaire, but there was little variability in the ages of the bitches at the time the first blood sample was taken This would make age differences in the white blood cells difficult to detect. The age distribution of the bitches at the time of the first questionnaire can be found in Figure 1, chapter two. There

were no significant associations between whether the blood samples were taken before or after the procedure of ovariohysterectomy.

3.3.2. Other factors associated with significant changes in leukocyte ratios

Dog owners who reported that they purchased books or videos on dog training in the last year were significantly less likely to have a dog with a ratio greater than 3.5 at the time of the second questionnaire (OR=0.061, P=0.021). There were no associations between attending training classes and having a ratio greater than 3.5 when evaluated by univariable analysis.

Owners that reported walking their bitches more than once a day were significantly less likely to have a dog with a ratio greater than 3.5 (OR=0.062, P=0.037). Dog owners who used head halters to take their dogs for walks were 26.28 times more likely to have a dog with a ratio greater than 3.5 (P=0.011).

Table 1.
Multivariable logistic regression model to explain risk factors for a neutrophil:lymphocyte ratio greater than 3.5 in ovariohysterectomized and intact bitches at the time of the second questionnaire (n=75)

Variable	Coefficient	Probability	Odds Ratio	95% CI*
Reproductive status (intact =1)	2.19	0.028	12.19	1.31-113.95
Age (months)	0.69	0.491	1.15	0.77-1.72
Health status[a]	1.77	0.076	5.53	0.83-36.88
Weight (kg)	-0.59	0.552	0.97	0.88-1.07
Owner purchased books or videos on dog training	-2.31	0.021	0.06	0.01-0.66
Dog wore head halter while on walks	2.56	0.011	26.27	2.14-322.16
Dog was walked more than once a day	-2.09	0.037	0.06	0.00-0.85

* 95 % Confidence Interval
[a] Health status was whether or not the owner reported that the dog had a health problem at the time the questionnaire was completed.

Table 2.
Questionnaire responses significantly associated with a neutrophil:lymphocyte ratio greater than 3.5 at the time of the first questionnaire (n=47)

Variable	*P*-value	Risk Ratio (RR)	Confidence Interval
Owner had reason for obtaining dog	0.055	2.51	0.908-6.926
Dog lived with another household dog	0.022	2.41	1.106-5.280
Dog spent time with another dog everyday	0.035	2.31	1.023-5.220
Dog was not walked at all	0.019	2.53	1.217-5.262
Dog was more work than owner expected	0.01	0.136	0.019-0.967
Dog was same amount of work as owner expected	<0.001	5.192	1.635-16.485
Dog was tied outside	0.025	0.35	0.127-0.961
Dog was judged to be in good condition by the veterinarian	<0.001	0.071	0.010-0.509
Dog was judged to be in excellent condition by the veterinarian	0.012	3.008	1.196-7.562

3.4. Discussion

3.4.1. Neutrophil to lymphocyte ratios in ovariohysterectomized versus intact bitches

Table 1 identifies risk factors for a neutrophil to lymphocyte ratio higher than 3.5 at the time of the second questionnaire. Ratio values from the blood sample taken at the time of the first questionnaire are summarized in Table 2, but the immune systems of the bitches would not have been fully developed at this time (Tizard, 1996), and the surgery also may have influenced white blood cell counts (Kreeger et al., 1990), therefore the white blood cell values would probably not predict an accurate ratio. Long-term stress in animals may be characterized by a marked decrease in lymphocytes as a result of long-term corticosteroid release in the body (Aiello and Mays, 1998). Inflammation, infectious or non-infectious diseases, and corticosteroid release as a result of stress are common causes of a decrease in lymphocytes. Characteristics of white blood cell counts are species specific. In dogs, normal neutrophil to lymphocyte ratios are 3.5:1. Dogs showing a marked decrease in lymphocytes will have a ratio larger than this which may be indicative of long-term stress (Aiello and Mays, 1998).

The prevalence of neutrophil to lymphocyte ratios higher than 3.5 in intact animals was higher than that of ovariohysterectomized animals at the time of the second questionnaire. Previous research indicates that animals that are stressed may be more likely to be aggressive (Liptrap and Raeside, 1978; Paroli et al., 1972). Based on this information, it might be expected that ovariohysterectomized animals, which are shown in this study to be more aggressive than intact animals (refer to section 3.8), would have a higher stress level than intact animals. The results of the neutrophil:lymphocyte ratio model in Table 1, however, indicate that intact bitches have a higher ratio. There appeared to be no association between these ratios and the incidence of

growling in this study. Other studies have correlated plasma corticosteroid concentrations with aggressive behaviours (Liptrap and Raeside, 1978; Paroli et al., 1972). Plasma corticosteroid concentrations are indicative of stress at approximately the time the serum sample was taken. Plasma cortisol concentrations may not be good indicators of long-term stress in animals. The release of cortisol after a stressor is applied is almost immediate (Norman and Litwack, 1987). In this study, white blood cell counts were evaluated to get a better idea of the long-term stress that may have been occurring in the bitches. However, other steroid hormones besides corticosteroids have been known to have an effect on lymphocyte values. Hormones associated with the estrous cycle such as estrogen, progesterone and prostaglandins may have a suppressive effect on the lymphocytes in serum (Kalland, 1980; Prabhala and Wira, 1995). The effect these hormones have on lymphocytes in serum may explain why intact bitches in this study are more likely to have ratios higher than 3.5. These hormones in bitches may have a suppressive effect on lymphocytes making it appear as though the animal has a white blood cell change as a result of some type of stressor. Another possibility for the difference in ratios between ovariohysterectomized and intact animals may be a difference in the way that the groups of bitches are managed, such as training methods, tools used for training and the environment in which the bitches are housed (Beerda et al., 1999; Rogerson, 1997; Rugbjerg et al., 2003).

Another concern regarding evaluating the white blood cell counts of the bitches at the time of the first questionnaire was that the blood samples were taken at the time of surgery. Kreeger et al., 1990 found that surgery may have an effect on the normal white blood cell counts. The time that the blood samples were taken was recorded by the veterinarian taking the blood. Blood samples were either taken before, during or after the surgery. There were no significant associations

between the time the blood samples were taken and the proportion of white blood cells found in the blood.

3.4.2. Other risk factors for neutrophil:lymphocyte ratios higher than 3.5 and association with reproductive status in female bitches

3.4.2.1. Training methods and devices and neutrophil:lymphocyte ratios

Owners that purchased training materials were less likely to have a dog with a neutrophil:lymphocyte ratio higher than 3.5. This may be because purchasing these materials informs owners of appropriate ways to train their bitches. Previous studies in rats indicate that an environment that is predictable and controllable may result in less gastric lesions that occur as a result of prolonged cortisol release in a stressful environment (Weiss, 1971). Training, if done effectively, may make the social environment of the dog more predictable and increase a dog's sense of control. There were no differences between ovariohysterectomized and intact dog owners for purchasing information on training when evaluated by univariable analysis.

The results regarding head halters and long-term stress in this study are in contrast to the results of Ogburn et al. (1998) who found that there were no differences in ACTH and cortisol concentrations in bitches that wore head halters when compared to bitches who wore traditional buckle collars. Ogburn et al. (1998), however, indicate that behavioural differences were noted between the bitches. Bitches wearing head halters pawed at their noses and watched their owners less, and bitches wearing buckle collars were noted to be more unruly and disobedient and more likely to pull on the leash. It is difficult to determine from our results whether the ratio increased as a result of the dog wearing the gentle leader, or if the head halter was used because the dog was

unruly or more stressed before the halter was introduced. Owners of ovariohysterectomized dog were slightly more likely to use a head halter (P=0.046, RR=1.275), although these same dogs were less likely to have a neutrophil to lymphocyte ratio higher than 3.5. Consequently, controlling for reproductive status (confounding) was necessary to identify the positive association between head halters and neutrophil to lymphocyte ratios.

3.4.2.2. Exercise and neutrophil to lymphocyte ratios

Exercise releases endorphins which may have a calming effect (Radosevich et al., 1989). Physiological responses to exercise vary depending on the intensity and duration of the exercise. In humans exercise has been shown to relieve depression when comparing physically active men who jogged routinely to sedentary men (Lobstein et al., 1989), on the other hand, Campbell et al. (1988) found that moderate exercise programs had no effect on cortisol and immune function in purpose-bred laboratory beagles. The laboratory environment is dissimilar to that of a dog living in a normal household environment. The differences in environment may result in differences in stress measures. Intense exercise over a long period of time has been shown in Olympic athletes to impair immunity (Nieman, 2000; Wigernaes et al., 2001). It is most likely that the bitches in this study which were walked more than once a day were not exposed to extremely intense exercise for long periods of time. It is also possible that bitches that are less stressed are more likely to be walked frequently. Bitches that are less stressed may be more well-behaved than bitches that are not and may therefore be walked more often by their owners.

3.5. Conclusions

Intact bitches are more likely to have neutrophil to lymphocyte ratios higher than 3.5 in this study when compared to ovariohysterectomized bitches. This effect, however, may be due to circulating estrous hormones or other factors in intact bitches causing them to have a decrease in lymphocytes, and not due to long-term stress. Training methods and devices appear to have an association with long-term stress in bitches. Owners who have more knowledge on training may be more likely to have a dog that does not exhibit indications of long-term stress. Dog owners who use head halters are more likely to have a dog that has a ratio higher than 3.5, however it is difficult to know which came first, the stress or the head halter. Bitches that are walked more than once a day appear to have a decreased incidence of neutrophil to lymphocyte ratios higher than 3.5. This may be as a result of the calming effects of endorphins that are released during exercise.

4. General Conclusions and Significance

The objective of this study was to determine if non-reproductive behavioural changes occur in association with ovariohysterectomy and if these changes were associated with white blood cell shifts or changes in testosterone concentrations. The telephone questionnaire used had a high response rate and was effective in determining detailed information regarding the behaviour and environment of bitches. Neutrophil to lymphocyte ratios and absolute lymphocyte values provided an insight as to whether these are effective methods of determining long-term stress in bitches. Compilation of the behavioural and physiological data in this study has provided significant information regarding the differences between ovariohysterectomized and intact bitches.

Ovariohysterectomizing was found to be a risk factor for growling in this study. Our findings are consistent with previous reports which have indicated that ovariohysterectomized bitches were more likely than intact bitches to show some form of aggressive behaviour (Voith and Borchelt, 1982; O'Farrell and Peachey, 1990; Podberscek and Serpell, 1996, Guy et al., 2001). Factors in the bitches' environment were controlled for in the multivariable statistical model to ensure that the behaviour changes were due to the ovariohysterectomy procedure and not due to differences in training, exercise, and other environmental factors.

Another risk factor for growling was if the owner had a particular reason for obtaining the dog. Owners that had a reason, other than companionship, were more likely to have a dog that growled. This may indicate that the owners' expectations for their dog may be associated with growling.

Consistent with the general principal reported by Beerda et al. (1998) who found that bitches that were switched from spacious group housing to smaller isolated kennels were more likely to be aggressive towards other bitches, our findings suggested that bitches that were restricted to part of the home were more likely to growl. It is not clear whether the aggression resulted from the confinement, or if the bitches were confined due to aggression. Frequency of exercise was also associated with aggression since bitches that were walked more than once a day were less likely to growl than bitches walked at lesser frequencies. It remains unclear whether the association between exercise and growling is due to the physiological changes that occur in the body as a result of exercise, or if the changes are due to social interactions that occur when a dog is walked.

Bitches were also found to be more likely to become excitable after the ovariohysterectomy procedure. Similar findings by Salmeri et al. (1991) who reported that ovariohysterectomized bitches were more likely to have a higher general activity level when compared to intact bitches support this finding. Bitches that wore a standard buckle collar in this study were less likely to become excitable after ovariohysterectomizing, as opposed to bitches wearing any other type of collar. It is difficult to establish which came first, the excitable behaviour, or the collar. Bitches reported to be highly excitable were more likely to be outgoing as puppies, contradicting the findings of Wilsson and Sungren (1997), who reported that willingness of eight week old puppies to approach people had no association with what they described as "nerve stability" when evaluating puppies for service training.

Reported appetite levels of ovariohysterectomized and intact bitches did not appear to differ in this study. There are conflicting results in the literature regarding appetite and weight gain in

bitches after ovariohysterectomy. O'Farrell and Peachey (1990) found that appetite increased after ovariohysterectomy, and Edney and Smith (1986) found that ovariohysterectomized bitches were more likely to be obese when compared to intact bitches, while Salmeri et al. (1991) reported no changes in appetite, weight gain, or food intake after ovariohysterectomizing.

Ovariohysterectomized bitches were more likely to destroy objects than intact bitches while left home alone. They were also were more likely to be fearful of vacuum cleaners and unusual noises. There were, however, no differences in fear of thunder between ovariohysterectomized and intact bitches. Owners of intact bitches were more likely to use crate training and less likely to use head halters for walking their bitches. Owners of intact bitches were also less likely to obtain information from their veterinarian regarding training, but were more likely to purchase books or videos on dog training than owners of ovariohysterectomized bitches. Intact bitches were more likely to spend time with another dog everyday. This time was spent with another household dog in all cases. Ovariohysterectomized bitches spent time with other dogs while visiting at neighbours' or friends' homes, or meeting up with other dogs while on walks.

All of the intact bitches in this study were purebred, and 58% were from the breeders' own litter. More than half of the ovariohysterectomized bitches were purebred (60.70%), and the majority originated from a breeder (45.23%), but only one ovariohysterectomized dog owner reported that the dog would be used for breeding purposes, but they chose not to.

Intact bitches were more likely to have neutrophil to lymphocyte ratio of greater than 3.5, which may be indicative of long-term stress. No other literature is presently available on stress levels of

ovariohysterectomized versus intact bitches. Too few animals had an absolute lymphocyte value indicative of lymphopenia to determine if prevalence of lymphopenia was significantly different between ovariohysterectomized and intact animals. Absolute lymphocyte values, however, may be a better indicator of stress than neutrophil to lymphocyte ratios (Latimer and Rakich, 1989; S. Burton, 2003, personal communication). There was, however, a strong correlation between the absolute lymphocyte values and the neutrophil to lymphocyte ratios. Blood results for testosterone were inconclusive due to the enzyme linked immunosorbent assay only being suitable for human blood and not dog blood. The results for testosterone are included in appendix 7 of this document.

The behavioural and physiological changes between ovariohysterectomized and intact bitches in this study have indicated significant outcomes associated with ovariohysterectomy. It is important that the outcomes of this procedure are determined due to the high number of owners that choose to ovariohysterectomize their bitches. Understanding the potential risks will help dog owners and veterinarians prevent unwanted behaviours associated with ovariohysterectomy, with the potential to improve human-animal interactions. Veterinarians and dog caretakers can use the information from this study to properly inform dog owners regarding changes in behaviour that may occur as a result of spaying. Dog owners in-turn can be aware of the behavioural changes that may occur as a result of the procedure and train their dog appropriately to adapt to any unwanted behaviours that may occur.

Future development in this area could include providing information sessions to inform veterinarians, animal-care workers and dog owners of the behavioural consequences which may be associated with ovariohysterectomy. Information regarding how to deal with any behavioural

problems that may occur as a result this procedure should be provided to the general public. Informing the public regarding what behavioural changes occur as a result of spaying, and providing means for them to properly handle these behavioural changes is a first step forward in developing a strong owner-dog relationship. Undesirable behaviours could then be resolved through appropriate training routines, and less dogs may be relinquished to animal shelters. This in turn may help develop a stronger human-animal bond between dogs and their owners.

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Appendix 1

Clinic Recruitment Form

DATE

Dear Dr. Smith,

The Atlantic Veterinary College (AVC) is currently undertaking a study of the behavioural consequences of ovariohysterectomy (OHE) in young female dogs. Although scientific information is available regarding the developmental and physical consequences of OHE, very little is known about the non-reproductive behavioural consequences of this procedure, or the effect of endogenous testosterone in young female dogs. This information would increase our understanding of behavioural changes which may occur after OHE, and would improve our knowledge of the relationship between hormones and non-reproductive behaviours in dogs. There is no evidence that neutering will adversely affect the performance of working dogs or the general activity level of dogs, but recent research suggests that OHE may be associated with an *increased* risk of aggression toward family members, especially in female dogs which were already showing some signs of aggression prior to being ovariohysterectomized. Aggression in dogs continues to be the most common and serious behaviour problem encountered by pet owners. A more thorough understanding of the motivation for problem behaviours will help veterinarians address these concerns more effectively.

If you should choose to be part of this study, your participation would involve the recruitment of approximately 20 of your clients over a 5 month period who have scheduled their dogs for a pre-pubertal ovariohysterectomize. Owners will be recruited on a first-come first serve basis, with no regard to their dog's current behaviour. In addition, the owners will be informed that the survey encompasses general dog behaviour, and is not particularly focussed on aggression in order to minimize problems with bias in the data. The owners will be required to participate in two telephone surveys , 1) when their dog is approximately 6 months of age (at the time of ovariohysterectomizing),

and 2) when their dog is 1 year of age. Our previous experience with telephone surveys has proven that owners definitely enjoy the opportunity to talk about their dog! We would also ask owners to allow us to obtain two blood samples from their dog, again at 6 months of age (during anaesthesia for the ovariohysterectomize if you prefer) and at 1 year of age (at the time of the first annual vaccination). Blood samples will be shipped pre-paid to the AVC for analysis (CBC, differential, serum cortisol, serum testosterone). There will be no charge to the client for participation in the study, and they may withdraw from the study at any time. In addition, the client will receive a $30.00 credit towards their dog's first annual vaccination at your practice, and your practice will receive a $10.00 monetary reward for each client successfully recruited into the study. Being believers in the effectiveness of positive reinforcement, we would suggest that the monetary reward be given directly to the staff member who is responsible for recruiting participants.

Thank you for taking the time to read this letter and for considering your participation in this multi-centre study. We will be in contact with you by phone within the next 10 days to discuss any questions or concerns you may have.

Sincerely,

Lynn McMullin BSc, MSc candidate
Dept. Anatomy and Physiology

Dr. Norma Guy DVM, MSc
Dept.Anatomy and Physiology

Appendix 2

Instructions for AVC

Ovariohysterectomize Project

1. The following dogs are eligible

- age (minimum 4 months, maximum12 months; **most importantly, must not have had a heat**)
- weight (minimum 4kg)
- must be in apparent good health and have no known congenital disorders or chronic disease states
- **only one dog from each household is eligible, and dog must be in a household environment, not a kennel environment**

2. The client must read the client information form and sign the client consent form. The client consent form should be returned to the AVC with the blood samples.

3. The following blood samples should be collected either before or during anaesthesia, but prior to surgery,

- 1 red top vacutainer (we will centrifuge)
- 1 purple top vaccutainer

(These should be sent to the Atlantic Veterinary College COD in the normal fashion that you send your clinic's blood samples for analysis. Just indicate that the sample is for Lynn McMullin's ovariohysterectomize project and the courier fees will be covered by us).

- **no samples should be sent on Friday or Saturday so that samples will not be in transit or sitting in lab for more than 2 days**
- **samples should not be frozen**

4. The veterinary log sheet must be completed and returned to the AVC with the blood samples.

5. Return the client consent form, the veterinary log sheet, and the blood samples to AVC.

6. Any questions??
Contact Lynn McMullin at

Thank You !

Appendix 3
Atlantic Veterinary College Ovariohysterectomy Project Log Sheet

Please check veterinarian completing log sheet,
Dr. Smith ☐ Other ☐ _____

Dog owners name_____, street name and number_____
_____,

city/town_____, postal code_____, phone number_____
_____

Dog's Name_____ Dog's Age_____ Dog's Body Weight_____kg
_____lbs

Date blood sample was taken,_____

Please rank the overall body condition of the dog, Poor ☐, Fair ☐, Good ☐, Excellent ☐

What type of preoperative medications and general anaesthetics were administered to the dog?

_____ _____

_____ _____

When was the blood sample collected?

before anaesthesia ☐
during anaesthesia ☐
after anaesthesia ☐

Please rate the dog on a scale of 1 to 5 (1 being not at all and 5 being extremely),

i) Aggressive towards clinical staff	1	2	3	4	5
ii)Aggressive towards animals in clinic	1	2	3	4	5
iii) Fearful towards clinical staff	1	2	3	4	5
iv) Fearful towards animals in clinic	1	2	3	4	5

Please return this log sheet along with the client consent form with the first blood samples you take from the patient (6 months of age) to the following address,

Lynn McMullin
Dept. of Anatomy and Physiology
Atlantic Veterinary College
550 University Ave.
Charlottetown, PEI
C1A 4P3

Appendix 4

Client Consent Form

I, _____, consent to the enrollment of my dog_____ in the project conducted by the Atlantic Veterinarian College (AVC) to study the behavioural consequences of ovariohysterectomy in dogs. I understand that I will be responsible for giving detailed, accurate information regarding the behaviour of my dog and that blood samples will be taken twice from my dog by a veterinarian. I give my permission for the results of the behaviour observations and the blood test results to be recorded and analyzed in s large study that will help benefit both the animals and owners involved. I understand that neither I nor my dog will be identified in the results.

I have completely read the client information form and understand the purpose of the study. I understand that the only invasive procedure being performed is blood collection for the purpose of routine blood analytical procedures performed by the staff at the AVC.

I understand that there will be no charges for the diagnostic services performed to analyze the blood. The only fees that I will be responsible for are the usual vet examination fee and any additional procedures I request during my normal office visit. I may withdraw from this study at any time if I feel it is necessary. For any further information regarding this study I may contact either Lynn McMullin, Anatomy and Physiology Dept., AVC (902) 628-4326, or Dr. Norma Guy, Clinical Behavioral Services, AVC (902) 628-0923.

Signed, _____ Date, _____

Witness, _____ Date, _____

Appendix 5

Client Information Form

Ovariohysterectomizing is the most effective method to control pet population and prevent unwanted diseases in the female dog. During this surgery the reproductive organs are removed, which not only prevents the animal from being able to reproduce, but eliminates or diminishes reproductive behaviours. Most female dogs are ovariohysterectomized at about 6 months of age, before they reach puberty. Puberty occurs when sex hormones are released into the blood stream, which leads to the first sexual behaviours and physical changes that are associated with puberty. Removal of sex hormones may also effect non-reproductive behaviours such as excitability or aggressiveness. Relatively little is known about how these other behaviours are affected when an animal is ovariohysterectomized. Because behaviour problems in dogs are extremely common, it would be useful to have better information about the potential effects or benefits of this surgical procedure.

The objective of this study is to compare the behviour of dogs both before ovariohysterectomizing and 6 months after ovariohysterectomizing. In addition we will compare the relative level of sex and stress hormones in 2 blood samples. Information on your dog's behaviour will be collected through two telephone surveys. Blood samples will be collected at the time of ovariohysterectomizing and 6 months after the surgery. If you have chosen to delay ovariohysterectomizing of your dog (perhaps for breeding reasons)

we are also very interested in including her in our study as a member of our "control" group. Please discuss this possibility with your veterinarian.

The results of this study will help improve our understanding of the behaviour of animals, which should strengthen the relationship between dogs and their owners. Your participation in this study would be greatly appreciated. In thanks for your participation we will provide a $30.00 credit towards your dog's first annual vaccination by your veterinarian. Please feel free to call myself, Lynn McMullin (Graduate Student, Deptartment of Anatomy and Physiology, AVC) (902) 628-4326, or Dr. Norma Guy (Clinical Behaviour Service, AVC) (902) 566-0950 with any questions or concerns you have regarding this study. Thank you for your time and cooperation.

Sincerely,

Lynn McMullin

Appendix 6
Questionnaire (Final)

Hello, my name is Lynn McMullin and I am Calling from the Atlantic Veterinary College. Do you have a dog named ----------? *If yes proceed to read rest of paragraph, if no check telephone number, if still no thank them for their time and terminate call.*

Several months ago your veterinarian asked you if you would participate in a behaviour study involving a telephone interview. Do you remember speaking to your veterinarian about this? *They may indicate if they were or were not the person who talked to the veterinarian.* That's fine, as long as I am speaking to an adult who helps to care for ----------.

We are trying to find out a lot more about how dogs and families get along together and what sort of advice veterinarians should give to people when they have a problem with the way their dog behaves. The interview should take approximately 20 minutes of your time and involves answering some questions regarding the health and behaviour of your dog. It would be great if we could do the questionnaire right now, but if that is a problem we could easily set up another time to talk to you. What do you think?

NOT A GOOD TIME - What time would be most convenient for you?

- *Try to set up an appointment for call back*

STILL UNWILLING

- The more people that we can talk to, the more likely it is that we can really have a positive impact on the welfare of dogs. We would really like to include your experiences.
- *if still no, thank them and terminate call*

If OKAY

- Thank you, your answers will be kept completely confidential. I'll ask questions about both your family and your dog. If there are any questions which you can not answer or do not wish to answer please let me know.

1. How old in months was --------- when you obtained her? . . . . . ---------- 1

2.1 Is ---------- purebred? 2.1

- no 0
- yes 1
- don't know 99

if yes:

2.2 What breed is she? . . . . . ---------- 2.2

2.3 Is she registered? 2.3

- no 0
- yes 1
- don't know 99

if not purebred:

2.4 What type of dog do you think ---------- is? . . . . . ---------- 2.4

2.5 What color is she? . . . . . ---------- 2.5

3. How old in months is she now? . . . . . ---------- 3

4. What is ---------- weight? . . . . . ---------- 4

5. Where did you get ----------?

5.1 humane society	1	5.1
5.2 breeder	2	5.2
5.3 pet shop	3	5.3
5.4 found as stray	4	5.4
5.5 family member or friend	5	5.5
5.6 own dog's litter	6	5.6
5.7 other (specify)	7	5.7
5.8 private shelter	8	5.8
5.9 don't know	99	5.9

6. Did you have to pay anything to obtain her? 6

- no 0
- yes 1
- don't know 99

7.1 Did you have a particular reason for getting ---------- other than just wanting a dog? 7.1

- no 0
- yes 1
- don' t know 99

if yes:

7.2 What was your reason?.....---------- 7.2

---

8. Did she have any previous owners other than her birth home? 8

- no 0
- yes 1
- don't know 99

9. Could you tell me the age and sexes of all of the people who are currently living in your home?

	Sex	Age	
1			9.1
2			9.2
3			9.3
4			9.4
5			9.5
6			9.6
7			9.7
8			9.8
9			9.9
10			9.10

10. Who is the primary caretaker of ---------- (primary caretaker is the person who feeds, walks and grooms ---------- on a regular basis)? . . ---------- 10

11. Has the primary caretaker ever owned a dog before? 11
- no 0
- yes 1
- don't know 99

12. Have you ever used crate training with ---------? 12
- no 0
- yes 1
- don't know 99

if yes proceed to question 13

13. Why did you start crate training ----------? ---------- 13

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14. How many times per week did ---------- interact with other dogs in the first two months you had her?

14.1 everyday	1	14.1
14.2 3-5 times a week	2	14.2
14.3 less than 2 times a week	3	14.3
14.4 rarely	4	14.4
14.5 never	5	14.5

15. Where has your dog spent time with other dogs?

15.1 with another household dog	1	15.1
15.2 at a neighbor or friends house	2	15.2
15.3 meeting up with other dogs on walks	3	15.3
15.4 at a dog play park	4	15.4
15.5 at puppy socialization classes	5	15.5

16. On a scale of 0%-100% how much time does your dog spend in the following areas (0 being no time spent at all, and 100 being all of the dogs time is primarily spent in this area)?

16.1 free access to all of home----------	16.1
16.2 free access to part of home----------	16.2
16.3 in crate----------	16.3
16.4 free roaming outdoors----------	16.4
16.5 tied outside----------	16.5
16.6 in pen outside----------	16.6
16.7 fenced in in back yard----------	16.7

17. How often is she taken for walks (a walk equals deliberate exercise with one or more people outside of the owner's yard, not just being outside the house in the presence of the owner)?

17.1 once a week	1	17.1
17.2 twice a week	2	17.2
17.3 three times a week	3	17.3
17.4 more than three times a week	4	17.4
17.5 more than once a day	5	17.5
17.6 not at all	6	17.6
17.7 don't know	99	17.7

if she is taken for walks proceed to question 18 and 19 if not skip to question 20

18. What type of collar do you use for ---------- when taking her for walks on leash?

18.1 leather or nylon with buckle	1	18.1
18.2 choke chain	2	18.2
18.3 prong collar/punch	3	18.3

18.4 head halter (gentle leader/halt) ..... 4 — 18.4
18.5 harness ..... 5 — 18.5
18.6 no pull (Lupi) ..... 6 — 18.6

19. Is your dog ever let off the leash when on walks? — 19
- no 0
- yes 1
- don't know 99

20. On a scale of 1-10 how would you describe your dog's attitude towards food? — 20
1 being very picky and 10 being ravenous? ..... ----------

21. Is your dog fed free choice, or does she have specified meal times? — 21
- specified meal times 0
- unspecified meal times 1
- free choice 2
- works for food 3

22. Has your dog ever had a serious illness that required hospitalization? — 22
- no 0
- yes 1
- don't know 99

23. Does your dog currently have any health problems? — 23
- no 0
- yes 1
- don't know 99

if yes proceed to question 24

24. What are the health problems? ..... — 24

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25. Is your dog currently on any medications besides flea products? — 25
- no 0
- yes 1
- don't know 99

if yes proceed to question 26

26. What are these medications?---------- — 26

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27. Has ---------- ever had a heat? 27
- no 0
- yes 1
- don't know 99

28. Has your dog ever attended any training classes? 28
- no 0
- yes 1
- don't know 99

if yes proceed to next four questions

29. When did she attend these classes? . . . . . ---------- 29

30. What kind of materials did they use in these classes(probe with food, clickers, types of collars)? . . . . . ---------- 30

31 Did your dog like these classes? 31
- no 0
- yes 1
- don't know 99

32. Was there a particular reason for attending these classes? 32
- no 0
- yes 1
- don't know 99

if yes proceed to question 33

33. What was the reason? 33

34.1 Have you purchased any books or videos on dog training in the past year? 34.1
- no 0
- yes 1
- don't know 99

if yes proceed to next two questions

34.2 Has your veterinarian given you any advice on dog training? 34.2
- no 0
- yes 1
- don't know 99

35. Where did you obtain this additional training information?

35.1 veterinarian	1	35.1
35.2 pet store	2	35.2
35.3 humane society	3	35.3
35.4 breeders	4	35.4
35.5 books, magazines or video tapes	5	35.5
35.6 relatives	6	35.6

36. Do you use treats to reward your dog for good behaviour? 36

- no	0
- yes	1
- don't know	99

37. What do you do when your dog misbehaves?---------- 37

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38. How often does your dog get special treats?

38.1 once a week	1	38.1
38.2 2-3 times a week	2	38.2
38.3 3-5 times a week	3	38.3
38.4 more than 5 times a week	4	38.4
38.5 never	5	38.5

39. Does your dog ever rest on your bed or other household furniture? 39

- no	0
- yes	1
- don't know	99

40. How often do you spend time grooming ----------?

40.1 once a month	1	40.1
40.2 once a week	2	40.2
40.3 every second day	3	40.3
40.4 every day	4	40.4
40.5 rarely	5	40.5
40.6 never	6	40.6
40.7 don't know	99	40.7

41. Has your dog had any housetraining accidents in the last month? 41

- no	0
- yes	1
- don't know	99

42. Has your dog done any of the following while left home alone? 42.1

42.1 scratched, chewed or destroyed things

-no 0
- yes 1
-don't know 99

42.2 whined

-no 0
- yes 1
- don't know 99

42.3 howled

-no 0
- yes 1
- don't know 99

42.4 defecated and urinated

-no 0
- yes 1
- don't know 99

43. Do you have any pictures of your dog?

- no 0
- yes 1
- don't know 99

44. Have you ever bought a gift for your dog?

- no 0
- yes 1
- don't know 99

45. Do you look for your dog's companionship when you are feeling lonely, frustrated, sad etc.?

- no 0
- yes 1
- don't know 99

only proceed to question 46 and 47 if owner had indicated earlier that he/she adopted animal earlier than 2 months:

46. Using a scale of 1-10 how would you describe your dogs behaviour in the first two months you owned her.

46.1 1 was very shy and 10 was very outgoing . . . . . ----------

46.2 1 was very calm and 10 was very excitable . . . . . ----------

46.3 1 was not at all interested in people and 10 was very interested in people . . . . . ----------

47. Using a scale of 1-10 how would you describe your dogs behaviour now.

47.1 1 is very shy and 10 is very outgoing ..... ---------- 47.1

47.2 1 is very calm and 10 is very excitable ..... ---------- 47.2

47.3 1 is not at all interested in people and 10 is very interested in people------ 47.3

48. In the first two months of owning your dog did she ever do any of the following:

48.1 growl, snap or guard food or toys 48.1

- no 0
- yes 1
- don't know 99

48.2 take food and hide with it 48.2

- no 0
- yes 1
- don' know 99

48.3 hide under furniture and resist being pulled out or coaxed out 48.3

- no 0
- yes 1
- don't know 99

49. Has your dog ever growled or snapped at anyone even if you think it was in play? 49

- no 0
- yes 1
- don't know 99

50. Has your dog ever bitten anyone even in play? (a bite is defined as the upper or lower teeth making contact with the victims skin with sufficient pressure to cause a visible injury such as an indentation, welt, scrape, bruise, puncture, or tear in the skin. A dog mouthing a person's skin without applying sudden pressure is not considered a bite). 50

- no 0
- yes 1
- don't know 99

if yes proceed to next question

51. What were the circumstances of the worst bite incident? 51

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52. Did --------- ever growl or snap in response to being disciplined or scolded? 52
- no 0
- yes 1
- don't know 2

53. Has ---------- ever been aggressive (barking/growling) towards other dogs? 53
- no 0
- yes 1
- don't know 99

54. Does your dog usually bark when people come to the door? 54
- no 0
- yes 1
- don't know 99

55. Does your dog seem afraid of any of the following things:

55.1 thunder 55.1
- no 0
- yes 1
- don' t know 99

55.2 vacuum cleaners 55.2
- no 0
- yes 1
- don't know 99

55.3 other unusual noises 55.3
- no 0
- yes 1
- don't know 99

55.4 riding in the car 55.4
- no 0
- yes 1
- don't know 99

55.5 children 55.5
- no 0
- yes 1
- don't know 99

55.6 men 55.6
- no 0
- yes 1
- don't know 99

55.7 women 55.7
- no 0
- yes 1
- don't know 99

55.8 unfamiliar visitors
- no 0
- yes 1
- don't know 99

55.9 delivery people
- no 0
- yes 1
- don't know 99

55.10 veterinarians
- no 0
- yes 1
- don't know 99

55.11 dog groomers
- no 0
- yes 1
- don't know 99

55.12 other dogs
- no 0
- yes 1
-don't know 99

56. Would you describe your dog as being generally fearful?
- no 0
- yes 1
- don't know 99

57. Does your dog ever respond to any of the following situations by growling, lifting a lip, snapping, lunging, or biting:

		yes	no	Don't Know
1	approaching her while eating			
2	taking away a bone, rawhide, pigs ear or toy			
3	taking back an object she has stolen			
4	touching or trying to move her in certain ways			
5	putting on her collar or taking it off			
6	staring at her eye to eye			
7	trying to lift her			

55.8
55.9
55.10
55.11
55.12
56
57.1
57.2
57.3
57.4
57.5
57.6
57.7

8	wiping her feet or cutting her toenails				57.8
9	holding her by the scruff				57.9
10	raising a voice to her				57.10
11	If someone raises their arm, holds up a newspaper, stick, etc.				57.11
12	grooming her				57.12

58. Does your dog ever mount other dogs (male or female) in play? 58

-no 0
-yes 1
- don' t know 99

59. What do you find most difficult about owning ----------? 59

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60. Is there anything about ----------'s behaviour that bothers you? 60

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61. Is having ---------- more, less or the same amount of work as you expected? . 61

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62. What do you like most about ----------?---------- 62

Thank you for taking the time to complete this questionnaire. We really appreciate it!! Do you have any questions or comments to add in response to the survey? (If yes address in space provided below:

If no:

Please feel free to contact either myself or Dr. Guy with any questions or concerns you may have at a later date. Our numbers and e-mail addresses are located on the client information form you have received from your veterinary clinic. You will be receiving a credit for a free vaccine when you go in to get your second blood work completed. I look forward to talking to you in the next interview. Thank you again and have a nice day.

Appendix 7

(Testosterone concentrations analyzed by Enzyme Linked Immunosorbent Assay; Neogen Co., KY)

Sample	Concentration Detected (ng/mL)	Standard Deviation
1 ng/mL of testosterone solution added to ovariohysterectomyd dog serum	0.014	0.001
0.1 ng/mL of testosterone solution added to ovariohysterectomyd dog serum	0.006	0.003
0.05 ng/mL of testosterone solution added to ovariohysterectomyd dog serum	0.008	0.01
male human blood diluted with 100 uL of ovariohyterectomized dog serum	0.058	0.026
male human blood diluted with 50 ul ovariohysterectomyd dog serum and 50 uL of buffer	0.024	0.003
male human blood diluted with 25 uL ovariohysterectomyd dog serum and 75 uL of buffer	0.015	0.006

* Ovariohysterectomyd dog serum was found to have a concentration of less than 0.19ng/ml when analyzed by radioimmunoassay, but this method will not detect levels less than 0.19ng/ml. It may be assumed that ovariohysterectomyd dog serum may be between 0 ng/ml and 0.19 ng/ml if measured by a test that could detect such a low level of testosterone.

R^2=0.982

The conclusion of the results of this test were that the testosterone ELISA assay for humans may not be compatible with dog serum. This may be because of interference in the assay of dog antibodies with the test. A monoclonal test developed for dogs may be more useful.