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Analysis of Relaxin and Acute-Phase Proteins in Urine and Feces for Canine Pregnancy Diagnosis

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By

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Measurements of relaxin and acute-phase proteins have not been validated for use in canine serum as a method of pregnancy diagnosis. This means that handling and anesthesia is still necessary to check the pregnancy status of most non-domestic canines. Therefore, the intention of this study was to determine whether relaxin and/or acute-phase proteins could be detected in the urine and/or feces of the domestic dog in order to evaluate the potential for a noninvasive pregnancy test in canines. Blood, urine and feces were collected from 18 domestic dogs and assayed for the presence of relaxin, fibrinogen, alpha-1 acid glycoprotein, and ceruloplasmin. Urinary relaxin appeared to be significant for detecting pregnancy of 30 Days or more in the domestic dog. Additionally, further research might shed light on the presence of relaxin in the feces and fibrinogen and AGP in the urine of the domestic dog and their significance for pregnancy diagnosis.

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CHAPTER I

INTRODUCTION

Diagnosing pregnancy and identifying pregnancy rates in non-domestic canines can be difficult because traditional methods such as abdominal palpation and ultrasound require multiple instances of handling and anesthesia that can be costly and inconvenient as well as overly stressful for the animal (Monfort, 2003). For many non-domestic species, the easiest method to detect and monitor pregnancy is by measuring progesterone in fecal samples since elevated concentrations of progesterone are usually indicative of pregnancy (Bauman et al., 2008; De Haas van Dorsser et al., 2006). However, this is not feasible in canines because of an obligate pseudopregnancy that affects many non-pregnant females and involves an elevation in progesterone that closely mimics the elevation seen during pregnancy (Bauman et al., 2008). Although pseudopregnancy is a natural process in canines, it can complicate pregnancy diagnosis by causing non-pregnant females to take on the physical, behavioral, and hormonal appearance of pregnancy (Gobello et al., 2001; Verstegen-Onclin and Verstegen, 2008). For this reason, pregnancy in non-domestic canines is most often determined by measuring relaxin, the only pregnancy-specific factor for canines. Relaxin has been used to diagnose pregnancy in the domestic dog (*Canis familiaris*), gray wolf (*Canis lupus*), coyote (*Canis latrans*), and many other canines (Bauman et al., 2008; Carlson and Gese, 2007; Verstegen-Onclin and Verstegen, 2008). Additionally, a number of acute-phase proteins are also capable of

determining pregnancy in the domestic dog (Hart, 1997; Ulutas et al., 2009; Vannucchi et al., 2002).

Statement of the Problem

In canines, the assays for relaxin and acute-phase proteins necessary for pregnancy diagnosis have only been validated for serum samples. Therefore, handling and anesthesia are still necessary for blood collection in non-domestic species (Bauman et al., 2008; Ulutas et al., 2009; Vannucchi et al., 2002). Chronic stress responses as a result of "unpleasant" handling have been linked to a decrease in pregnancy rates in pigs (Hemsworth et al., 1986). Blood collection is believed to cause stress in some nondomestic carnivores, and both acute and chronic stress is known to adversely affect reproduction in captive species (Goodrowe et al., 2000). This makes it difficult to monitor pregnancy in non-domestic canines as it would be unwise to compromise pregnancy by causing undue stress to the maternal body during handling and anesthesia. Not only can handling and anesthesia be costly for researchers and stressful for the animal, but an elevation in stress hormones of the animal may interfere with or mask normal hormonal patterns (Monfort, 2003).

Furthermore, blood collection in wild populations is extremely difficult during the period of gestation, and there are few samples available for wildlife biologists and researchers during this time. For many non-domestic canines, there is very little information on their reproductive physiology, including even basic parameters (Bauman et al., 2004). This lack of information may be the result of the excessive handling that is required to take serial serum samples for the study of reproductive hormones in canines. In fact, what reproductive parameters that have been recorded for non-domestic canines

are the result of postmortem examinations of reproductive tissues taken during predator control programs (Bauman et al., 2004; McNay et al., 2006). The best method to monitor the reproductive status in non-domestic canines, both captive and wild, would be to measure relaxin and/or acute-phase proteins via urine or feces. A urinary relaxin assay has recently been developed for felines, and one study has shown that a urinary relaxin assay is possible for identifying pregnancy in maned wolves (*Chrysocyon brachyurus*; De Haas van Dorsser et al., 2006; Harris et al., 2008; Steinetz et al., 2009). Additionally, urine concentrations of ceruloplasmin, an acute-phase protein, were used for pregnancy diagnosis in the giant panda (Willis et al., 2011). However, to date, there have been no studies to determine the presence of relaxin in the feces of pregnant canines, and the urine and feces of canines have yet to be investigated for the presence of acute-phase proteins capable of pregnancy diagnosis.

Objectives of the Study

The intent of this study was 1) to determine if relaxin and acute-phase proteins could be detected in the urine and feces of the pregnant domestic bitch, 2) to compare urine and fecal concentrations of relaxin and acute-phase protein with their respective serum concentrations, and 3) to ultimately establish whether there was any potential for developing a canine pregnancy test with either relaxin or acute-phase proteins in either urine or feces. In light of the studies done by De Haas van Dorsser et al. (2006) on urinary relaxin in felids, it was hypothesized that urinary relaxin would be capable of diagnosing pregnancy in the domestic dog with elevations comparable to serum concentrations. Likewise, since Willis et al. (2011) were able to use ceruloplasmin for pregnancy diagnosis in the giant panda, it was also hypothesized that urine concentrations

of the acute-phase proteins fibrinogen, alpha-1 acid glycoprotein, and ceruloplasmin would be capable of diagnosing pregnancy in the domestic dog as long as health could be accounted for.

Significance of the Study

If urine and fecal concentrations of relaxin and acute-phase protein are comparable to the concentrations found in serum, the results should validate the proposed assays for use as a pregnancy test in the domestic bitch. These methods can then be used as a basis from which to test the effectiveness of urinary and fecal measurements of relaxin and acute-phase proteins in non-domestic canines. Being able to detect elevated concentrations of relaxin or acute-phase proteins in urine or fecal samples of pregnant bitches will allow for a noninvasive means of identifying and monitoring pregnancy that would be highly advantageous for non-domestic canines. Due to overly zealous control programs in addition to problems such as disease and habitat loss, several species of non-domestic canines have experienced declining populations that put them at risk for extinction (Sillero-Zubiri et al., 2004). Many of these species depend on captive breeding efforts to help restore their populations, and captive breeding often depends on being able to identify and monitor pregnancy while causing the least amount of stress possible. In some cases, such as the maned wolf, breeding success is low, and potential pregnancies need to be monitored more strictly. Fortunately, a urinary relaxin assay has already been shown to identify pregnancy in maned wolves, thus allowing for further insight into maned wolf reproduction (Steinetz et al., 2009). It is, therefore, feasible that such methods can be equally beneficial to other non-domestic canines.

Furthermore, since progesterone can be measured in fecal samples, it would be possible to noninvasively determine the reproductive status, whether pregnant or pseudopregnant or anovulatory, of a canine if relaxin or acute-phase proteins could be measured via urine or feces (Bauman et al., 2008). There have been very few studies done on pseudopregnancy or anovulation in non-domestic canines, and the occurrence of anovulatory cycles is unknown for most species. Being able to detect relaxin or acute-phase proteins in the urine or feces of canines may open up many new research opportunities into the reproductive processes of non-domestic canines since most research occurs in zoo populations where urine and feces are often easily accessible. Routine collection of urine and feces could allow for weekly or daily analysis of the reproductive hormones of captive canines, furthering the development of species-specific hormonal profiles for captive breeding purposes.

Lastly, most studies on the reproductive biology of non-domestic canines are almost exclusively from captive populations due to the difficulty of obtaining serum samples from wild populations. Obtaining multiple serum samples from an individual canine in the wild is practically impossible. However, since collection of fecal samples is a fairly routine practice in wildlife biology, studies into the reproductive biology of wild populations could benefit significantly if relaxin or acute-phase proteins can be measured in the feces. Reproductive factors, such as pregnancy rates and parturition rates, are essential to understanding the population growth of wildlife species and, therefore, play an important role in the balancing act of managing for non-domestic canines that are detrimental to livestock species (McNay et al., 2006).

Summary

In summary, pregnancy diagnosis in canines can be slightly more complicated due to the obligate pseudopregnancy that is part of the canine reproductive cycle. While progesterone is a sufficient means of pregnancy diagnosis for many other mammals, it is not feasible in canines. To date, the hormone relaxin as well as certain acute-phase proteins have been the only good indicators of pregnancy in the canine. Unfortunately, a non-invasive method of detecting relaxin and the acute-phase proteins has not yet been developed for the domestic dog or other non-domestic canines. Because breeding programs and reproductive studies are often crucial for the management of many non-domestic species, this study has been designed to investigate the potential for less invasive methods of detecting relaxin and acute-phase proteins in the domestic dog. The intent is to determine whether or not relaxin and acute-phase proteins can be measured in urine or feces and, if so, whether or not there is enough correlation with serum concentrations to be used in pregnancy detection.

CHAPTER II

THE CANINE REPRODUCTIVE PROCESS

The canine reproductive process, despite its distinctive characteristics, is a fairly simple and straightforward form of reproduction. In fact, canine reproduction is thought to resemble that of early placental mammals (Concannon et al., 2009). Like most other mammals, canines have four stages in their reproductive cycle, each distinguished by hormonal and physiological changes (Pineda, 2003). The luteal phase of their reproductive cycle is the phase during which pregnancy and pseudopregnancy occur (Pineda, 2003). The obligate pseudopregnancy experienced by non-pregnant females is one of the more unique aspects of canine reproduction. Many of the same hormonal and physiological changes that occur during canine pregnancy also occur during this obligate pseudopregnancy, causing pregnant and non-pregnant females to exhibit similar physical and behavioral signs (Gobello et al., 2001; Verstegen-Onclin and Verstegen, 2008). Because of the similarities exhibited by pregnant and pseudopregnant canines, developing accurate diagnostic methods for detection of pregnancy in canines has been challenging, especially for non-domestic canines (Bauman et al., 2008). To date, relaxin is the only pregnancy-associated factor found to reliably detect pregnancy in canines although there has been some studies showing that certain acute-phase proteins can be used in pregnancy diagnosis as well (Bauman et al., 2008; Hart, 1997; Vannucchi et al., 2002). The following is an overview of the canine reproductive process comparing and

contrasting pregnancy and pseudopregnancy and outlining current methods used in canine pregnancy diagnosis.

Review of the Canine Estrous Cycle

In canines, there are four phases of the female reproductive cycle: proestrus, estrus, diestrus, and anestrus (Pineda, 2003). These phases are differentiated by changes in hormone concentrations and in vaginal epithelium, which coincide with characteristic behavioral patterns (Kreeger, 2003; Packard, 2003; Pineda, 2003). Most canines are monoestrous, having only one estrous cycle per year, and do not return to estrus even if they fail to become pregnant (Asa and Valdespino, 1998; Kennelly and Johns, 1976). The domestic bitch can sometimes cycle twice per year. However, this is usually considered a result of a loss of seasonality instead of a true diestrous cycle (Pineda, 2003). In contrast, non-domestic canines exhibit a seasonal breeding cycle, and some are considered highly photoperiodic (Carlson and Gese, 2009; Mech, 1970).

Proestrus

Proestrus is characterized by rising concentrations of serum estrogen that, in conjunction with vaginal bleeding, cause increased attractiveness and proceptive behavior in bitches (Kreeger, 2003). This is due to the pheromones that are secreted in urine and vaginal discharge. Onset of proestrus is considered to be the first day that blood is discharged from the vulva. During this stage, follicular development occurs as a result of gonadotropic stimulation. Follicular stimulating hormone (FSH) and luteinizing hormone (LH) experience nearly parallel increases and then peak late in proestrus. Follicular development causes increasing concentrations of estrogen, which peak during the last two days of proestrus. In the Beagle bitch, estradiol-17β averaged 58 ± 7 pg/ml (Pineda,

2003). Similarly, estradiol-17β initially rises to 10-20 pg/ml in the gray wolf but then later peaks in late proestrus at 30-50 pg/ml (Kreeger, 2003).

Proestrus averages 9 days in the domestic bitch with a range of 2−15 days, but it generally lasts longer in non-domestic canines since most non-domestic canines experience true monoestrum (Kreeger, 2003; Pineda, 2003). Captive wolves average a proestrus length of 15.7 ± 1.6 days, but it can last much longer in the wild, potentially up to 45 days (Kreeger, 2003). In the coyote, proestrus is well known for its extremely long duration, lasting about 2−3 months on average (Amoss and Hodges, 1995).

Estrus

Estrus is considered the period of sexual receptivity and begins when the female accepts the male for mating (Pineda, 2003). Acceptance of mating is characterized by a lordosis-like stance and deflection of the tail to one side to allow mounting (Kreeger, 2003; Pineda, 2003). A copulatory lock-and-tie is then performed during mating (Pineda, 2003). Estrus is characterized by a decline in estrogen and a rise in progesterone that causes this sexual behavior and leads up to the preovulatory LH surge (Kreeger, 2003). Follicular luteinization and progesterone production occur as a result of increasing concentrations of LH. Therefore, canines experience a unique preovulatory luteinization. The preovulatory LH surge occurs on either the last two days of proestrus or the first two days of estrus, and ovulation typically follows 48 hours after the preovulatory LH surge (Pineda, 2003). Wolves demonstrate preovulatory LH surges of 5−15 ng/ml that can last 1−3 days. Two or three large tertiary follicles are generally present on the ovaries and are lined by stratified layers of elongated granulosa cells that penetrate the antral space (Sundeep and Adler, 2008).

In response to the LH surge, ovulation occurs, and estrogen-secreting ovarian follicles further enlarge and luteinize, becoming the progesterone-secreting corpus luteum (CL). In the gray wolf, estradiol-17β decreases abruptly to around 10−20 pg/ml after the LH surge while progesterone increases rapidly above its baseline concentration of 1−3 ng/ml (Kreeger, 2003). Formation of the CL and initiation of progesterone production, a process commonly referred to as metestrus, takes about 3-5 days in the bitch (Pineda, 2003). In the coyote, ovulation can occur anywhere from the first day of estrus until the ninth day of estrus, while it is thought to occur toward the end of estrus in the gray wolf (Amoss and Hodges, 1995; Kreeger, 2003). Estrus is most easily identifiable through vaginal cytology because the cells of the vaginal epithelium are almost entirely keratinized superficial cells with few or no nuclei (Pineda, 2003). Estrus lasts approximately 10 days in the domestic dog, gray wolf, and coyote (Amoss and Hodges, 1995; Kreeger, 2003; Pineda, 2003).

Diestrus

The diestrus phase composes most of the luteal phase, during which the CL and elevated concentrations of progesterone are at their most functional. Behaviorally, the beginning of diestrus is considered to be when the bitch refuses to mate on two consecutive days (Pineda, 2003). Since canines are spontaneous ovulators, both pregnant and non-pregnant bitches develop CLs and enter the luteal phase unless non-pregnant bitches are anovulatory (Kreeger, 2003; Pineda, 2003). Most bitches will develop at least two large CLs in each ovary during early diestrus, but these CLs will regress into cytoplasmic vacuoles in late diestrus (Sundeep and Adler, 2008). Due to the similarity in hormonal concentrations and in length of diestrus between pregnant and non-pregnant

bitches, non-pregnant bitches in diestrus are often referred to as being physiologically pseudopregnant (Asa and Valdespino, 1998). Diestrus ends with the return of progesterone to its basal concentrations of 1 ng/ml and below (Pineda, 2003).

Generally, the diestrus phase is considered to last between 50-70 days, based upon progesterone secretion, but it can vary depending upon the presence of pregnancy or the intensity of a pseudopregnant reaction. Gestation in the bitch is consistently an average of 57 days; whereas, pseudopregnancy can last as long as 70−80 days (Pineda, 2003). In the gray wolf, progesterone elevation generally lasts between 56−68 days, while gestation is 60−65 days (Kreeger, 2003). Gestation in the coyote is 60−63 days long (Carlson and Gese, 2007).

Anestrus

Anestrus is the period of sexual quiescence following diestrus. Because of the sexual inactivity during this stage, anestrus was thought to be a period of endocrine inactivity as well, but recent studies have shown that many reproductive hormones rise during late anestrus. Concentrations of FSH, in fact, are higher in the last month of anestrus than they are during proestrus. In response, estradiol-17β increases to as high as 46 pg/ml during late anestrus (Pineda, 2003). This causes early follicular development to occur, with several primary and secondary follicles seen in the ovaries in late anestrus (Sundeep and Adler, 2008). The reason why there are no reproductive behaviors that correlate with the ovarian activity during anestrus is not yet understood although it is suspected that the expression of estrous behaviors is inhibited by low concentrations of progesterone during anestrus. Furthermore, lactation occurs at the beginning of anestrus

in postpartum bitches and lasts for 6−9 weeks, during which concentrations of prolactin are increased (Pineda, 2003).

In the domestic bitch, anestrus can range anywhere from 40−270 days. Because the domestic bitch is aseasonal, anestrus is extremely variable, not only between breeds but also between individuals within the same breed and between cycles within the same individual (Pineda, 2003). In wild canines, the period of anestrus is more consistent, varying slightly depending upon region. For example, June to December is considered to be the period of anestrus in the gray wolf (Kreeger, 2003).

Anovulatory Cycles

When bitches fail to reach the threshold value of 2 ng/ml during the period of cytological estrus and therefore fail to enter into diestrus, they instead become anestrous. This abnormality in the canine estrous cycle is referred to as anovulation. Anovulation may be caused by an insufficient secretion of hormones to stimulate normal ovulation or the failure of the ovary to respond to stimulation. For example, the ovary may not produce a strong enough estrogen signal to initiate the LH surge, or the ovary may simply not respond to a normal LH surge. Anovulatory cycles are uncommon in the domestic dog, one study reporting an occurrence as little as 1.2% among bitches in a breeding management program (Meyers-Wallen, 2007). However, anovulation has been known to persist for over two years in some adult captive wolves (Kreeger, 2003).

Review of the Canine Luteal Phase: Pregnancy versus Pseudopregnancy

One of the major challenges in canine reproduction has been to distinguish the differences between pregnancy and pseudopregnancy. Physiological pseudopregnancy refers to a naturally extended luteal phase that closely mimics the physical, behavioral,

and hormonal characteristics of pregnancy (Gobello et al., 2001; Verstegen-Onclin and Verstegen, 2008). In canines, bitches that do not become pregnant after ovulation enter this extended luteal phase (Pineda, 2003). It is thought that psuedopregnancy originally evolved in social canines as a function of cooperative breeding since it offered advantages in rearing young and maintaining social cohesion (Creel et al., 1991; Concannon et al., 2009). However, in veterinary medicine, clinical pseudopregnancy is considered an endocrine disorder of the domestic dog due to the problematic behaviors associated with pseudopregnancy (Gobello et al., 2001). Despite the similarities between pregnancy and pseudopregnancy, there are a number of endocrine and physiological differences to distinguish one from the other (Concannon et al., 2009; Bauman et al., 2008; Verstegen-Onclin and Verstegen, 2008; Vannucchi et al., 2002).

Endocrine Characteristics

Progesterone

Both pregnancy and pseudopregnancy are characterized by an elevation of plasma progesterone beyond its threshold value of 1−2 ng/ml that peaks around 20−30 days after the LH surge (Concannon et al., 2009; Verstegen-Onclin and Verstegen, 2008). Peak concentrations of plasma progesterone are similar between pregnant and pseudopregnant bitches (Concannon et al., 2009; Concannon et al., 1975). This elevation in progesterone causes the mammary development and the development and enlargement of the uterus that is seen in pregnancy and often in pseudopregnancy (Gobello et al., 2001; Pineda, 2003).

Pregnancy-associated progesterone is actually produced by the CL at significantly higher concentrations than progesterone in pseudopregnant bitches, but these higher

concentrations dwindle rapidly as they are metabolized in the periphery and consumed by the placenta. This results in a 25% or more decrease in circulating progesterone concentration (Concannon et al., 2009). For this reason, concentrations of plasma progesterone appear similar between pregnant and pseudopregnant bitches. The difference in concentrations of progesterone can only be seen when progesterone metabolites are measured from the feces and compared with plasma progesterone (Concannon et al., 2009; Verstegen-Onclin and Verstegen, 2008).

After its peak, progesterone begins to decline in both pregnant and pseudopregnant bitches around 30 days after the LH surge (Verstegen-Onclin and Verstegen, 2008). In pregnant bitches, this decline culminates in parturition, during which progesterone drops rapidly and becomes undetectable within a day (Pineda, 2003). In pseudopregnant bitches, progesterone declines more slowly, possibly as a result of gradual luteal regression (Concannon et al., 2009; Pineda, 2003). This is true for wolves as well, as one pseudopregnant wolf was documented with a luteal phase of 70 days based on elevated concentrations of progesterone (Seal et al., 1979). Overall, progesterone elevation is shorter in duration and more abrupt in its decline during pregnancy than during pseudopregnancy (Verstegen-Onclin and Verstegen, 2008).

Estrogen

Some studies have shown that estrogen undergoes a pregnancy-specific rise in luteal secretion in the domestic bitch (Concannon et al., 2009; Concannon et al., 1975). Although estrogens decrease at the end of estrus, estradiol-17β experiences a significant increase about 10−15 days after the LH surge and remains elevated during pregnancy until Day 64. It is suggested that this rise in estradiol is related to estradiol's ability to

sustain luteal function and to stimulate progesterone secretion (Verstegen-Onclin and Verstegen, 2008). However, there is no prepartum rise in estradiol-17β in the gray wolf. Instead, concentrations of estradiol remain between 10−30 pg/ml throughout diestrus (Seal et al., 1979). Furthermore, other studies of the domestic bitch have found no significant difference in concentrations of estrogen between pregnant and pseudopregnant bitches (Verstegen-Onclin and Verstegen, 2008).

Prolactin

After Day 30, prolactin is the main luteotrophic factor necessary for supporting the CL in both pregnant and pseudopregnant bitches (Gobello et al., 2001; Verstegen-Onclin and Verstegen, 2008). Prolactin forms a complex with LH, whose function in diestrus is still under speculation (Pineda, 2003; Verstegen-Onclin and Verstegen, 2008). Additionally, prolactin has also been known to play a role in the development and differentiation of mammary tissue (Kooistra and Okkens, 2002). Prolactin rises slightly above basal concentrations in mid-gestation and remains elevated throughout gestation and lactation (Verstegen-Onclin and Verstegen, 2008). In the pseudopregnant bitch, concentrations of prolactin are slightly lower, but not significantly low enough to distinguish pregnancy from pseudopregnancy (Concannon et al., 2009). Furthermore, there is much individual variation in prolactin concentration in the domestic bitch (Verstegen-Onclin and Verstegen, 2008).

Prolactin is likely the hormone responsible for the maternal behavior exhibited during pregnancy and pseudopregnancy, as it has been linked to parental behavior in many other species (Asa and Valdespino, 1998). In the domestic bitch, the unwanted nesting behaviors of pseudopregnant bitches are often treated with dopamine agonists,

such as bromocriptine, to inhibit prolactin secretion, further supporting the link between increased prolactin secretion and maternal behaviors (Pineda, 2003; Verstegen-Onclin and Verstegen, 2008). All wolves, regardless of gender and reproductive status, exhibit a circannual rhythm of prolactin, with peaks just prior to summer solstice, suggesting that prolactin may be involved in the reestablishment of gonadotropin secretion and may essentially serve as a cue for breeding activity (Kreeger et al., 1991). Furthermore, elevations of prolactin in wolves during spring may cause the priming of parental behavior, such as den construction and pup feeding, which has been observed in both pregnant and pseudopregnant pack members (Kreeger et al., 1991; Mech et al., 1996; Packard, 2003).

Relaxin

Although relaxin can be produced by both the ovary and the placenta of the bitch, it is secreted primarily by the trophoblasts of the placenta (Klonisch and Hombach-Klonisch, 2000; Klonisch et al., 1999). In carnivores like the dog and cat, placentation is endotheliochorial and zonary, and trophoblasts actively pervade the uterine endometrium into the basal lamina (Klonisch and Hombach-Klonisch, 2000). During pregnancy, concentrations of relaxin rise to 8−10 μg/ml in the domestic bitch, which has one of the highest reported concentrations of serum relaxin for any species (Steinetz et al., 1996). Relaxin is detectable within serum around Day 18 of gestation and peaks around Day 35 in the bitch (Klonisch et al., 1999; Steinetz et al., 1996). In contrast, serum relaxin is undetectable in bitches that are pseudopregnant, anovulatory, or hysterectomized, making relaxin a pregnancy-specific hormone for canines (Klonisch et al., 1999).

In placental mammals, relaxin is probably most well known for its effects on the cervix and vagina that allow for the passage of young. However, relaxin has a myriad of functions during pregnancy that differ depending upon species (Sherwood, 2004; Steinetz et al., 1989; Swabe and Büllesbach, 1994). Relaxin is thought to act through autocrine or paracrine mechanisms to prepare the uterine endometrium for implantation since it is involved in uterine growth and development as well as myometrial contractility (Sherwood, 2004). Although the hormone's specific function in the placenta has yet to be determined, it is suspected that relaxin is a placental growth factor, as it is associated with highly proliferative fetal tissues in the domestic bitch. For this reason, relaxin may also be used to identify the onset of parturition or to predict unexpected abortion, as it decreases to undetectable concentrations during both incidents (Klonisch and Hombach-Klonisch, 2000; Klonisch et al., 1999). Similarly, it has been linked to an increase in fetal weights in rats (Sherwood, 2004). Though it has yet to be fully proven, it has been suggested that relaxin plays a role in follicular growth in the pig (Sherwood, 2004).

Parturition and Luteal Regression

When the fetal pituitary-adrenal axis matures at the end of pregnancy, concentrations of fetal glucocorticoids rise, causing increased prostaglandin production. In response to elevated prostaglandins, the CL undergoes luteolysis, and progesterone sharply declines to concentrations less than 2 ng/ml. Generally, parturition occurs a few days after this sharp decline in progesterone (Verstegen-Onclin and Verstegen, 2008).

In contrast, luteal regression in the non-pregnant bitch is a slow process. Without fetuses to initiate prostaglandin production, endogenous prostaglandin is not involved in luteal regression, as there is no evidence of expression of PGF synthetase on the CL

(Concannon et al., 2009; Verstegen-Onclin and Verstegen, 2008). Furthermore, the uterus of the non-pregnant bitch does not appear to be a factor in luteolysis (Pineda, 2003). Because the bitch's reproductive system seems to be lacking in luteolytic factors, it is likely that luteal regression is simply a result of the aging of the CL and that luteal cells are preprogrammed to endure for two months to ensure potential pregnancy is successful (Concannon et al., 2009; Pineda, 2003). Therefore, concentrations of plasma progesterone, while decreasing markedly at parturition in the pregnant bitch, may persist longer than two months in the non-pregnant bitch (Pineda, 2003).

Lactation

Milk secretion occurs when progesterone, which suppresses lactation, declines at parturition (Verstegen-Onclin and Verstegen, 2008). However, since progesterone also declines during luteal regression in pseudopregnant bitches, milk secretion is commonly seen among pseudopregnant bitches, and it has been documented in some wolves (Asa and Valdespino, 1998; Gobello et al., 2001). There has been much speculation as to the role of lactation in pseudopregnant canines since pseudopregnant dwarf mongooses have been known to use spontaneous lactation for communal nursing (Concannon et al., 2009; Creel et al., 1991; Packard, 2003). However, most researchers seem to believe that milk secretion from pseudopregnant wolves does not offer any function in communal nursing. All cases of cooperative nursing in wolves have only been observed among postpartum bitches (Packard, 2003).

Review of Pregnancy Diagnosis in Canines

While pregnancy diagnosis in most mammals can be performed by monitoring fecal or serum progesterone values, this is not possible with canines because both

pregnant and pseudopregnant females have elevated progesterone values (Bauman et al., 2008). Although it is fairly routine to perform pregnancy tests in domestic bitches, pregnancy diagnos can be more difficult in non-domestic canines, who are subject to greater stress during handling. In the past, pregnancy rates in wolves and other non-domestic canines were mainly calculated from post mortem examinations. Ultrasound has also been used in wolves as a reliable means of pregnancy diagnosis, although it requires anesthesia (McNay et al., 2006). However, the relaxin assay is by far the most common method of diagnosing pregnancy in both domestic and non-domestic canines due to its accuracy and reliability (Bauman et al., 2008). Additionally, some acute-phase proteins have gained increasing recognition for their ability to indicate early pregnancy in the domestic bitch (Hart, 1997; Vannucchi et al., 2002).

Relaxin

The relaxin molecule is a 6-kDa polypeptide that is structurally similar to insulin (Steinetz et al., 1996; Steinetz et al., 1989). The diverse functions of relaxin across species can be attributed to the variability of its structure between species and even between breeds, its sequences differing an average of 50% between relaxin molecules of different species (Stewart et al., 1992; Schwabe and Büllesbach, 1994). However, the receptor-binding region on relaxin is specifically located at the mid-region of the B chain helix in most species. For that reason, there is a high degree of biological cross-reactivity among different species (Schwabe and Büllesbach, 1994).

Relaxin has been found in ovarian tissues, seminal fluid, prostatic tissues, and various connective tissues of many species (Gelsleichter et al., 2003; Niebauer et al., 2005; Ryan et al., 1997; Sherwood, 2004; Steinetz et al., 1989; Weiss, 1989). During

pregnancy, the main source of relaxin production is the ovary for the sow, cow, and rat and the placenta for the bitch, queen, mare, and rabbit (Anderson et al., 1973; Anderson and Long, 1978; Addiego et al., 1987; Fields and Larkin, 1980; Fields et al., 1980; Tsutsui and Stewart, 1991; Stewart et al., 1982). Because it is produced primarily by the placenta in canines, relaxin has long been considered the only hormonal marker of pregnancy in canines (Bauman et al., 2008; Carlson and Gese, 2007; Verstegen-Onclin and Verstegen, 2008). It has been used to diagnose pregnancy in the coyote, gray wolf, Mexican gray wolf (*Canis lupus baileyi*), fennec fox (*Vulpes zerda*), and island fox (*Urocyon littoralis*; Bauman et al., 2008; Carlson and Gese, 2007; De Haas van Dorsser et al., 2007; DiGangi et al., 2010).

Urinary Relaxin Assays

Due to its small size and molecular weight, it has been suggested that the relaxin might pass through the glomeruli of the kidney and be excreted relatively unaltered in the urine (De Haas van Dorsser et al., 2006). The potential of measuring relaxin in urine for wildlife conservation was first reported by Steinetz et al. (2005) in a study on the giant panda (*Ailuropoda melanoleuca*) in which relaxin was measured in paired serum and urine samples. Although urinary relaxin appeared to be pregnancy-specific, it was determined to be unreliable for pregnancy diagnosis in the giant panda due to sporadic fluctuations in urinary relaxin combined with complicating physiological factors. However, the significance of this study was that it demonstrated the ability to detect relaxin in urine as a possible means of non-invasive pregnancy diagnosis in other species (Steinetz et al., 2005).

Subsequently, De Haas van Dorsser et al. (2006) validated the use of a urinary relaxin radioimmunoassay for pregnancy diagnosis in felines. Paired serum and urine samples were taken from pregnant and non-pregnant domestic cats and leopards (*Panthera pardus*) and tested for concentrations of relaxin using the double-antibody canine relaxin radioimmunoassay (RIA). Urinary relaxin became detectable in the pregnant cat around 21−28 Days and in the pregnant leopard around 25−28 Days. For both species, the concentrations of urinary relaxin compared favorably to those seen in serum, allowing pregnancy diagnosis via urine as early as the third or fourth week of gestation (De Haas van Dorsser et al., 2006). Further studies on urinary relaxin showed that it could indicate late gestation pregnancy in the Iberian lynx (*Lynx pardinus*) and was likely capable of indicating pregnancy in the Pallas cat (*Otocolobus manul*) and the maned wolf (Braun et al., 2009; Harris et al, 2008; Steinetz et al., 2009).

Acute-Phase Proteins

Recent studies on acute-phase proteins have unveiled their potential in pregnancy diagnosis of the domestic bitch, even in the development of an early pregnancy test (Ulutas et al., 2009; Vannucchi et al., 2002). Acute-phase proteins are proteins produced by the liver during acute-phase response to inflammation or infection (Evans and Anderton, 1992). They are known to be involved in the immune response and the recovery of damaged tissue, but they can also be released under normal physiological conditions, such as pregnancy (Evans and Anderton, 1992; Ulutas et al., 2009). In cyclic, non-pregnant bitches, concentrations of acute-phase proteins remain stable, resulting in significant differences in concentrations of acute-phase protein between pregnant and pseudopregnant bitches. Furthermore, acute-phase proteins do not seem to be affected by

variations in sexual hormones or by external stimuli, such as excitement or stress (Ulutas et al., 2009). The rise in acute-phase proteins during pregnancy is, therefore, attributed to an inflammatory reaction of the maternal body to embryonic implantation, occurring around 16−18 days after the LH surge, and subsequent placental growth (Ulutas et al., 2009; Vannucchi et al., 2002). This inflammatory response stimulates acute inflammatory response mediators to produce acute-phase proteins (Vannucchi et al., 2002). In the domestic dog, seven acute-phase proteins have been evaluated for their usefulness in pregnancy diagnosis: fibrinogen, ceruloplasmin, haptoglobin, C reactive protein, seromucoid, alpha-1 acid glycoprotein, and α_2 globulin. Of these, fibrinogen, ceruloplasmin, and alpha-1 acid glycoprotein appear to have the most consistently reliable results (Ulutas et al., 2009; Vannucchi et al., 2002).

Although acute-phase protein panels have been identified in the domestic dog as having 97% accuracy rate in pregnancy diagnosis, it is important to realize that acute-phase proteins are not pregnancy-specific factors (Evans and Anderton, 1992). Pregnancy diagnosis by acute-phase protein panels is only effective in healthy animals since acute-phase proteins will respond to inflammation and infection. Unhealthy, infected animals may cause false-positives (Ulutas et al., 2009; Vannucchi et al., 2002).

Fibrinogen

Fibrinogen has been recognized in numerous studies as a method for diagnosing pregnancy in the domestic bitch. Fibrinogen peaks in mid-gestation, usually around Day 28 (Concannon et al., 1995; Concannon et al., 1996; Hart, 1997; Ulutas et al., 2009; Vannucchi et al., 2002). However, Ulutas et al. reported that there was a significant rise in fibrinogen during the first half of pregnancy as well (2009). Most studies show that

fibrinogen is significantly elevated throughout pregnancy until parturition (Ulutas et al., 2009; Vannucchi et al., 2002). However, Concannon et al. reported that fibrinogen peaked between Days 25−50, declining before parturition (1995). The variation seen among these studies may be due to the different assays that were used to measure fibrinogen (Concannon et al., 1995; Concannon et al., 1996; Ulutas et al., 2009; Vannucchi et al., 2002). Bitches are classified as pregnant with 100% accuracy if concentrations of fibrinogen are over 280 mg/dl, and they are classified as non-pregnant if concentrations of fibrinogen are below 230 mg/dl (Root Kustritz, 2005; Vannucchi et al., 2002).

Ceruloplasmin

Ceruloplasmin is an α2-glycoprotein that protects host tissues during inflammatory processes, transports copper, and functions in antioxidant defense (Cerón and Martínez-Subiela, 2004). Its role in the metabolism and transportation of copper makes ceruloplasmin important to parturition, prostaglandin biosynthesis, and gonadotropin synthesis and release (Ulutas et al., 2009). Ceruloplasmin is known to increase by 140% in response to an induced inflammatory process (Vannucchi et al., 2002). In gestation, concentrations of ceruloplasmin increase by 100% during the second week and have been shown in some studies to increase during the first week as well (Ulutas et al., 2009; Vannucchi et al., 2002). These concentrations remain significantly elevated throughout gestation, and increasing estradiol-17β towards the end of gestation causes a second increase in concentrations of ceruloplasmin (Ulutas et al., 2009; Vannucchi et al., 2002). Values above 7.47 U/l between Days 21−42 of gestation can be significant for diagnosing pregnancy (Vannucchi et al., 2002). Furthermore,

ceruloplasmin was examined in the urine of the giant panda as a potential marker of pregnancy. Urinary ceruloplasmin was found to be elevated in pregnant pandas from the first week of pregnancy until 20−24 days before parturition but was not found to increase in pseudopregnant pandas (Willis et al., 2011).

Alpha-1 Acid Glycoprotein

Glycoproteins, also known as inflammatory proteins, offer an overall profile of an inflammatory reaction. Essentially, they represent the sum of the inflammatory reactions seen from the acute-phase proteins. Alpha-1 acid glycoprotein specifically is considered a fetal protein for most species since it is not usually found in large concentrations in the serum of adult animals (Gruys et al., 2005). Vannucchi et al. found that concentrations of alpha-1 acid glycoprotein (AGP) increased significantly around Day 21 of gestation in pregnant bitches and peaked during the 6th week of gestation (2002).

CHAPTER III

MATERIALS AND METHODS

Animals

Domestic dogs used in this study were from local animal shelters in northern Mississippi and enrolled in the Spay-and-Neuter Program offered by the Mississippi State University College of Veterinary Medicine (Mississippi State, MS, USA). They were temporarily housed aboard the College of Veterinary Medicine's mobile veterinary unit for routine castration or ovariohysterectomy. Animals were deprived of food and water about 12 hours before surgery. Dogs were of various breeds, ages, and weights. Dogs were divided into four weight classes: < 9 kg, 9−18 kg, 18−32 kg, and > 32 kg.

Specimen Collection

Blood, urine, and feces were collected once from 13 pregnant bitches, 3 non-pregnant bitches, and 2 male dogs presented for routine castration or ovariohysterectomy. Bitches were assessed by a veterinarian for pregnancy based on observation and palpation of the uterus. All animals were routinely anesthetized intramuscularly or intravenously with 35 mcg/kg of Dexmedetomidine, 0.35 mg/kg of Butorphanol, and 3.5 mg/kg of Ketamine before surgery. Additionally, 4.4 mg/kg of Carprofen was administered subcutaneously after blood collection. A saphenous venipuncture with a 20- or 22-gauge needle was performed to collect 7 ml of blood in red top and purple top blood separator tubes. Urine was collected by free catch or manual expression of the bladder, and feces was collected by free catch or sanitized fecal loop. Following ovariohysterectomy,

ovaries and uteri of all 16 bitches were saved for additional analysis. Blood was centrifuged into serum, which was stored at -20˚C. Feces were stored at -20˚C, and urine was stored at -80˚C. Reproductive tissues were fixed in neutral buffered 10% formalin. Individual serum and urine samples were dispensed by pipette into four sets of microcentrifuge tubes for use with each assay to prevent freeze-thaw cycles. Since the fibrinogen assay had to be run twice on serum and fecal samples and the AGP assay had to be run twice on fecal samples, some thawing and refreezing did occur with these sets of samples.

Pregnancy and Health Assessment

Pregnancy was confirmed by evaluating uteri for the presence of embryos or fetuses. Ovaries and uteri of the non-pregnant bitches were assessed histologically for signs of pregnancy. Crown-rump length, head diameter, and body diameter of each fetus was measured to the nearest millimeter. Body diameter was measured as an average of two measurements, one caudal to the shoulders and one cranial to the hips. Based on these measurements, average fetal measurements for each litter were calculated. Gestational age (GA), defined as number of days from the LH surge, was calculated from litter measurements according to the equation and chart developed by Yeager et al. (1992) for estimation of gestational age in Beagle fetuses during ultrasound. Since Kutzler et al. (2003) found that small and giant breeds deviate from normal fetal growth trend, the suggested correction factors were used for dogs weighing outside of 9−32 kg. Each litter was classified according to gestational age: early post-implantation gestation (Day 20-30), mid-gestation (Day 30-50), and late gestation (over Day 50).

Because acute-phase proteins also become elevated due to inflammation and infection, it was necessary to assess the health status of animals used in this study (Evans and Anderton, 1992). At sample collection, a brief health assessment was performed by the veterinarian, and any observable health problems were recorded on a health assessment form. All animals were visually evaluated by the veterinarian for mange, flea and tick problems, fever, wounds, inflammation, abnormally high stress response, and other observable issues. Complete blood counts (CBC) were performed by the clinical pathology laboratory at Mississippi State University College of Veterinary Medicine, and results were assessed by a clinical pathologist to screen for disease. For simplicity's sake in assessing health against pregnancy for the acute-phase proteins, each individual animal was assigned a health grade from 0−4 based on the number of abnormalities within their white blood cell counts. Grade 0 was indicative of white blood cell counts within normal limits; grade 1 was indicative of one abnormality within white blood cells counts; grade 2 was indicative of two abnormalities within white blood cell counts, etc.

Laboratory Assays

Relaxin

Relaxin was measured by means of the double-antibody canine relaxin radioimmunoassay (RIA; Steinetz et al., 1996). This method involves synthetic canine relaxin for the standards and iodination of synthetic canine relaxin with ^{125}I as the label. Anti-canine relaxin is used as the primary antibody, and goat anti-rabbit IgG is used as the secondary antibody. Radioactivity was measured through a gamma counter. Samples were run in triplicate in the Endocrinology Laboratory at the Nelson Institute of Environmental Medicine, NYU School of Medicine (Tuxedo, NY, USA).

Fecal Extraction

Extraction of relaxin from feces was modeled following the procedure by Schwabe et al. (1978) for extraction of relaxin from the hyena placenta. Feces were allowed to partially thaw and then weighed on a gram scale. For every 1 g of feces, 10 ml of H_2O and 1 ml of ice cold concentrated HCl was added and mixed thoroughly with the feces. The mixture was placed on a magnetic stirrer at 4˚C for approximately 4 hours. Then 84 ml of acetone was added to the mixture, placed on the magnetic stirrer, and stored overnight at 4˚C. The following day, the mixture was centrifuged at 1000xg for 30 minutes to create a precipitate. The clear supernatant was removed and added to 5 volume acetone that had been pre-cooled to -15˚C. The supernatant-acetone solution was placed on a magnetic stirrer and stored overnight again at 4˚C. The following day, precipitate was collected through centrifugation and was decanted and dried using nitrogen gas. This precipitate was used in the relaxin RIA (Schwabe et al., 1978).

Acute-Phase Proteins

Fibrinogen, alpha-1 acid glycoprotein, and ceruloplasmin were measured in serum, urine, and feces. Fibrinogen was measured using a quantitative two-site enzyme linked immunoassay (ELISA) from MyBioSource (San Diego, CA, USA) following the manufacturer's instructions. In this assay, fibrinogen from the samples forms complexes with the anti-fibrinogen antibodies that have been adsorbed to the surface of polystyrene microtitre wells. Anti-fibrinogen antibodies conjugated with horseradish peroxidase are then added to form complexes with previously bound fibrinogen. Finally, a chromogenic substrate is added to assay the enzyme bound to the immunosorbent. Optical densities were calculated using an ultraviolet-visible spectrophotometer, and concentrations were automatically calculated from the optical density by the SoftMax Pro software.

Alpha-1 acid glycoprotein was also measured using a quantitative two-site enzyme linked immunoassay from MyBioSource (San Diego, CA, USA) following the manufacturer's instructions. As with the fibrinogen ELISA, AGP from the samples forms complexes with the anti-AGP antibodies that have been adsorbed to the polystyrene microtitre wells. Anti-AGP antibodies conjugated with horseradish peroxidase are then added to form complexes with previously bound AGP. Finally, a chromogenic substrate is added to assay the enzyme bound to the immunosorbent. Optical densities were calculated using an ultraviolet-visible spectrophotometer, and concentrations were automatically calculated from the optical density by the SoftMax Pro software.

A trial set of serum, urine, and fecal extracts from four dogs (3 pregnant females, 1 male) was sent to the Memphis Zoo (Memphis, TN) for ceruloplasmin testing. Ceruloplasmin was measured through the oxidasic activity method proposed by Sunderman and Nomoto (1970) for human serum and modified by Willis et al. (2011) for use in urine in the giant panda. This assay determines the concentration of ceruloplasmin based on the rate of formation of a colored product from ceruloplasmin and the substrate N,N-dimethyl-p-phenylenediamine. Ceruloplasmin concentration was proportional to the rate of colorimetric change in each sample (Willis et al., 2011).

Fecal Extraction

Acute-phase proteins were extracted from feces using a wash buffer (Fischer Scientific) according to the fecal extraction process used in the Human Alpha-1-Antitrypsin ELISA from BioVendor (Candler, NC, USA). A mixture of 100 mg of feces and 5 ml of wash buffer was created and homogenized using a vortex mixer. Then 1 ml of the mixture was added to a polystyrene centrifuge tube and centrifuged for 10 minutes

at 10000xg. The resulting supernatant was then diluted using wash buffer according to the dilution protocol for each assay.

Urinary Creatinine

Creatinine assays were performed for each urine sample in the diagnostic laboratory at the Mississippi State University College of Veterinary Medicine (Mississippi State, MS) in order to express concentrations of relaxin and acute-phase protein relative to creatinine. Creatinine was measured using a quantitative absorbance assay from Alfa Wassermann Diagnostic Technologies, LLC (West Caldwell, NJ). In an alkaline medium, creatinine and picric acid react to create a red-orange colored complex that strongly absorbs at 505 nm. The rate of absorbance over a fixed time interval is directly proportional to creatinine concentration. Urine protein to creatinine ratios were then calculated for each set of urine data to standardize urine values.

Data Analysis

Data was evaluated by histograms and Q-Q plots for distribution and the possibility of outliers. The data did not appear to be normally distributed. Therefore, Spearmans rank correlation was used to determine statistical dependence between serum and urine and between serum and feces for each assay. Spearmans rank correlation was also used to determine statistical dependence of concentrations of relaxin, fibrinogen, and AGP with gestational age in pregnant bitches. Spearmans rank correlation is a nonparametric measurement of statistical dependence and was performed using the CORR procedure from SAS for Windows. A probability of $P < 0.05$ was used to indicate significance of the correlation coefficient for each set of data.

The independent t-test was used to determine whether there was a statistical difference between mean concentrations of relaxin, fibrinogen, and AGP in pregnant bitches and mean concentrations of relaxin, fibrinogen, and AGP in non-pregnant bitches. The Satterthwaite method was used with serum and urinary relaxin, urinary AGP, and urinary fibrinogen since their variances were found to be unequal. The pooled method was used for serum and fecal AGP and serum and fecal fibrinogen since their variances were found to be equal. A probability of $P < 0.05$ for the two-sided P value was used to indicate a significant difference in mean concentrations between pregnant bitches and non-pregnant bitches with the expectation that pregnant bitches should have higher mean concentrations. Mean concentrations were expressed as $\bar{x}$ ± SD. An alpha level of 0.05 was used for all statistical tests.

CHAPTER IV

RESULTS

Blood, urine, and feces were collected from 13 pregnant bitches, 3 non-pregnant bitches, and 2 male dogs. All 13 bitches assessed as pregnant by the veterinarian were carrying fetuses. In contrast, no fetuses were present in the uteri of the three bitches assessed by the veterinarian as non-pregnant. Based upon histological observations of the ovaries and uteri, two non-pregnant bitches (Dogs 04 and 06) were physiologically pseudopregnant due to the presence of corpora lutea. The third non-pregnant bitch (Dog 03) had very little activity within her ovaries and uterus and was determined to be anovulatory. In pregnant bitches, number of fetuses per litter ranged from 1−12 and averaged 6 fetuses per little. Gestational age of each litter, as calculated from fetal measurements, ranged from 26−57 days and averaged 43 days (see Table 1). Most pregnant bitches were in mid-gestation between Days 30−50, with two bitches classified as early gestation and three classified as late gestation (see Table 2). The gestational age of each litter was consistent with the physical development of the fetuses according to the chart adapted from Pineda (2003) and Pretzer (2008; see Appendix A).

Table 1 Gender, weight, breed, litter size, and gestational age for eighteen omestic dogs sampled from February to July 2011 in northern Mississippi

Animal ID	Gender	Weight	Breed	Litter Size	GA (days)
Dog 03	F	9−18 kg	Cocker Spaniel	–	–
Dog 04	F	< 9 kg	Beagle	–	–
Dog 05	F	< 9 kg	Beagle	–	–
Dog 06	M	9−18 kg	Rat Terrier	–	–
Dog 07	F	< 9 kg	Chihuahua	5	34 ± 3
Dog 08	F	18−32 kg	Hound Mix	11	43 ± 3
Dog 09	F	18−32 kg	Mix	12	26 ± 3
Dog 10	F	18−32 kg	Lab Mix	6	57 ± 3
Dog 11	F	18−32 kg	Pointer Mix	10	48 ± 3
Dog 12	F	9−18 kg	Terrier Mix	5	45 ± 3
Dog 13	F	18−32 kg	Mix	8	29 ± 3
Dog 14	F	9−18 kg	Husky Mix	7	38 ± 3
Dog 15	F	< 9 kg	Rat Terrier	5	58 ± 3
Dog 16	F	9−18 kg	Mix	1	44 ± 3
Dog 17	F	< 9 kg	Rat Terrier	3	38 ± 3
Dog 19	F	9−18 kg	Mix	6	52 ± 3
Dog 20	M	9−18 kg	Pit Bull Mix	–	–
Dog 21	F	9−18 kg	Mix	3	40 ± 3

NOTE: GA = gestational age, defined as days from the surge of luteinizing hormone

Of the 18 domestic dogs samples, 3 were indicated by the veterinarian as having observable health problems: one with wounds/inflammatory sites and flea infestation (Dog 09), one with tick infestation (Dog 10), and one with flea infestation (Dog 11). According to CBC results, 9 animals presented for possible inflammation and infection due to elevated white blood cell counts. The remaining 9 animals did not appear to be experiencing significant inflammation or infection based upon their white blood counts. Dog 07 presented for an inflamed leukogram, which was likely due to stress, since inflamed leukograms are often seen in small breed dogs such as Chihuahuas. The sample population also contained 2 animals presenting for mild dehydration, 4 presenting for anemia, and 6 presenting for internal and/or external parasites. Of all 18 dogs, 4 had

blood cell counts that were within normal limits as determined by the clinical pathologist and assigned a health grade of 0. Only two dogs (Dogs 09 and 20) were classified with the highest health grade of 4. The remaining 12 dogs were either grade 1 or grade 2 (see Appendix D)

Table 2 Classification of thirteen pregnant bitches as early, mid, or late gestation

Early Gestation (20-50 Days)	Mid-Gestation (30-50 Days)	Late Gestation (>50 Days)
	Dog 07	
	Dog 08	
Dog 09		
		Dog 10
	Dog 11	
	Dog 12	
Dog 13		
	Dog 14	
		Dog 15
	Dog 16	
	Dog 17	
		Dog 19
	Dog 21	

Relaxin

Samples from 17 of the 18 animals were used in the relaxin radioimmunoassay since specimen collection on Dog 21 was not complete when the assay was performed. Due to time constraints, fecal relaxin was only calculated for two animals. The relaxin RIA was performed three times on serum relaxin at quantities of 100 µl, 10 µl, and 2 µl due to the high serum concentrations of relaxin found in the dog. The percent of specific binding for serum concentrations was 18.62%, 17.25%, and 15.35% respectively, and the percent of nonspecific binding was 8.62%, 13.14%, and 13.77% respectively. The relaxin

RIA was performed twice on urine relaxin at quantities of 100 µl and 10 µl. The percent of specific binding for urine concentrations was 11.80% and 10.43% respectively, and the percent of nonspecific binding was 13.02% and 9.16% respectively. The two fecal extracts were run once at 100 µl. The percent of specific binding for fecal extracts was 4.47%, and the percent of nonspecific binding was 16.66%. The average intra-assay coefficient of variation was 4.4% for all relaxin RIAs performed, and the overall interassay coefficient of variation was 19.5%.

Serum concentrations of relaxin ranged from 0.02−11 µg/ml in pregnant bitches with a mean concentration of 3.5 ± 2.4 µg/ml. In contrast, relaxin was undetectable in the serum of non-pregnant bitches and males. Urine concentrations of relaxin ranged from 0 to 24.1 ng/mg creatinine in non-pregnant bitches and from 0.78−480 ng/mg creatinine in pregnant bitches, with mean concentrations of 10.8 ± 30.5 ng/mg creatinine and 108 ± 100 ng/mg creatinine respectively. Relaxin was also detected in the urine of the two male dogs with a mean concentration of 7.2 ± 58.0 ng/mg creatinine. Urine concentrations of relaxin were 3.6% of their respective serum concentrations. Concentrations of relaxin for the two fecal samples were reported as 13.86 ng/ml for the pregnant bitch (Dog 09) and 20.94 ng/ml for the male (Dog 20).

Serum concentrations of relaxin correlated with urine concentrations for the total sample population ($r_{15} = 0.74$, $P < 0.001$), for pregnant and non-pregnant bitches ($r_{13} = 0.78$, $P < 0.001$), and for pregnant bitches only ($r_{10} = 0.82$, $P = 0.001$). Among pregnant bitches, both serum ($r_{10} = 0.69$, $P = 0.01$) and urine concentrations of relaxin ($r_{11} = 0.66$, $P = 0.01$) increased with gestational age (see Appendix B).

Serum concentrations of relaxin were higher in pregnant bitches ($\bar{x} = 3546$, SD = 3735) than in non-pregnant bitches ($\bar{x} = 0$, SD = 0; $t_{11} = 3.29$, $P = 0.007$) since serum

relaxin was undetectable in non-pregnant bitches. Urine concentrations of relaxin were 94% higher in pregnant bitches ($\bar{x}$ = 165, SD = 45.9) than in non-pregnant bitches ($\bar{x}$ = 10.8, SD=12.3; $t_{13} = 2.09$, $P = 0.05$; see Appendix C). The highest concentration of urinary relaxin among the non-pregnant group, both males and females, was 24.1 ng/mg creatinine (see Table 3). There were four pregnant bitches whose concentrations of urinary relaxin also fell below this number: Dog 09, Dog 13, Dog 16, and Dog 11. Dog 09 and Dog 13 were the only two bitches in early gestation within the sample population, and Dog 16 represented the only pregnant bitch to be carrying a litter of one fetus. Dog 11 was likely a true false negative. Therefore, a urine concentration of 25 ng/mg creatinine of relaxin was capable of indicating pregnancy over 30 days in 9 out of 10 pregnant bitches as long as they were carrying multiple fetuses. This equals to a 90% accuracy rate in this study.

Table 3 Serum, urine, and fecal concentrations of relaxin in eighteen domestic dogs as sorted by gestational age (GA)

Animal ID	Pregnant	Gender	Litter Size	GA (days)	Serum (ng/ml)	Urine (ng/mg creatinine)	Feces (ng/ml)
Dog 03	N	F	–	–	0.00	0.00	–
Dog 04	N	F	–	–	0.00	8.18	–
Dog 05	N	F	–	–	0.00	24.14	–
Dog 06	N	M	–	–	0.00	11.78	–
Dog 20	N	M	–	–	0.00	2.65	20.94
Dog 09	Y	F	12	26 ± 3	19.97	0.78	13.86
Dog 13	Y	F	8	29 ± 3	87.44	2.38	–
Dog 07	Y	F	5	34 ± 3	785.70	27.48	–
Dog 14	Y	F	7	38 ± 3	2954.59	53.38	–
Dog 17	Y	F	3	38 ± 3	1894.87	16.14	–
Dog 21	Y	F	3	40 ± 3	–	27.98	–
Dog 08	Y	F	11	43 ± 3	11649.03	163.25	–
Dog 16	Y	F	1	44 ± 3	1219.44	22.73	–
Dog 12	Y	F	5	45 ± 3	10291.32	59.17	–
Dog 11	Y	F	10	48 ± 3	2578.39	1.11	–
Dog 19	Y	F	6	52 ± 3	4277.39	452.41	–
Dog 10	Y	F	6	57 ± 3	3773.34	480.28	–
Dog 15	Y	F	5	58 ± 3	3023.37	97.31	–

Fibrinogen

Samples from all 18 animals were used to measure concentrations of fibrinogen. The urine value on Dog 19 was excluded as an outlier since it was over 200 times higher than the upper quartile for urine concentrations of fibrinogen. There is also no reported fecal value for fibrinogen on Dog 19 because there was not an adequate amount of feces remaining for the fibrinogen assay. The fibrinogen ELISA had to be performed twice on serum and fecal samples due to initially poor results. Since the first set of serum and fecal data reported as zero for all samples, this set of data was excluded from analysis. Since assays were performed on different days, serum and fecal aliquots for fibrinogen

underwent one freeze-thaw cycle during testing. A quantity of 2 µl was adequate for performing the fibrinogen ELISA on serum, urine, and feces. The average intra-assay coefficient of variance for the fibrinogen ELISA was 6.4%, and the overall interassay coefficient of variance was 23.2%.

Serum concentrations of fibrinogen ranged from 4.3−15 µg/ml in non-pregnant bitches and from 4.7−46 µg/ml in pregnant bitches, with mean concentrations of 8.8 ± 13 µg/ml and 18 ± 8.6 µg/ml respectively. Urine concentrations of fibrinogen ranged from 35−83 ng/mg creatinine in non-pregnant bitches and from 33−17000 ng/mg creatinine in pregnant bitches, with mean concentrations of 67 ± 67 ng/mg creatinine and 3161.4 ± 3860.2 ng/mg creatinine respectively. Fecal concentrations of fibrinogen ranged from 5.5− 26 ng/ml in non-pregnant bitches and from 5.3−140 ng/ml in pregnant bitches, with mean concentrations of 15 ± 26 ng/ml and 23 ± 24 ng/ml respectively. Mean concentrations of fibrinogen for the two male dogs were 24 ± 84 µg/ml for serum, 1400 ±16000 ng/mg creatinine for urine, and 380 ± 470 ng/ml for feces. Urine concentrations of fibrinogen were 19.8% of their respective serum concentration, and fecal concentration were 0.7% of their respective serum concentrations.

Serum concentrations of fibrinogen did not correlate with urine concentrations for the total sample population ($r_{15} = 0.36$, $P = 0.16$), for pregnant and non-pregnant bitches ($r_{13} = 0.44$, $P = 0.10$), or for pregnant bitches only ($r_{10} = 0.25$, $P = 0.43$). Serum concentrations of fibrinogen also did not correlate with fecal concentrations for the total sample population ($r_{15} = 0.05$, $P = 0.84$), for pregnant and non-pregnant bitches ($r_{13} = 0.09$, $P = 0.75$), or for pregnant bitches only ($r_{10} = -0.02$, $P = 0.95$). However, urine concentrations of fibrinogen did correlate with fecal concentrations for the total sample population ($r_{15} = 0.51$, $P = 0.04$), for pregnant and non-pregnant bitches ($r_{13} = 0.53$, $P =$

0.04), and for pregnant bitches only (r_{10} = 0.69, P = 0.01). For pregnant bitches, serum (r_{10} = -0.06, P = 0.85) and urine concentrations of fibrinogen (r_{11} = -0.46, P = 0.14) were unrelated to gestational age. Fecal concentrations decreased with gestational age (r_{10} = -0.60, P = 0.04; see Appendix B).

Serum concentrations of fibrinogen did not differ between pregnant bitches ($\bar{x}$ = 17599, SD = 14266) and non-pregnant bitches ($\bar{x}$ = 8818, SD = 5316; t_{10} =1.75, P = 0.11). Urine concentrations of fibrinogen did not differ between pregnant bitches ($\bar{x}$ = 3161, SD = 6075) and non-pregnant bitches ($\bar{x}$ = 66.53, SD = 26.90; t_{11} = 1.76, P = 0.11). Fecal concentrations of fibrinogen did not differ between pregnant bitches ($\bar{x}$ = 23.18, SD = 37.80) and non-pregnant bitches ($\bar{x}$ = 15.49, SD = 10.39; t_{13} = 0.62, P = 0.55; see Appendix C).

Urine concentrations of < 90 ng/mg creatinine were associated with all non-pregnant bitches, males, early gestation bitches, and late gestation bitches except for one male (Dog 09) and one early gestation bitch (Dog 20). Since both Dogs 09 and 20 were the only two animals to receive a grade 4 health status for elevations in multiple white blood cells, their elevated urine concentrations could be attributed to inflammation or infection. All mid-gestation bitches had urine concentrations of fibrinogen > 90 ng/mg creatinine except for one (Dog 21; see Table 4).

Table 4 Serum, urine, and fecal concentrations of fibrinogen in eighteen domestic dogs as sorted by gestational age (GA)

Animal ID	Pregnant	Gender	Litter Size	GA (days)	Serum (ng/ml)	Urine (ng/mg creatinine)	Feces (ng/ml)
Dog 03	N	F	–	–	4341.04	35.49	14.76
Dog 04	N	F	–	–	14693.64	83.06	26.24
Dog 05	N	F	–	–	7419.83	81.03	5.49
Dog 06	N	M	–	–	12348.99	85.47	415.76
Dog 20	N	M	–	–	7075.18	2651.83	341.18
Dog 09	Y	F	12	26 ± 3	9557.45	17114.24	140.69
Dog 13	Y	F	8	29 ± 3	8875.16	54.27	6.31
Dog 07	Y	F	5	34 ± 3	6187.75	3986.30	23.53
Dog 14	Y	F	7	38 ± 3	32741.58	704.14	14.76
Dog 17	Y	F	3	38 ± 3	10192.77	345.29	8.49
Dog 21	Y	F	3	40 ± 3	10481.12	33.01	12.62
Dog 08	Y	F	11	43 ± 3	37077.77	14703.32	18.29
Dog 16	Y	F	1	44 ± 3	15300.93	562.36	29.06
Dog 12	Y	F	5	45 ± 3	33450.52	112.69	5.72
Dog 11	Y	F	10	48 ± 3	46096.66	206.92	5.84
Dog 19	Y	F	6	52 ± 3	7182.16	–	–
Dog 10	Y	F	6	57 ± 3	4665.34	34.22	7.57
Dog 15	Y	F	5	58 ± 3	6989.53	79.49	5.27

Alpha-1 Acid Glycoprotein

Samples from all 18 animals were used to measure concentrations of AGP. The serum value on Dog 16 was excluded as an outlier since it was over 5 times higher than the upper quartile for serum concentrations of AGP. There is also no reported fecal value for fibrinogen on Dog 19 because there was not an adequate amount of feces remaining for the AGP assay. The AGP ELISA was performed twice on fecal samples, and the first set of fecal concentrations were excluded due to a high intra-assay coefficient of variation. Since assays were performed on different days, fecal aliquots for AGP underwent one freeze-thaw cycle during testing. A quantity of 5 µl was adequate for

performing the AGP ELISA on serum, urine, and feces. The average intra-assay coefficient of variance for the AGP ELISA was 5.9%, and the overall interassay coefficient of variance was 33.6%.

Serum concentrations of AGP ranged from 25.1−1790 µg/ml in non-pregnant bitches and from 185−1710 µg/ml in pregnant bitches, with mean concentrations of 805 ± 2120 µg/ml and 949 ± 320 µg/ml respectively. Urine concentrations of AGP ranged from 0−6470 ng/mg creatinine in non-pregnant bitches and from 0−336000 ng/mg creatinine in pregnant bitches, with mean concentrations of 3310 ± 8050 ng/mg creatinine and 47700 ± 61800 ng/mg creatinine respectively. Fecal concentrations of AGP ranged from 0−131 ng/ml in non-pregnant bitches and from 0−239 ng/ml in pregnant bitches, with mean concentrations of 43.6 ± 188 ng/ml and 51.9 ± 53.0 ng/ml respectively. Mean concentrations of AGP for the two male dogs were 1150 ± 922 µg/ml for serum and 2580 ± 3270 ng/mg creatinine for urine. Alpha-1 acid glycoprotein was detectable in the feces of only 7 out of the 17 animals sampled. Urine concentrations of AGP were 2.8% of their respective serum concentrations.

Serum concentrations of AGP correlated with urine concentrations for the total sample population (r_{15} = 0.57, P = 0.02), for pregnant and non-pregnant bitches (r_{13} = 0.65, P = 0.01), and for pregnant bitches only (r_{10} = 0.73, P = 0.01). Serum concentrations of AGP did not correlate with fecal concentrations for either the total sample population (r_{15} = -0.02, P = 0.10), for pregnant and non-pregnant bitches (r_{13} = 0.01, P = 0.98), or for pregnant bitches only (r_{10} = -0.36, P = 0.27). For pregnant bitches, both serum (r_{10} = 0.65, P = 0.02) and urine concentrations of AGP (r_{11} = 0.50, P = 0.08) increased with gestational age; whereas, fecal concentrations of AGP were unrelated to gestational age (r_{10} = -0.35, P = 0.27; see Appendix B).

Serum concentrations of AGP did not differ between pregnant bitches ($\bar{x}$ = 949194, SD = 504228) and non-pregnant bitches ($\bar{x}$ = 804559, SD = 854557; $t_2 = 0.28$, $P = 0.80$). Urine concentrations of AGP did not differ between pregnant bitches ($\bar{x}$ = 47695, SD = 102189) and non-pregnant bitches ($\bar{x}$ = 3313, SD = 3238; $t_{12} = 1.56$, $P = 0.14$). Fecal concentrations of AGP did not differ between pregnant bitches ($\bar{x}$ = 51.90, SD = 84.99) and non-pregnant bitches ($\bar{x}$ = 43.59, SD = 75.50; $t_4 = 0.17$, $P = 0.88$; see Appendix C).

Urine concentrations of AGP were undetectable in all but one pregnant bitch under Day 40 of gestation. That exception was again Dog 09, which had a grade 4 health status. All pregnant bitches over Day 40 and one pregnant bitch at Day 38 had urine concentrations of AGP of 715 ng/mg creatinine or higher. Of the non-pregnant bitches, only one had undetectable concentrations of AGP in urine; the other two had concentrations > 3000 ng/mg creatinine (see Table 5).

Table 5 Serum, urine, and fecal concentrations of alpha-1 acid glycoprotein in eighteen domestic dogs as sorted by gestational age (GA)

Animal ID	Pregnant	Gender	Litter Size	GA (days)	Serum (ng/ml)	Urine (ng/mg creatinine)	Feces (ng/ml)
Dog 03	N	F	–	–	1788778.11	3466.86	130.76
Dog 04	N	F	–	–	373700.41	6472.28	0.00
Dog 05	N	F	–	–	251198.58	0.00	0.00
Dog 06	N	M	–	–	1072651.27	5153.04	0.00
Dog 20	N	M	–	–	1217737.05	0.00	0.00
Dog 09	Y	F	12	26 ± 3	656306.97	18388.96	129.34
Dog 13	Y	F	8	29 ± 3	248792.48	0.00	0.00
Dog 07	Y	F	5	34 ± 3	184794.69	0.00	239.35
Dog 14	Y	F	7	38 ± 3	787744.78	0.00	0.00
Dog 17	Y	F	3	38 ± 3	1280638.55	29846.69	11.05
Dog 21	Y	F	3	40 ± 3	500490.92	739.84	0.00
Dog 08	Y	F	11	43 ± 3	1148643.00	5189.16	187.11
Dog 16	Y	F	1	44 ± 3	–	568.07	51.09
Dog 12	Y	F	5	45 ± 3	1554468.96	8593.58	0.00
Dog 11	Y	F	10	48 ± 3	1449405.90	19370.29	0.00
Dog 19	Y	F	6	52 ± 3	1120545.90	200515.86	–
Dog 10	Y	F	6	57 ± 3	751638.16	714.91	4.92
Dog 15	Y	F	5	58 ± 3	1706857.35	336113.41	0.00

Ceruloplasmin

A trial set of serum, urine, and fecal extracts from four dogs (3 pregnant bitches, 1 male) was sent to the Memphis Zoo (Memphis, TN) for ceruloplasmin testing (see Table 6). There was only low amount of ceruloplasmin present in the urine and feces, and duplicate values may be indicative of baseline values in the assay. Negative concentrations for the urine suggest that those samples needed to be rerun at different dilutions, but unfortunately, there was not enough sample remaining to do so.

Table 6 Serum, urine, and fecal concentrations of ceruloplasmin in four domestic dogs

Animal ID	Gender	Litter Size	GA (days)	Serum (ng/ml)	Urine (ng/mg creatinine)	Feces (ng/ml)
Dog 06	M	–	–	3.86	-0.7	0.43
Dog 10	F	6	57 ± 3	4.29	-0.7	0
Dog 13	F	8	29 ± 3	2.57	0.56	0
Dog 21	F	3	40 ± 3	4.29	0	0.43

CHAPTER V

DISCUSSION

Relaxin, fibrinogen, and alpha-1 acid glycoprotein were detected in the urine of the domestic dog. Relaxin and fibrinogen were also detected in the feces of the domestic dog although fecal fibrinogen was low compared to serum and urine concentrations. This is the first reported instance in which relaxin, the pregnancy-specific hormone for canines, was detected in the urine of the domestic dog and is also the first reported study to evaluate the presence of relaxin in fecal samples. Although serum, urine, and fecal concentrations of fibrinogen and AGP were not significant for detecting pregnancy in this study, urinary relaxin appeared to be capable of detecting pregnancy at concentrations of 25 ng/mg creatinine or higher in bitches over 30 days past the LH surge as long as they were carrying multiple fetuses.

Relaxin

Serum concentrations of relaxin can typically elevate to 8−10 μg/ml during pregnancy in the domestic dog, generally reaching these higher concentrations around Day 40 to 50 after the LH surge (Steinetz et al., 1996; Steinetz et al., 1987). This was true among the pregnant bitches within our sample population, two of which reached serum concentrations > 10 μg/ml. Both of these bitches were close to Day 45 of gestation. Although serum and urine concentrations of relaxin correlated well, urine concentrations were markedly lower than serum concentrations and did not reach the high concentrations seen with serum relaxin in domestic bitches. This was somewhat unexpected as the study

by De Haas van Dorsser et al. (2006) showed similar mean concentrations of relaxin for serum and urine, differing by only 1−3 ng/ml, in pregnant felids. The difference could be attributed to a difference in renal physiology and degradation and reabsorption rates between the two species. Relaxin is believed to have profound effects upon the renal system during pregnancy in humans and rats, increasing glomerular filtration rate and renal plasma flow (Bogzil et al., 2005; Conrad and Novak, 2004; Smith et al., 2006). If relaxin has similar functions in the domestic dog, it could be possible that the high concentrations of relaxin in the circulating blood are being partially consumed and metabolized by the renal system prior to excretion.

Because this study was limited to a one-time sample collection of each animal, there was little means to investigate whether or not there was any lag time involved in the excretion rates of urinary relaxin. While serum concentrations are representative of concentrations of relaxin in the circulating blood at the time of blood collection, urine concentrations would likely be representative of concentrations of relaxin in circulating blood days prior to the time of urine collection. In the study on urinary relaxin in felids, serum concentrations of relaxin became elevated around Day 21; whereas, urine concentrations did not become elevated until a week later (De Haas van Dorsser et al., 2006). This is likely why serum relaxin was capable of identifying pregnancy in the two early gestation bitches in this study while urinary relaxin was not. If urinary relaxin is to be used for pregnancy diagnosis in canines, it would be beneficial to develop an overall profile of urinary relaxin during pregnancy in the domestic bitch in order to isolate the exact day at which urinary relaxin becomes elevated. Based upon the results of this study, urinary relaxin appears to become elevated between 30-35 days after the LH surge.

Urinary Relaxin in the Non-Pregnant Bitch and Male Dog

It was interesting to note that in this study, while relaxin was undetectable in the serum of non-pregnant bitches and males, it was present in the urine of both males and two of the three non-pregnant bitches. This differs from the studies on felids and maned wolves where urinary relaxin, like serum relaxin, was undetectable in non-pregnant females and in males (De Haas van Dorsser et al., 2006; Steinetz et al., 2009). It could be possible that something within the urine is causing interference with the relaxin radioimmunoassay. If that is the case, an extraction process might be needed. However, De Haas van Dorsser et al. (2006) found that an extraction process for relaxin on cat urine resulted in 10-fold losses in concentration and, therefore, favored using unextracted cat urine. The other possibility is that trace amount of relaxin are excreted in the urine of non-pregnant bitches and males. In non-pregnant bitches, the source of these low amounts of relaxin could be attributed to the ovary. While the placenta is the primary source for relaxin production in the domestic dog, relaxin is also produced in smaller amounts by the ovary (Klonisch et al., 1999; Steinetz et al., 1989). In the mare, the corpus luteum is thought to be the source of relaxin production by the ovary since relaxin gene expression and production in the ovary are greatest during the luteal phase, declining after luteolysis (Ryan et al., 1997). Since both of the two non-pregnant bitches with traces of relaxin immunoreactivity in their urine (Dog 04 and 06) had several corpus lutea present on their ovaries, it is possible that this is also true for the domestic dog. In contrast, the one non-pregnant bitch for which relaxin was undetectable in the urine (Dog 03) showed very little ovarian activity histologically. Serum concentrations of relaxin are known to be significantly lower in ovariectomized bitches as compared to

unaltered bitches, and it has been suggested that ovarian relaxin contributes to total circulating concentrations of relaxin (Steinetz et al., 1989).

Additionally, relaxin has been found in the testis and seminal fluid of many species including chickens, pigs, humans, baboons, cattle, goats, guinea pigs, and rats (Sherwood, 2004; Weiss, 1989). Relaxin produced in the male reproductive tract is excreted primarily in the seminal fluid and is thought to function in sperm motility (Sherwood, 2004). Relaxin in seminal fluid can reach concentrations as high as 40 ng/ml in bulls (Kohsaka et al., 2003). Neither of the male dogs in this study exhibited concentrations of urinary relaxin as high as this, both having urine concentrations below 12 ng/ml. Studies on perineal hernias in male dogs have shown that relaxin is expressed in the prostate and paraprostatic tissues of the domestic dog (Niebauer et al., 2005). Whether or not the relaxin in the urine of the two male dogs is actually urinary relaxin or whether it is simply residual concentrations left by seminal fluid is uncertain considering both male dogs were intact prior to sample collection. It is interesting to note that the urine sample from Dog 06, which was collected in late February during the typical spring breeding period for many domestic dogs, had a higher concentration of relaxin than the urine sample from Dog 20, which was collected in mid-June. Seminal relaxin has been demonstrated to exhibit monthly patterns in bonnethead sharks (Gelsleichter et al., 2003). Therefore, it may be of interest to perform a more in-depth study of urinary relaxin in male dogs to evaluate its origin and determine if male dogs exhibit seasonal reproductive patterns for relaxin.

Fecal Relaxin

Relaxin immunoreactivity was found in the fecal extracts of two domestic dogs, one a pregnant bitch and the other a male dog. Based upon the results from these two samples, it was originally determined that fecal relaxin would not be useful for pregnancy diagnosis since the male dog had a higher fecal concentration of immunoreactive relaxin than did the pregnant bitch, and due to time constraints, no further investigation was made. However, upon further review of the data, the particular bitch (Dog 09) for which fecal relaxin was measured was found to be one of the early gestation bitches that presented as a false negative with urinary relaxin. Therefore, a lower concentration in Dog 09 might not necessarily reflect on fecal relaxin in other pregnant bitches, as originally thought, and it might be worthwhile to further examine the potential of fecal relaxin for pregnancy testing. If it is indeed relaxin being measured in the feces, this would be the first reported evidence of fecal relaxin to date.

The two fecal concentrations of immunoreactive relaxin were higher in concentration than their respective urine concentrations and, therefore, may be more comparable to serum concentrations. Since relaxin is a protein hormone and not a steroid hormone, there is some concern in regards to degradation and reabsorption within the gastrointestinal tract. The mechanism by which relaxin is degraded is not very well understood, but studies have shown that relaxin can act as a substrate for and is rapidly degraded by insulin-degrading enzyme. However, relaxin has a relatively low affinity for binding with insulin-degrading enzyme, insulin being the more preferable substrate (Bennett et al., 2009; Pilistine and Varandani, 1986). Therefore, it may be possible that relaxin is excreted in the feces with minimal losses in concentration. Further study into the degradation mechanism for relaxin is needed, however, to support this suggestion.

Additionally, another issue that needs to be addressed in regards to fecal relaxin, especially if it is to be used with wild populations, is the degradation of relaxin by fecal bacteria. Chemical changes caused by bacteria or by improper storage procedures could potentially skew concentrations due to cross-reactivity with other molecules. Such problems have been well documented and addressed with steroid hormones, such as estrogen and glucocorticoids, which can decompose hours after defecation (Khan et al., 2002; Moestl et al., 1999). However, these issues have not yet been addressed with protein hormones.

Acute-Phase Proteins

Since the study by Vannucchi et al. (2002) on serum AGP used a different method of measuring AGP than this study, it was difficult to determine if our serum AGP concentrations were comparable. However, serum concentrations of fibrinogen were extremely low as compared to other studies in which fibrinogen was found to range as high as 300−800 mg/dl in the serum of pregnant bitches (Root Kustritz, 2005; Ulutas et al., 2009; Vannucchi et al., 2002). The highest serum concentration of fibrinogen within the whole sample population for this study was 11.52 mg/dl. Why serum concentrations of fibrinogen were so low in this study is uncertain. However, the serum aliquots for fibrinogen did undergo a freeze-thaw cycle during the testing process. Acute-phase proteins appear to be particularly sensitive to freeze-thaw cycles. For example, concentrations of ceruloplasmin have been known to decrease by 33% by the second thawing and by 50% by the fourth thawing (Willis et al., 2011). Furthermore, while urine and fecal concentrations of fibrinogen did not correlate with serum concentrations, urine and fecal concentrations did show a positive correlation with each other. This could be

further indication that the serum concentrations of fibrinogen were negatively affected by the extra freeze-thaw cycle during testing and might not necessarily be a reliable representation.

Neither fibrinogen nor AGP were found to be statistically significant for pregnancy in serum, urine, or feces. This could be due in part to the questionable health status of the sample population. Since the sample population was a random assortment of dogs from local animal shelters, there was no medical history available beyond the veterinarian's one-time health assessment at the time of sample collection. Furthermore, it was virtually impossible to differentiate elevations in fibrinogen and AGP that were caused by pregnancy from those elevations caused by inflammation based solely on CBC results. However, there were a few tentative observations that could be made about urine concentrations of fibrinogen and AGP. First, urine concentrations of fibrinogen appeared to be elevated in pregnant bitches between Days 30−50 as compared to lower concentrations seen in non-pregnant bitches and bitches in early and late gestation. If a lag time of a few days is assumed for urine concentrations, then this is fairly consistent with the elevation of serum concentrations of fibrinogen reported as being between Day 25-50 by Concannon et al. (1995). Likewise, urine concentrations of AGP appeared to become elevated beyond Day 40 as compared to mostly undetectable concentrations for bitches earlier in gestation. This coincides fairly conveniently with the peak in serum concentrations of AGP noted to occur around the 6th week of gestation (Vannucchi et al., 2002). However, 2 of the 3 non-pregnant bitches had urine concentrations of AGP as high or higher than the concentrations seen among pregnant bitches, and there was no way of differentiating between the two groups. Furthermore, as stated earlier, there was also no way to confirm whether or not these elevations in fibrinogen and AGP could be attributed

solely to pregnancy status. Even so, the urine concentrations for both fibrinogen and AGP were more explicable in relation to pregnancy than were their respective serum concentrations. This could be due to a reduction of sporadic patterns of secretion caused by pooling of the urine in the bladder (Monfort, 2003).

While this study does provide evidence for the presence of fibrinogen and AGP in the urine of the domestic dog, a more controlled study in which the health of the sample population can be reliably monitored and assessed is needed before their usefulness for noninvasive pregnancy testing can be fully determined. If anything, the results on fibrinogen and AGP are witness to the difficulty of using acute-phase proteins for pregnancy diagnosis with animals of limited or unknown medical histories. Therefore, even if a noninvasive pregnancy test could be validated for fibrinogen or AGP in the domestic dog, it would not be applicable for use on canines within wild populations where diseases and infections are likely even more problematic than with our sample population of stray dogs. Any application for pregnancy diagnosis with acute-phase proteins would be limited to zoo populations, where health can actually be monitored. Acute-phase proteins like fibrinogen and AGP typically increase around 8 hours after an inflammatory stimulus and then remain elevated between 24 and 48 hours (Gruys et al., 2005). Therefore, in a zoo setting where urine samples can be routinely collected and tested, it may be much easier to rule out the possibility of elevations due to infection.

Implications of the Study

While serum relaxin would still be the more preferable method of diagnosing pregnancy in the domestic dog due to its 100% accuracy rate even in early gestation bitches, the benefit of urinary relaxin is that it is noninvasive and eliminates stress caused

by blood collection. Therefore, urinary relaxin would be the more preferable method in nondomestic canines, especially captive populations that are more susceptible to captivity stress and its negative effects on reproduction. Additionally, the ability to measure relaxin in urine may provide wildlife biologists the opportunity to study pregnancy rates and other reproductive factors in wild populations of some nondomestic canines since it is possible to collect frozen urine samples from snow for analysis. Most nondomestic canines experience gestation around late winter to early spring, so it would be possible to collect frozen urine samples for pregnancy diagnosis on species found in areas of long periods of snow cover, such as with the wolf (Mech et al., 1987).

Furthermore, this is the first study to detect relaxin in feces of the domestic dog. Although further study is needed to support the finding of fecal relaxin, the implications of this for wildlife biologists studying reproduction in nondomestic canines would be enormous considering that scat samples are far more easily obtained from free-ranging populations than serum samples or even urine samples. With noninvasive genetic sampling possible with feces, wildlife biologists could potentially measure relaxin and determine the specific individual to which the scat belongs and the gender of that individual (Waits and Paetkau, 2005). Furthermore, since progesterone can be measured from fecal samples, it would also be possible to evaluate pregnancy rates, pseudopregnancy rates, and anovulatory rates in wild populations, which could greatly benefit the study of canine reproductive biology.

Conclusion

Due to the diverse nature of the sample population, this study was not able to evaluate factors such as breed, maternal weight, or litter size and their relationship to

concentrations of relaxin and acute-phase proteins. Breed, maternal weight, and litter size are thought to influence serum concentrations of relaxin in the domestic bitch (Synbiotics Corporation, 2012). Additionally, large differences in concentrations of relaxin have been seen between breeds in the mare (Stewart et al., 1992). In contrast, no such studies have been done to assess the effects of such factors on concentrations of acute-phase protein. Therefore, it might be beneficial to characterize the relationship of breed, maternal weight, and litter size with concentrations of relaxin and acute-phase proteins. Suggestions for future research also include evaluating 1) lag times for urine and fecal concentrations, 2) recovery rates within urine and fecal samples, 3) the source of urinary and fecal relaxin in the male dog, 4) seasonality of urinary relaxin within the male dog, 5) hormonal profiles for pregnant bitches, 6) effects of freeze-thaw cycles on fibrinogen and AGP, 7) storage methods for fecal samples, and 8) the effect of degradation by fecal bacteria upon relaxin.

Although this study could not reliably confirm the ability of urinary fibrinogen or urinary AGP to detect pregnancy in the domestic dog, these two acute-phase proteins warrant further research with a more accessible sample population for which health factors can be controlled and evaluated. Such a study may well unveil the potential of acute-phase proteins in noninvasive pregnancy diagnosis for canines. Further research is also needed to confirm the presence of relaxin within the feces of the domestic dog and determine whether or not fecal relaxin is capable of indicating pregnancy in the domestic dog. However, this study presents the first evidence for the use of urinary relaxin as a method of noninvasive pregnancy diagnosis in the domestic dog, which may be used as a basis for the development of similar noninvasive pregnancy tests in other nondomestic canine.

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APPENDIX A

CANINE FETAL DEVELOPMENT

Table 7 Fetal development chart for the domestic dog adapted from Pineda (2003) and Pretzer (2008)

Day of Gestation	Length	Noticeable Physical Development
Day 16−18	—	Implantation occurs.
Day 23	0.5−1.0 cm	First limb buds are visible.
Day 24	0.5−1.4 cm	Thoracic limb buds are present; eyes are first visible.
Day 25	1.0−1.4 cm	Mammary ridge is present.
Day 28	1.2−1.7 cm	First ossification is seen in mandible, maxilla, frontal bone, and clavicle.
Day 30	1.9 cm	Eyelids and external ears are forming; distinct forelimb digits are present.
Day 32	2.3−2.7 cm	Sexual differentiation occurs.
Day 33	2.7 cm	Ossification is developing in facial bones, ribs, and the mid-shaft of several long bones; the palatal shelves have fused; and there are distinct digits on the hind paws.
Day 35	2.8−4.1 cm	Developing eyelids cause the eye to be almost closed; the pinna covers the opening of the ear; claws are developing.
Day 36	—	Testicular differentiation occurs.
Day 40	—	Eyes are closed, and the eyelids are fused; claws have developed on all digits.
Day 42	4.0−9.0 cm	Fetus is fully developed.
Day 45	—	Color markings appear on the body; hair begins to form; scrotal swellings are large in males, and labia are present in females.
Day 49	9.0−10.0 cm	Hair is present.
Day 55	—	All deciduous teeth are calcified.

APPENDIX B

SCATTER PLOTS FOR SERUM, URINE, AND FECAL CONCENTRATIONS OF RELAXIN, FIBRINOGEN, AND AGP WITH GESTATIONAL AGE OF THIRTEEN PREGNANT BITCHES

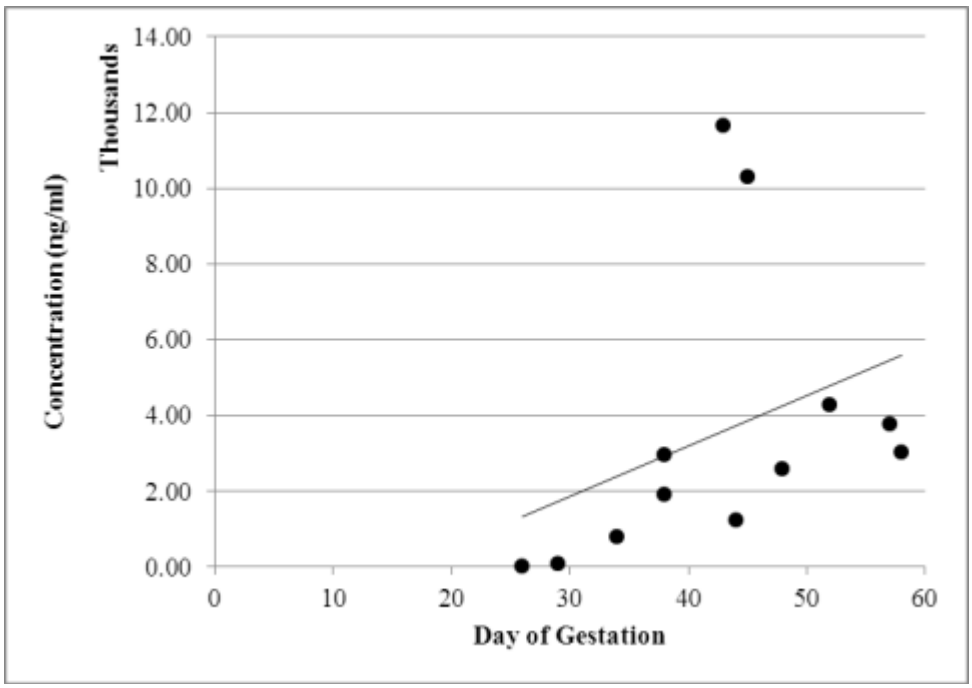

Figure 1 Scatter plot between serum relaxin and day of gestation in thirteen pregnant bitches ($r = 0.69$, $P = 0.01$)

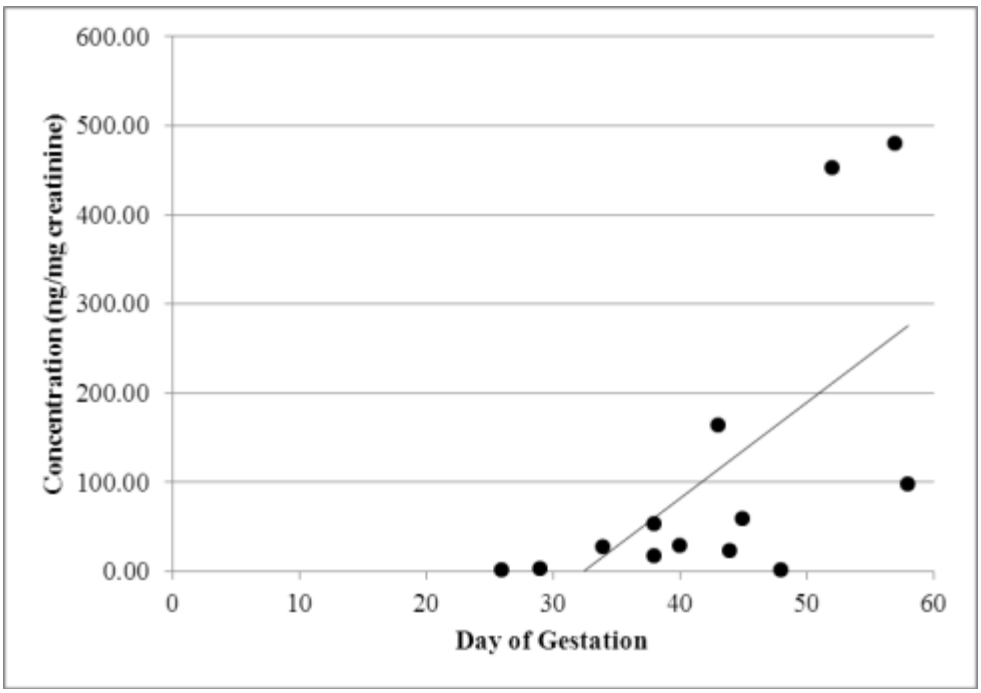

Figure 2 Scatter plot between urinary relaxin and day of gestation in thirteen pregnant bitches ($r = 0.66$, $P = 0.01$)

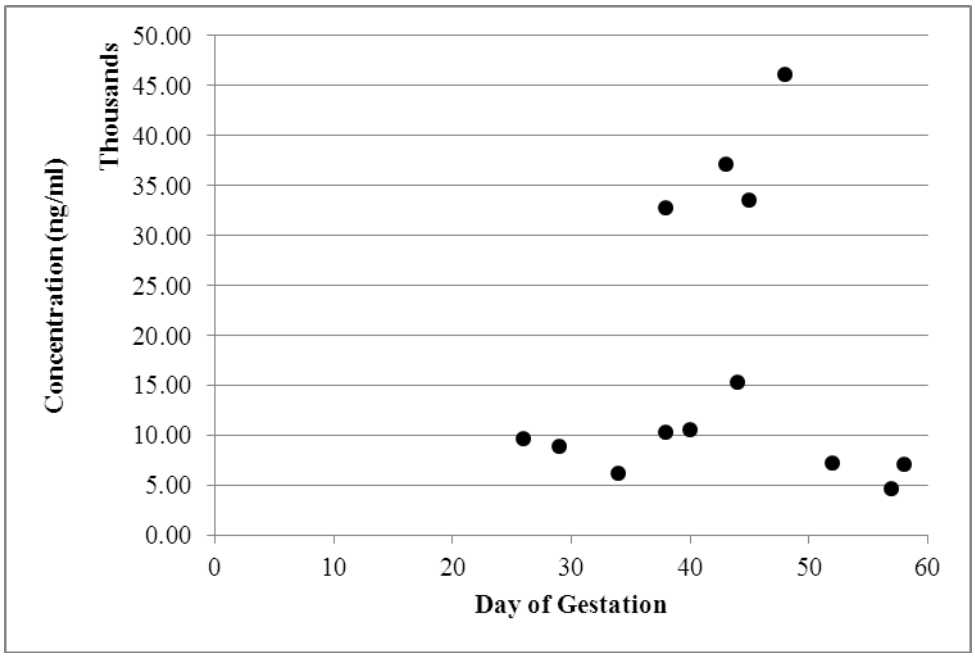

Figure 3 Scatter plot between serum fibrinogen and day of gestation in thirteen pregnant bitches (r = -0.06, P = 0.85)

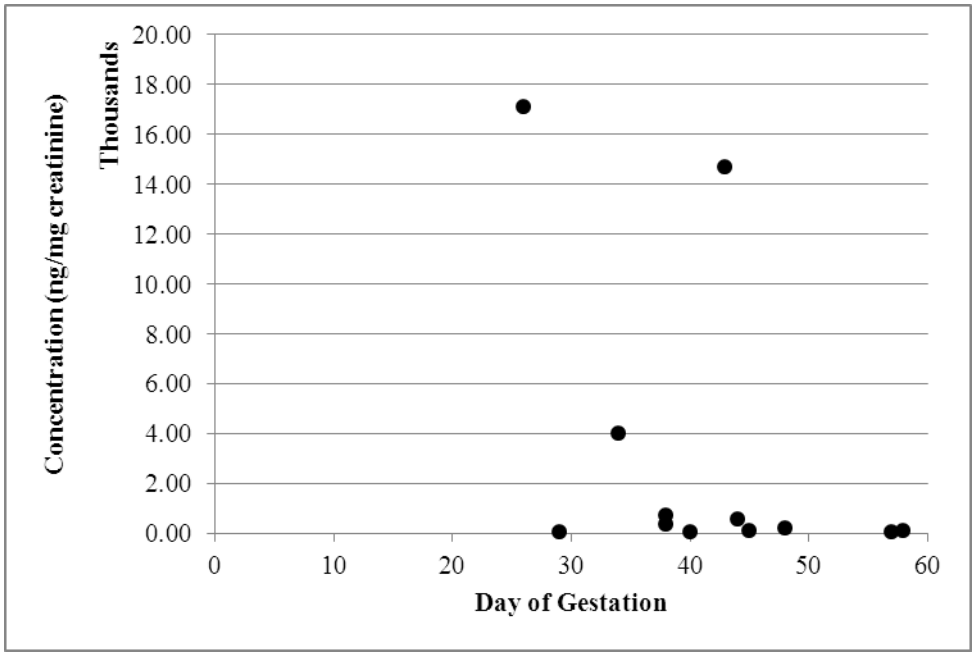

Figure 4 Scatter plot between urinary fibrinogen and day of gestation in thirteen pregnant bitches (r = -0.46, P = 0.14)

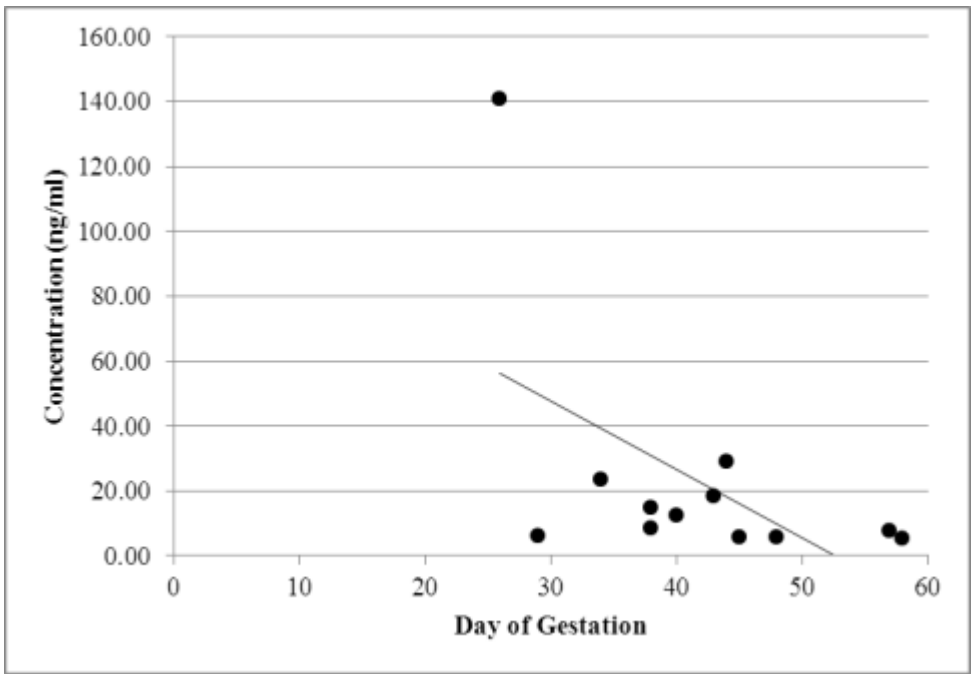

Figure 5 Scatter plot between fecal fibrinogen and day of gestation in thirteen pregnant bitches (r = -0.60, P = 0.04)

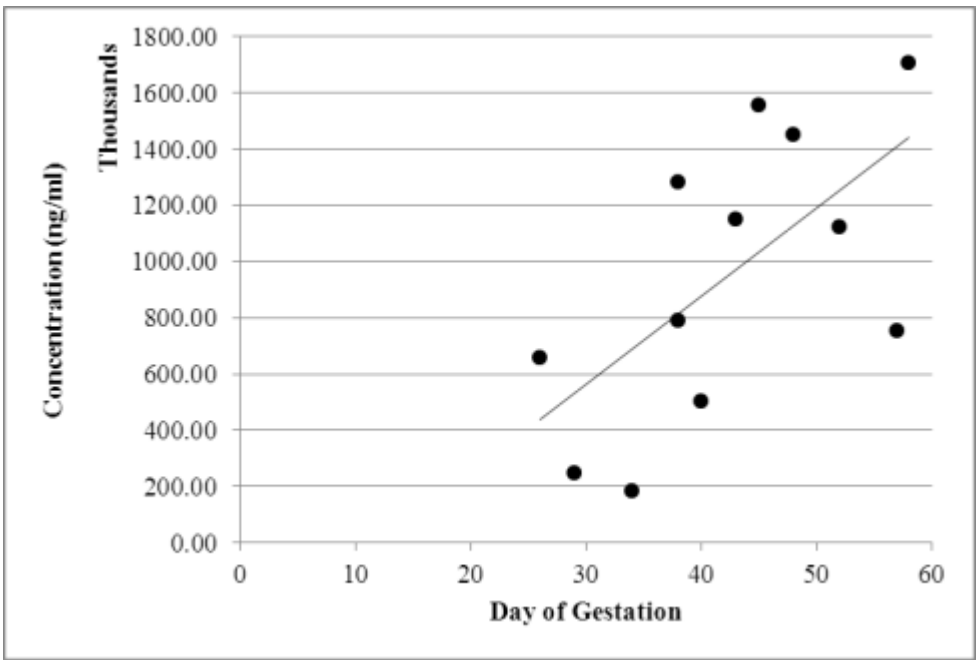

Figure 6 Scatter plot between serum AGP and day of gestation in thirteen pregnant bitches (r = 0.65, P = 0.02)

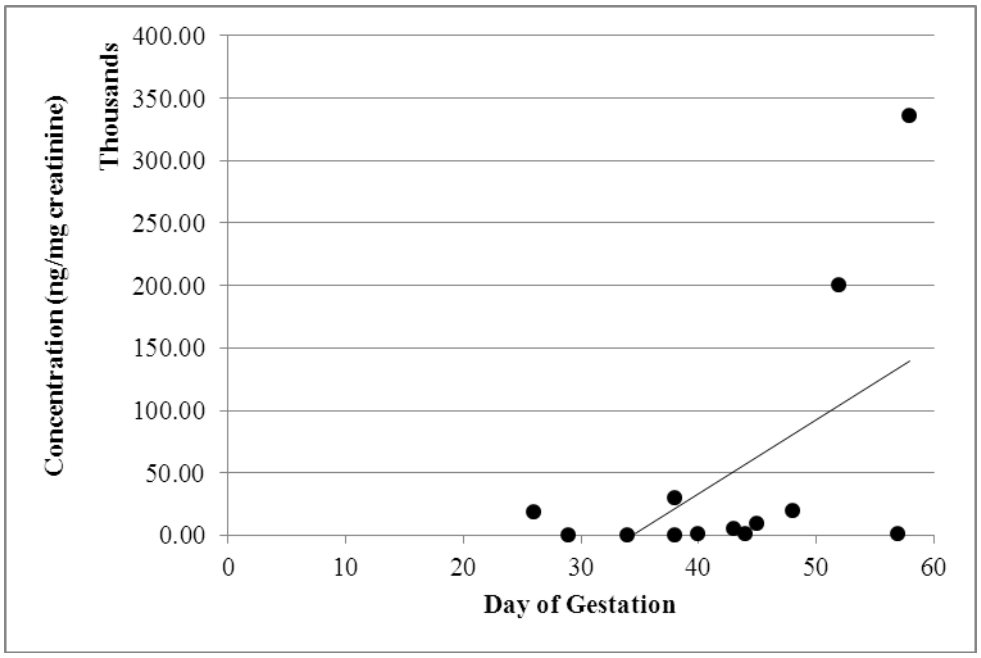

Figure 7 Scatter plot between urinary AGP and day of gestation in thirteen pregnant bitches (r = 0.50, P = 0.08)

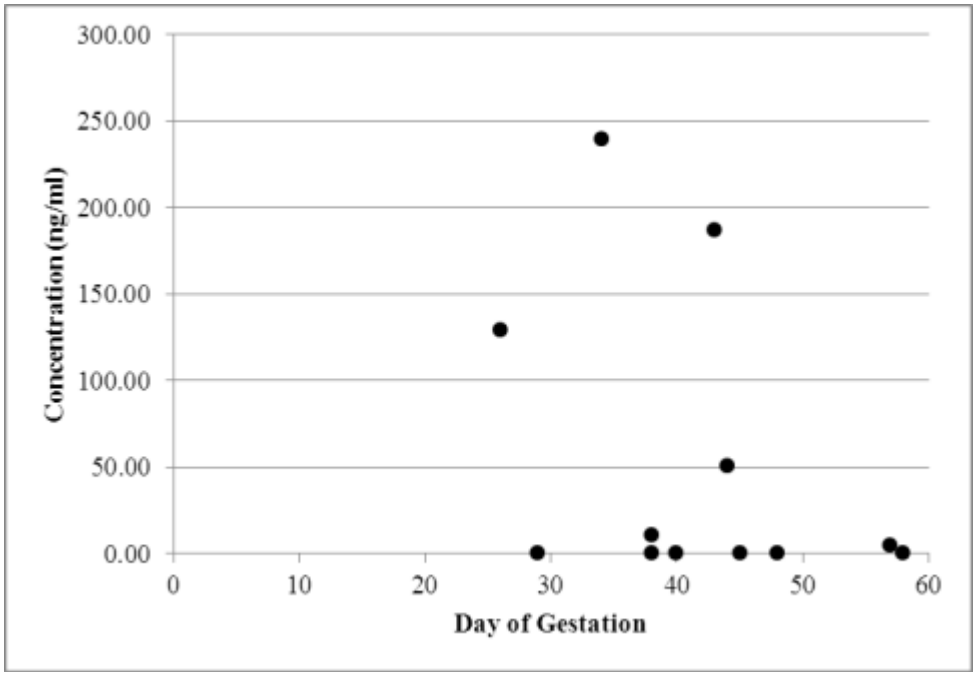

Figure 8 Scatter plot showing no correlation between fecal AGP and day of gestation in thirteen pregnant bitches (r = -0.35, P = 0.27)

APPENDIX C

T TEST RESULTS FOR SERUM, URINE, AND FECAL CONCENTRATIONS OF RELAXIN, FIBRINOGEN, AND AGP FOR PREGNANCY STATUS IN EIGHTEEN DOMESTIC DOGS

Table 8 Independent t-test on serum concentrations of relaxin to evaluate mean differences in concentrations between pregnant and non-pregnant bitches

Pregnant	N	Mean	Std Dev	Std Err	Minimum	Maximum
N	3	0	0	0	0	0
Y	12	3546.2	3735.1	1078.2	19.97	11649
Diff (1-2)		-3546.2	3435.8	2217.8		

Pregnant	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
N		0	0	0	0	–	–
Y		3546.2	1173.1	5919.4	3735.1	2645.9	6341.7
Diff (1-2)	Pooled	-3546.2	-8337.4	1245	3435.8	2490.8	5535.1
Diff (1-2)	Satterthwaite	-3546.2	-5919.4	-1173.1			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	13	-1.6	0.1338
Satterthwaite	Unequal	11	-3.29	0.0072

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	11	2	Infty	<.0001

Table 9 Independent t-test on urine concentrations of relaxin to evaluate mean differences in concentrations between pregnant and non-pregnant bitches

Pregnant	N	Mean	Std Dev	Std Err	Minimum	Maximum
N	3	10.7719	12.2744	7.0866	0	24.135
Y	13	108	165.4	45.8828	0.7848	480.3
Diff (1-2)		-972588	153.2	98.1466		

Pregnant	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
N		10.7719	-19.7193	41.2632	12.2744	6.3908	77.1413
Y		108	8.0606	208	165.4	118.6	273.1
Diff (1-2)	Pooled	-972588	-307.8	113.2	153.2	112.2	241.7
Diff (1-2)	Satterthwaite	-972588	-197.9	3.4186			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	14	-0.99	0.3385
Satterthwaite	Unequal	12.537	-2.09	0.0571

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	12	2	181.65	0.011

Table 10 Independent t-test on serum concentrations of fibrinogen to evaluate mean differences in concentrations between pregnant and non-pregnant bitches

Pregnant	N	Mean	Std Dev	Std Err	Minimum	Maximum
N	3	8818.2	5316.1	3069.2	4341	14693.6
Y	13	17599.9	14266.1	3956.7	4665.3	46096.7
Diff (1-2)		-8781.7	13359.8	8557.1		

Pregnant	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
N		8818.2	-4387.7	22024	5316.1	2767.9	33410.1
Y		17599.9	8979	26220.8	14266.1	10230	23549.5
Diff (1-2)	Pooled	-8781.7	-27134.9	9571.4	13359.8	9781	21069.7
Diff (1-2)	Satterthwaite	-8781.7	19985.5	2422.1			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	14	-1.03	0.3222
Satterthwaite	Unequal	9.7043	-1.75	0.1109

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	12	2	7.2	0.2565

Table 11 Independent t-test on urine concentrations of fibrinogen to evaluate mean differences in concentrations between pregnant and non-pregnant bitches

Pregnant	N	Mean	Std Dev	Std Err	Minimum	Maximum
N	3	66.526	26.8958	15.5283	35.4915	83.0578
Y	12	3161.4	6075.6	1753.9	33.0097	17114.2
Diff (1-2)		-3094.8	5588.8	3607.5		

Pregnant	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
N		66.526	-0.2869	133.3	26.8958	14.0035	169
Y		3161.4	-698.9	7021.6	6075.6	4303.9	10315.7
Diff (1-2)	Pooled	-3094.8	-10888.4	4698.8	5588.8	4051.6	9003.7
Diff (1-2)	Satterthwaite	-3094.8	-6955.2	765.5			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	13	-0.86	0.4065
Satterthwaite	Unequal	11.002	-1.75	0.1054

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	11	2	51028.3	<.0001

Table 12 Independent t-test on fecal concentrations of fibrinogen to evaluate mean differences in concentrations between pregnant and non-pregnant bitches

Pregnant	N	Mean	Std Dev	Std Err	Minimum	Maximum
N	3	15.4947	10.3917	5.9997	5.492	26.236
Y	12	23.1787	37.8028	10.9127	5.265	140.7
Diff (1-2)		-7.684	35.0116	22.5999		

Pregnant	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
N		15.4947	-10.3198	41.3091	10.3917	5.4105	65.3091
Y		23.1787	-0.8401	47.1975	37.8028	26.7794	64.1846
Diff (1-2)	Pooled	-7.684	-56.5081	41.1401	35.0116	25.3818	56.4052
Diff (1-2)	Satterthwaite	-7.684	-34.7169	19.3489			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	13	-0.34	0.7393
Satterthwaite	Unequal	12.416	-0.62	0.5484

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	11	2	13.23	0.1446

Table 13 Independent t-test on serum concentrations of AGP to evaluate mean differences in concentrations between pregnant and non-pregnant bitches

Pregnant	N	Mean	Std Dev	Std Err	Minimum	Maximum
N	3	804559	854557	493379	251199	1788778
Y	12	949194	504228	145558	184795	1706857
Diff (1-2)		-144635	572258	369391		

Pregnant	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
N		804559	-1318277	2927395	854557	444932	5370662
Y		949194	628823	1269565	504228	357192	856117
Diff (1-2)	Pooled	-144635	-942656	653386	572258	414861	921932
Diff (1-2)	Satterthwaite	-144635	-2063852	1774583			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	13	-0.39	0.7017
Satterthwaite	Unequal	2.3601	-0.28	0.8014

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	2	11	2.87	0.1983

Table 14 Independent t-test on urine concentrations of AGP to evaluate mean differences in concentrations between pregnant and non-pregnant bitches

Pregnant	N	Mean	Std Dev	Std Err	Minimum	Maximum
N	3	3313	3238.9	1870	0	6472.3
Y	13	47695.4	102189	28342.2	0	336113
Diff (1-2)		-44382.4	94616.9	60603.3		

Pregnant	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
N		3313	-4732.8	11358.9	3238.9	1686.4	20355.5
Y		47695.4	-14057	109448	102189	73278.6	168688
Diff (1-2)	Pooled	-44382.4	-174364	85598.8	94616.9	69271.6	149220
Diff (1-2)	Satterthwaite	-44382.4	-10621	17445.8			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	14	-0.73	0.476
Satterthwaite	Unequal	12.103	-1.56	0.1439

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	12	2	995.45	0.002

Table 15 Independent t-test on fecal concentrations of AGP to evaluate mean differences in concentrations between pregnant and non-pregnant bitches

Pregnant	N	Mean	Std Dev	Std Err	Minimum	Maximum
N	3	43.5873	75.4955	43.5873	0	130.8
Y	12	51.9043	84.9883	24.534	0	239.4
Diff (1-2)		-8.3169	83.5981	53.9623		

Pregnant	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
N		43.5873	-144	231.1	75.4955	39.3073	474.5
Y		51.9043	-2.0947	105.9	84.9883	60.2053	144.5
Diff (1-2)	Pooled	-8.3169	-124.9	108.3	83.5981	60.6047	134.7
Diff (1-2)	Satterthwaite	-8.3169	-157.3	140.7			

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	13	-0.15	0.8799
Satterthwaite	Unequal	3.4059	-0.17	0.8773

Equality of Variances

Method	Num DF	Den DF	F Value	Pr > F
Folded F	11	2	1.27	1

APPENDIX D

HEALTH ASSESSMENT AND COMPLETE BLOOD COUNTS FOR EIGHTEEN DOMESTIC DOGS

Table 16 Health assessment of eighteen domestic dogs from northern Mississippi based upon physical observations and results from complete blood counts

Animal ID	Elevated Eosinophils	Elevated Lymphocytes	Elevated Neutrophils	Elevated Monocytes	Elevated Plasma Proteins	Reactive Lymphocytes	Anemia	Heartworms	External Parasites	WNL	Health Grade
Dog 03	X										1
Dog 04		X									1
Dog 05										X	0
Dog 06	X							X			2
Dog 07										X	0
Dog 08								X			1
Dog 09	X		X		X				X		4
Dog 10	X								X		2
Dog 11								X	X		2
Dog 12		X	X								2
Dog 13						X					1
Dog 14			X								1
Dog 15							X	X			2
Dog 16										X	0
Dog 17							X				1
Dog 19										X	0
Dog 20	X			X	X		X				4
Dog 21						X	X				2

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240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Owner: RES-DEWBERRY, MIKE
MAILSTOP 9825

MISSISSIPPI STATE, MS 39762

To: .
RES--RELAXIN REPRO (MCMILLAN)
PATHOBIOLOGY AND POPULATION MEDICINE
CVM 9825
MISSISSIPPI STATE UNIVERSITY, MS 39762

Accession Number: C1604-11

Case Coordinator: Missy Bolin
Received: 02/24/2011 Finalized: 02/25/2011
Sampled: 02/24/2011

Phone # 662-325-1104

Case Summary			
Species	Animals	Tests	Completed
CANINE	3	3	3

Final Report

Animal ID: DOG 03
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: SPANIEL AMERICAN COCKER

Completed 70 of 70 Results

Requested: 02/24/2011 03:26pm
Received: 02/24/2011 03:41pm
Status: 02/24/2011 04:31pm

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	15.9		K/ul	7 - 22	02/24/2011 04:29pm
RBC	6.34		M/ul	4.3 - 8.77	02/24/2011 04:29pm
Hgb	15.1		g/dl	11 - 19	02/24/2011 04:29pm
HCT	42.1		%	34 - 60	02/24/2011 04:29pm
MCV	66.4		fL	63 - 77	02/24/2011 04:29pm
MCH	23.8		pg	15 - 29	02/24/2011 04:29pm
MCHC	35.9		g/dl	32 - 37	02/24/2011 04:29pm
RDW	14.4		%	No Ref Range	02/24/2011 04:29pm
Platelets	568		K/ul	160 - 650	02/24/2011 04:29pm
Plasma Protein	6.6		g/dl	6 - 8	02/24/2011 04:29pm
Technologist: Instrument	N. MCBRAYER, MT(ASCP)			No Ref Range	02/24/2011 04:29pm
Segs %	55		%	No Ref Range	02/24/2011 04:29pm
Segs	8745		/ul	3000 - 11500	02/24/2011 04:30pm
Lymph %	21		%	12 - 30	02/24/2011 04:29pm
Lymph	3339		/ul	1000 - 4800	02/24/2011 04:30pm
Mono %	2	L	%	3 - 10	02/24/2011 04:29pm
Mono	318		/ul	150 - 1350	02/24/2011 04:30pm
Eosinophil %	22	H	%	2 - 10	02/24/2011 04:29pm
Eosinophil	3498	H	/ul	100 - 1250	02/24/2011 04:30pm
Platelet Estimate	APPEARS ADEQUATE			No Ref Range	02/24/2011 04:29pm
Platelet Estimate Number	480		K/ul	No Ref Range	02/24/2011 04:29pm
RBC Morphology	1+ POIKILOCYTOSIS, FEW TARGET CELLS, FEW CRENATED RBC			No Ref Range	02/24/2011 04:29pm
Technologist Diff	H. PEAVY, MT(ASCP)			No Ref Range	02/24/2011 04:29pm

Container

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Animal ID: DOG 04
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: BEAGLE
Completed 70 of 70 Results

Accession Number: C1604-11
Requested: 02/24/2011 03:26pm
Received: 02/24/2011 03:41pm
Status: 02/24/2011 04:31pm

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	17.3		K/ul	7 - 22	02/24/2011 04:27pm
RBC	6.46		M/ul	4.3 - 8.77	02/24/2011 04:27pm
Hgb	15.0		g/dl	11 - 19	02/24/2011 04:27pm
HCT	44.0		%	34 - 60	02/24/2011 04:27pm
MCV	68.1		fL	63 - 77	02/24/2011 04:27pm
MCH	23.1		pg	15 - 29	02/24/2011 04:27pm
MCHC	34.0		g/dl	32 - 37	02/24/2011 04:27pm
RDW	17.3		%	No Ref Range	02/24/2011 04:27pm
Platelets	575		K/ul	160 - 650	02/24/2011 04:27pm
Plasma Protein	7.0		g/dl	6 - 8	02/24/2011 04:27pm
Technologist: Instrument	N. MCBRAYER, MT(ASCP)			No Ref Range	02/24/2011 04:27pm
Segs %	53		%	No Ref Range	02/24/2011 04:27pm
Segs	9169		/ul	3000 - 11500	02/24/2011 04:28pm
Lymph %	32	H	%	12 - 30	02/24/2011 04:27pm
Lymph	5536	H	/ul	1000 - 4800	02/24/2011 04:28pm
Mono %	3		%	3 - 10	02/24/2011 04:27pm
Mono	519		/ul	150 - 1350	02/24/2011 04:28pm
Eosinophil %	12	H	%	2 - 10	02/24/2011 04:27pm
Eosinophil	2076	H	/ul	100 - 1250	02/24/2011 04:28pm
Platelet Estimate	APPEARS ADEQUATE			No Ref Range	02/24/2011 04:27pm
Platelet Estimate Number	640		K/ul	No Ref Range	02/24/2011 04:27pm
Platelet Morphology	MODERATE MEGA PLATELETS			No Ref Range	02/24/2011 04:27pm
RBC Morphology	SLIGHT ANISOCYTOSIS, SLIGHT POIKILOCYTOSIS			No Ref Range	02/24/2011 04:27pm
Technologist Diff	H. PEAVY, MT(ASCP)			No Ref Range	02/24/2011 04:27pm

Container

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Animal ID: DOG 05
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: BEAGLE
Completed 70 of 70 Results

Accession Number: C1604-11
Requested: 02/24/2011 03:26pm
Received: 02/24/2011 03:41pm
Status: 02/24/2011 04:31pm

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	10.5		K/ul	7 - 22	02/24/2011 04:25pm
RBC	5.55		M/ul	4.3 - 8.77	02/24/2011 04:25pm
Hgb	13.8		g/dl	11 - 19	02/24/2011 04:25pm
HCT	39.4		%	34 - 60	02/24/2011 04:25pm
MCV	71.0		fL	63 - 77	02/24/2011 04:25pm
MCH	24.8		pg	15 - 29	02/24/2011 04:25pm
MCHC	35.0		g/dl	32 - 37	02/24/2011 04:25pm
RDW	16.7		%	No Ref Range	02/24/2011 04:25pm
Platelets	357		K/ul	160 - 650	02/24/2011 04:25pm
Plasma Protein	6.8		g/dl	6 - 8	02/24/2011 04:25pm
Technologist: Instrument	N. MCBRAYER, MT(ASCP)			No Ref Range	02/24/2011 04:25pm
Segs %	58		%	No Ref Range	02/24/2011 04:25pm
Segs	6090		/ul	3000 - 11500	02/24/2011 04:26pm
Lymph %	28		%	12 - 30	02/24/2011 04:25pm
Lymph	2940		/ul	1000 - 4800	02/24/2011 04:26pm
Mono %	3		%	3 - 10	02/24/2011 04:25pm
Mono	315		/ul	150 - 1350	02/24/2011 04:26pm
Eosinophil %	11	H	%	2 - 10	02/24/2011 04:25pm
Eosinophil	1155		/ul	100 - 1250	02/24/2011 04:26pm
Platelet Estimate	APPEARS ADEQUATE			No Ref Range	02/24/2011 04:25pm
Platelet Estimate Number	416		K/ul	No Ref Range	02/24/2011 04:25pm
RBC Morphology	SLIGHT ANISOCYTOSIS, SLIGHT POIKILOCYTOSIS			No Ref Range	02/24/2011 04:25pm
Technologist Diff	H. PEAVY, MT(ASCP)			No Ref Range	02/24/2011 04:25pm

Container

Report Date
04/16/2012 12:14 PM

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Owner: RES-DEWBERRY, MIKE
MAILSTOP 9825

MISSISSIPPI STATE, MS 39762

Accession Number: C1707-11

Case Coordinator: Missy Bolin
Received: 02/28/2011 Finalized: 02/28/2011
Sampled: 02/28/2011

To: .
RES--RELAXIN REPRO (MCMILLAN)
PATHOBIOLOGY AND POPULATION MEDICINE
CVM 9825
MISSISSIPPI STATE UNIVERSITY, MS 39762

Phone # 662-325-1104

Case Summary			
Species	Animals	Tests	Completed
CANINE	1	1	1

Final Report

Animal ID: DOG 06
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex: MALE
Weight: 0lb
Breed: TERR RAT

Completed 24 of 24 Results

Requested: 02/28/2011 04:36pm
Received: 02/28/2011 04:40pm
Status: 02/28/2011 05:11pm

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	13.5		K/ul	7 - 22	02/28/2011 05:09pm
RBC	7.19		M/ul	4.3 - 8.77	02/28/2011 05:07pm
Hgb	17.2		g/dl	11 - 19	02/28/2011 05:07pm
HCT	50.4		%	34 - 60	02/28/2011 05:07pm
MCV	70.1		fL	63 - 77	02/28/2011 05:07pm
MCH	23.9		pg	15 - 29	02/28/2011 05:07pm
MCHC	34.1		g/dl	32 - 37	02/28/2011 05:07pm
RDW	16.7		%	No Ref Range	02/28/2011 05:07pm
Platelets	111	L	K/ul	160 - 650	02/28/2011 05:07pm
Plasma Protein	8.1	H	g/dl	6 - 8	02/28/2011 05:07pm
Technologist: Instrument	H. PEAVY, MT(ASCP)			No Ref Range	02/28/2011 05:07pm
Segs %	73		%	No Ref Range	02/28/2011 05:07pm
Segs	9855		/ul	3000 - 11500	02/28/2011 05:10pm
Lymph %	12		%	12 - 30	02/28/2011 05:07pm
Lymph	1620		/ul	1000 - 4800	02/28/2011 05:10pm
Mono %	4		%	3 - 10	02/28/2011 05:07pm
Mono	540		/ul	150 - 1350	02/28/2011 05:10pm
Eosinophil %	11	H	%	2 - 10	02/28/2011 05:07pm
Eosinophil	1485	H	/ul	100 - 1250	02/28/2011 05:10pm
Platelet Estimate	APPEARS ADEQUATE			No Ref Range	02/28/2011 05:07pm
Platelet Estimate Number	240		K/ul	No Ref Range	02/28/2011 05:07pm
Platelet Morphology	MODERATE MEGA PLATELETS			No Ref Range	02/28/2011 05:07pm
RBC Morphology	SLIGHT ANISOCYTOSIS, SLIGHT POIKILOCYTOSIS, FEW MICROFILARIA SP			No Ref Range	02/28/2011 05:07pm
Technologist Diff	H. PEAVY, MT(ASCP)			No Ref Range	02/28/2011 05:07pm

Report Date
04/16/2012 12:14 PM

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Animal ID: DOG 06
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex: MALE
Weight: 0lb
Breed: TERR RAT

Completed 24 of 24 Results

Accession Number: C1707-11

Requested: 02/28/2011 04:36pm
Received: 02/28/2011 04:40pm
Status: 02/28/2011 05:11pm

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

Comments:
WBC Result value for test WBC previously reported as 4.2 on 02/28/2011 05:07 p.m.

Container

Report Date
04/16/2012 12:16 PM

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Owner: **RES-DEWBERRY, MIKE**
MAILSTOP 9825

MISSISSIPPI STATE, MS 39762

To: .
RES--RELAXIN REPRO (MCMILLAN)
PATHOBIOLOGY AND POPULATION MEDICINE
CVM 9825
MISSISSIPPI STATE UNIVERSITY, MS 39762

Accession Number: C2339-11

Case Coordinator: Missy Bolin
Received: 03/21/2011 Finalized: 03/22/2011
Sampled: 03/21/2011

Phone # 662-325-1104

Case Summary

Species	Animals	Tests	Completed
CANINE	1	1	1

Final Report

Animal ID: DOG 07
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: CHIHUAHUA
Completed 23 of 23 Results

Requested: 03/21/2011 04:29pm
Received: 03/21/2011 04:32pm
Status: 03/22/2011 09:32am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	23.1	H	K/ul	7 - 22	03/22/2011 09:30am
RBC	4.87		M/ul	4.3 - 8.77	03/22/2011 09:30am
Hgb	11.9		g/dl	11 - 19	03/22/2011 09:30am
HCT	35.2		%	34 - 60	03/22/2011 09:30am
MCV	72.3		fL	63 - 77	03/22/2011 09:30am
MCH	24.4		pg	15 - 29	03/22/2011 09:30am
MCHC	33.8		g/dl	32 - 37	03/22/2011 09:30am
RDW	15.2		%	No Ref Range	03/22/2011 09:30am
Platelets	425		K/ul	160 - 650	03/22/2011 09:30am
Plasma Protein	7.0		g/dl	6 - 8	03/22/2011 09:30am
Technologist: Instrument	H. PEAVY, MT(ASCP)			No Ref Range	03/22/2011 09:31am
Segs %	85		%	No Ref Range	03/22/2011 09:30am
Segs	19635	H	/ul	3000 - 11500	03/22/2011 09:32am
Lymph %	10	L	%	12 - 30	03/22/2011 09:30am
Lymph	2310		/ul	1000 - 4800	03/22/2011 09:32am
Mono %	3		%	3 - 10	03/22/2011 09:30am
Mono	693		/ul	150 - 1350	03/22/2011 09:32am
Eosinophil %	2		%	2 - 10	03/22/2011 09:30am
Eosinophil	462		/ul	100 - 1250	03/22/2011 09:32am
Platelet Estimate	APPEARS ADEQUATE			No Ref Range	03/22/2011 09:30am
Platelet Estimate Number	464		K/ul	No Ref Range	03/22/2011 09:30am
RBC Morphology	SLIGHT ANISOCYTOSIS, SLIGHT POIKILOCYTOSIS			No Ref Range	03/22/2011 09:31am
Technologist Diff	M. BOLIN, MT(ASCP)			No Ref Range	03/22/2011 09:31am

Container

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Owner: RES-DEWBERRY, MIKE
MAILSTOP 9825

MISSISSIPPI STATE, MS 39762

To: .
RES--RELAXIN REPRO (MCMILLAN)
PATHOBIOLOGY AND POPULATION MEDICINE
CVM 9825
MISSISSIPPI STATE UNIVERSITY, MS 39762

Accession Number: C2489-11

Case Coordinator: Missy Bolin
Received: 03/25/2011 Finalized: 03/25/2011
Sampled: 03/25/2011

Phone # 662-325-1104

Case Summary			
Species	Animals	Tests	Completed
CANINE	1	1	1

Final Report

Animal ID: DOG 08
Species: CANINE
Age: >1Y
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: MIXED BREED DOG
Completed 26 of 26 Results

Requested: 03/25/2011 04:17pm
Received: 03/25/2011 04:19pm
Status: 03/25/2011 04:46pm

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	12.1		K/ul	7 - 22	03/25/2011 04:44pm
RBC	4.98		M/ul	4.3 - 8.77	03/25/2011 04:44pm
Hgb	11.6		g/dl	11 - 19	03/25/2011 04:44pm
HCT	37.5		%	34 - 60	03/25/2011 04:44pm
MCV	75.2		fL	63 - 77	03/25/2011 04:44pm
MCH	23.3		pg	15 - 29	03/25/2011 04:44pm
MCHC	31.0	L	g/dl	32 - 37	03/25/2011 04:44pm
RDW	14.9		%	No Ref Range	03/25/2011 04:44pm
Platelets	419		K/ul	160 - 650	03/25/2011 04:44pm
Plasma Protein	8.0		g/dl	6 - 8	03/25/2011 04:44pm
Technologist: Instrument	M. BOLIN, MT(ASCP)			No Ref Range	03/25/2011 04:45pm
Segs %	70		%	No Ref Range	03/25/2011 04:44pm
Segs	8470		/ul	3000 - 11500	03/25/2011 04:46pm
Band %	1		%	0 - 3	03/25/2011 04:44pm
Band	121		/ul	0 - 300	03/25/2011 04:46pm
Lymph %	19		%	12 - 30	03/25/2011 04:44pm
Lymph	2299		/ul	1000 - 4800	03/25/2011 04:46pm
Mono %	4		%	3 - 10	03/25/2011 04:44pm
Mono	484		/ul	150 - 1350	03/25/2011 04:46pm
Eosinophil %	6		%	2 - 10	03/25/2011 04:44pm
Eosinophil	726		/ul	100 - 1250	03/25/2011 04:46pm
Platelet Estimate	APPEARS ADEQUATE			No Ref Range	03/25/2011 04:44pm
Platelet Estimate Number	400		K/ul	No Ref Range	03/25/2011 04:44pm
Platelet Morphology	FEW MEGA PLATELETS			No Ref Range	03/25/2011 04:44pm
RBC Morphology	SLIGHT ANISOCYTOSIS, FEW MICROFILARIA SP			No Ref Range	03/25/2011 04:45pm
Technologist Diff	M. BOLIN, MT(ASCP)			No Ref Range	03/25/2011 04:45pm

Report Date
04/16/2012 12:17 PM

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Animal ID: DOG 08
Species: CANINE
Age: >1Y
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: MIXED BREED DOG

Completed 26 of 26 Results

Accession Number: C2489-11
Requested: 03/25/2011 04:17pm
Received: 03/25/2011 04:19pm
Status: 03/25/2011 04:46pm

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

Container

Report Date
04/16/2012 12:15 PM

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Owner: **RES-DEWBERRY, MIKE**
MAILSTOP 9825

MISSISSIPPI STATE, MS 39762

To: .
RES--RELAXIN REPRO (MCMILLAN)
PATHOBIOLOGY AND POPULATION MEDICINE
CVM 9825
MISSISSIPPI STATE UNIVERSITY, MS 39762

Accession Number: C1966-11

Case Coordinator: Missy Bolin
Received: 03/09/2011 Finalized: 03/09/2011
Sampled: 03/08/2011

Phone # 662-325-1104

Case Summary			
Species	Animals	Tests	Completed
CANINE	1	1	1

Final Report

Animal ID: DOG 09
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: MIXED BREED DOG
Completed 23 of 23 Results

Requested: 03/09/2011 08:42am
Received: 03/09/2011 09:06am
Status: 03/09/2011 09:47am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	18.1		K/ul	7 - 22	03/09/2011 09:46am
RBC	7.02		M/ul	4.3 - 8.77	03/09/2011 09:46am
Hgb	16.9		g/dl	11 - 19	03/09/2011 09:46am
HCT	47.7		%	34 - 60	03/09/2011 09:46am
MCV	68.0		fL	63 - 77	03/09/2011 09:46am
MCH	24.0		pg	15 - 29	03/09/2011 09:46am
MCHC	35.3		g/dl	32 - 37	03/09/2011 09:46am
RDW	16.3		%	No Ref Range	03/09/2011 09:46am
Platelets	363		K/ul	160 - 650	03/09/2011 09:46am
Plasma Protein	8.4	H	g/dl	6 - 8	03/09/2011 09:46am
Technologist: Instrument	N. MCBRAYER, MT(ASCP)			No Ref Range	03/09/2011 09:46am
Segs %	80		%	No Ref Range	03/09/2011 09:46am
Segs	14480	H	/ul	3000 - 11500	03/09/2011 09:47am
Lymph %	8	L	%	12 - 30	03/09/2011 09:46am
Lymph	1448		/ul	1000 - 4800	03/09/2011 09:47am
Mono %	5		%	3 - 10	03/09/2011 09:46am
Mono	905		/ul	150 - 1350	03/09/2011 09:47am
Eosinophil %	7		%	2 - 10	03/09/2011 09:46am
Eosinophil	1267	H	/ul	100 - 1250	03/09/2011 09:47am
Platelet Estimate	APPEARS ADEQUATE			No Ref Range	03/09/2011 09:46am
Platelet Estimate Number	320		K/ul	No Ref Range	03/09/2011 09:46am
RBC Morphology	SLIGHT ANISOCYTOSIS, SLIGHT POIKILOCYTOSIS			No Ref Range	03/09/2011 09:46am
Technologist Diff	N. MCBRAYER, MT(ASCP)			No Ref Range	03/09/2011 09:46am

Container

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Owner: RES-DEWBERRY, MIKE
MAILSTOP 9825

MISSISSIPPI STATE, MS 39762

To: .
RES--RELAXIN REPRO (MCMILLAN)
PATHOBIOLOGY AND POPULATION MEDICINE
CVM 9825
MISSISSIPPI STATE UNIVERSITY, MS 39762

Accession Number: C2285-11

Case Coordinator: Missy Bolin
Received: 03/18/2011 Finalized: 03/21/2011
Sampled: 03/18/2011

Phone # 662-325-1104

Case Summary

Species	Animals	Tests	Completed
CANINE	1	1	1

Final Report

Animal ID: DOG 10
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: RET LABRADOR

Completed 25 of 25 Results

Requested: 03/18/2011 05:32pm
Received: 03/18/2011 06:06pm
Status: 03/18/2011 07:02pm

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	15.1		K/ul	7 - 22	03/18/2011 06:58pm
RBC	5.37		M/ul	4.3 - 8.77	03/18/2011 06:58pm
Hgb	12.3		g/dl	11 - 19	03/18/2011 06:58pm
HCT	37.4		%	34 - 60	03/18/2011 06:58pm
MCV	69.7		fL	63 - 77	03/18/2011 06:58pm
MCH	23.0		pg	15 - 29	03/18/2011 06:58pm
MCHC	33.0		g/dl	32 - 37	03/18/2011 06:58pm
RDW	16.8		%	No Ref Range	03/18/2011 06:58pm
Platelets	379		K/ul	160 - 650	03/18/2011 06:58pm
Plasma Protein	6.7		g/dl	6 - 8	03/18/2011 06:58pm
Technologist: Instrument	ELT - JENNY SPROTT			No Ref Range	03/18/2011 06:59pm
Segs %	58		%	No Ref Range	03/18/2011 06:59pm
Segs	8758		/ul	3000 - 11500	03/18/2011 07:01pm
Lymph %	12		%	12 - 30	03/18/2011 06:59pm
Lymph	1812		/ul	1000 - 4800	03/18/2011 07:01pm
Mono %	3		%	3 - 10	03/18/2011 06:59pm
Mono	453		/ul	150 - 1350	03/18/2011 07:01pm
Eosinophil %	27	H	%	2 - 10	03/18/2011 06:59pm
Eosinophil	4077	H	/ul	100 - 1250	03/18/2011 07:01pm
Nucleated RBC	1		/100 WBC	.5 - 1	03/18/2011 06:59pm
Platelet Estimate	APPEARS ADEQUATE			No Ref Range	03/18/2011 06:59pm
Platelet Estimate Number	394		K/ul	No Ref Range	03/18/2011 06:59pm
Platelet Morphology	RARE MEGA PLATELETS			No Ref Range	03/18/2011 06:59pm
RBC Morphology	SLIGHT POLYCHROMASIA, SLIGHT ANISOCYTOSIS, SLIGHT POIKILOCYTOSIS			No Ref Range	03/18/2011 06:59pm
Technologist Diff	ELT - JENNY SPROTT			No Ref Range	03/18/2011 07:00pm

Report Date
04/16/2012 12:16 PM

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Animal ID: DOG 10
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: RET LABRADOR
Completed 25 of 25 Results

Accession Number: C2285-11
Requested: 03/18/2011 05:32pm
Received: 03/18/2011 06:06pm
Status: 03/18/2011 07:02pm

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

Container

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Owner: RES-DEWBERRY, MIKE
MAILSTOP 9825

MISSISSIPPI STATE, MS 39762

To: .
RES--RELAXIN REPRO (MCMILLAN)
PATHOBIOLOGY AND POPULATION MEDICINE
CVM 9825
MISSISSIPPI STATE UNIVERSITY, MS 39762

Accession Number: C3829-11

Case Coordinator: Missy Bolin
Received: 05/09/2011 Finalized: 05/10/2011
Sampled: 05/09/2011

Phone # 662-325-1104

Case Summary			
Species	Animals	Tests	Completed
CANINE	2	2	2

Final Report

Animal ID: DOG 11
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: MIXED BREED DOG
Completed 47 of 47 Results

Requested: 05/09/2011 04:49pm
Received: 05/09/2011 04:52pm
Status: 05/10/2011 09:16am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	11.3		K/ul	7 - 22	05/10/2011 09:14am
RBC	4.81		M/ul	4.3 - 8.77	05/10/2011 09:14am
Hgb	11.0		g/dl	11 - 19	05/10/2011 09:14am
HCT	34.0		%	34 - 60	05/10/2011 09:14am
MCV	70.7		fL	63 - 77	05/10/2011 09:14am
MCH	22.9		pg	15 - 29	05/10/2011 09:14am
MCHC	32.4		g/dl	32 - 37	05/10/2011 09:14am
RDW	15.7		%	No Ref Range	05/10/2011 09:14am
Platelets	392		K/ul	160 - 650	05/10/2011 09:14am
Plasma Protein	8.0		g/dl	6 - 8	05/10/2011 09:14am
Technologist: Instrument	M. BOLIN, MT(ASCP)			No Ref Range	05/10/2011 09:14am
Segs %	57		%	No Ref Range	05/10/2011 09:14am
Segs	6441		/ul	3000 - 11500	05/10/2011 09:16am
Lymph %	37	H	%	12 - 30	05/10/2011 09:14am
Lymph	4181		/ul	1000 - 4800	05/10/2011 09:16am
Mono %	4		%	3 - 10	05/10/2011 09:14am
Mono	452		/ul	150 - 1350	05/10/2011 09:16am
Eosinophil %	2		%	2 - 10	05/10/2011 09:14am
Eosinophil	226		/ul	100 - 1250	05/10/2011 09:16am
Nucleated RBC	2	H	/100 WBC	.5 - 1	05/10/2011 09:14am
Platelet Estimate	APPEARS ADEQUATE			No Ref Range	05/10/2011 09:14am
Platelet Estimate Number	400		K/ul	No Ref Range	05/10/2011 09:14am
RBC Morphology	FEW MICROFILARIA SP, 1+ ANISOCYTOSIS, SLIGHT POIKILOCYTOSIS			No Ref Range	05/10/2011 09:14am
Technologist Diff	M. BOLIN, MT(ASCP)			No Ref Range	05/10/2011 09:14am

Container

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Animal ID: DOG 12
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: TERRIER

Completed 47 of 47 Results

Accession Number: C3829-11

Requested: 05/09/2011 04:49pm
Received: 05/09/2011 04:52pm
Status: 05/10/2011 09:16am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	25.0	H	K/ul	7 - 22	05/10/2011 09:08am
RBC	5.41		M/ul	4.3 - 8.77	05/10/2011 09:08am
Hgb	12.7		g/dl	11 - 19	05/10/2011 09:08am
HCT	37.4		%	34 - 60	05/10/2011 09:08am
MCV	69.1		fL	63 - 77	05/10/2011 09:08am
MCH	23.6		pg	15 - 29	05/10/2011 09:08am
MCHC	34.1		g/dl	32 - 37	05/10/2011 09:08am
RDW	16.2		%	No Ref Range	05/10/2011 09:08am
Platelets	376		K/ul	160 - 650	05/10/2011 09:08am
Plasma Protein	6.4		g/dl	6 - 8	05/10/2011 09:08am
Technologist: Instrument	M. BOLIN, MT(ASCP)			No Ref Range	05/10/2011 09:09am
Segs %	64		%	No Ref Range	05/10/2011 09:09am
Segs	16000	H	/ul	3000 - 11500	05/10/2011 09:11am
Lymph %	21		%	12 - 30	05/10/2011 09:09am
Lymph	5250	H	/ul	1000 - 4800	05/10/2011 09:11am
Mono %	5		%	3 - 10	05/10/2011 09:09am
Mono	1250		/ul	150 - 1350	05/10/2011 09:11am
Eosinophil %	10		%	2 - 10	05/10/2011 09:09am
Eosinophil	2500	H	/ul	100 - 1250	05/10/2011 09:11am
Platelet Estimate	APPEARS ADEQUATE			No Ref Range	05/10/2011 09:09am
Platelet Estimate Number	384		K/ul	No Ref Range	05/10/2011 09:09am
RBC Morphology	SLIGHT POLYCHROMASIA, SLIGHT ANISOCYTOSIS			No Ref Range	05/10/2011 09:09am
Technologist Diff	M. BOLIN, MT(ASCP)			No Ref Range	05/10/2011 09:09am

Container

Report Date
04/16/2012 12:18 PM

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Owner: **RES-DEWBERRY, MIKE**
MAILSTOP 9825

MISSISSIPPI STATE, MS 39762

To: .
RES--RELAXIN REPRO (MCMILLAN)
PATHOBIOLOGY AND POPULATION MEDICINE
CVM 9825
MISSISSIPPI STATE UNIVERSITY, MS 39762

Accession Number: C3648-11

Case Coordinator: Missy Bolin
Received: 05/03/2011 Finalized: 05/04/2011
Sampled: 05/03/2011

Phone # 662-325-1104

Case Summary

Species	Animals	Tests	Completed
CANINE	3	3	3

Final Report

Animal ID: DOG 13
Species: CANINE
Age: >1Y
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: MIXED BREED DOG
Completed 70 of 70 Results

Requested: 05/03/2011 06:45pm
Received: 05/03/2011 07:10pm
Status: 05/04/2011 09:47am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT	UNITS	REF RANGE	RESULT DATETIME
WBC	12.9	K/ul	7 - 22	05/04/2011 09:44am
RBC	7.38	M/ul	4.3 - 8.77	05/04/2011 09:44am
Hgb	16.7	g/dl	11 - 19	05/04/2011 09:44am
HCT	50.6	%	34 - 60	05/04/2011 09:44am
MCV	68.6	fL	63 - 77	05/04/2011 09:44am
MCH	22.7	pg	15 - 29	05/04/2011 09:44am
MCHC	33.1	g/dl	32 - 37	05/04/2011 09:44am
RDW	16.5	%	No Ref Range	05/04/2011 09:44am
Platelets	194	K/ul	160 - 650	05/04/2011 09:44am
Plasma Protein	7.6	g/dl	6 - 8	05/04/2011 09:44am
Technologist: Instrument	ELT - JAIMEE BUMGARNER		No Ref Range	05/04/2011 09:45am
Segs %	61	%	No Ref Range	05/04/2011 09:44am
Segs	7869	/ul	3000 - 11500	05/04/2011 09:46am
Lymph %	23	%	12 - 30	05/04/2011 09:44am
Lymph	2967	/ul	1000 - 4800	05/04/2011 09:46am
Mono %	8	%	3 - 10	05/04/2011 09:44am
Mono	1032	/ul	150 - 1350	05/04/2011 09:46am
Eosinophil %	8	%	2 - 10	05/04/2011 09:44am
Eosinophil	1032	/ul	100 - 1250	05/04/2011 09:46am
Platelet Estimate	APPEARS ADEQUATE		No Ref Range	05/04/2011 09:44am
Platelet Estimate Number	192	K/ul	No Ref Range	05/04/2011 09:45am
WBC Morphology	FEW REACTIVE LYMPHOCYTES		No Ref Range	05/04/2011 09:46am
RBC Morphology	SLIGHT POIKILOCYTOSIS		No Ref Range	05/04/2011 09:45am
Technologist Diff	N. MCBRAYER, MT(ASCP)		No Ref Range	05/04/2011 09:45am

Container

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Animal ID: DOG 14
Species: CANINE
Age: >1Y
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: SIBERIAN HUSKY
Completed 70 of 70 Results

Accession Number: C3648-11
Requested: 05/03/2011 06:45pm
Received: 05/03/2011 07:10pm
Status: 05/04/2011 09:47am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	19.8		K/ul	7 - 22	05/04/2011 09:17am
RBC	6.23		M/ul	4.3 - 8.77	05/04/2011 09:17am
Hgb	15.4		g/dl	11 - 19	05/04/2011 09:17am
HCT	45.4		%	34 - 60	05/04/2011 09:17am
MCV	72.9		fL	63 - 77	05/04/2011 09:17am
MCH	24.7		pg	15 - 29	05/04/2011 09:17am
MCHC	33.9		g/dl	32 - 37	05/04/2011 09:17am
RDW	14.9		%	No Ref Range	05/04/2011 09:17am
Platelets	424		K/ul	160 - 650	05/04/2011 09:17am
Plasma Protein	6.6		g/dl	6 - 8	05/04/2011 09:17am
Technologist: Instrument	ELT - JAIMEE BUMGARNER			No Ref Range	05/04/2011 09:18am
Segs %	72		%	No Ref Range	05/04/2011 09:17am
Segs	14256	H	/ul	3000 - 11500	05/04/2011 09:20am
Lymph %	17		%	12 - 30	05/04/2011 09:17am
Lymph	3366		/ul	1000 - 4800	05/04/2011 09:20am
Mono %	2	L	%	3 - 10	05/04/2011 09:17am
Mono	396		/ul	150 - 1350	05/04/2011 09:20am
Eosinophil %	9		%	2 - 10	05/04/2011 09:17am
Eosinophil	1782	H	/ul	100 - 1250	05/04/2011 09:20am
Platelet Estimate	APPEARS ADEQUATE			No Ref Range	05/04/2011 09:17am
Platelet Estimate Number	400		K/ul	No Ref Range	05/04/2011 09:17am
Platelet Morphology	OCCASIONAL PLATELET CLUMPING			No Ref Range	05/04/2011 09:18am
RBC Morphology	1+ POIKILOCYTOSIS, FEW CRENATED RBC			No Ref Range	05/04/2011 09:18am
Technologist Diff	H. PEAVY, MT(ASCP)			No Ref Range	05/04/2011 09:18am

Container

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Animal ID: DOG 16
Species: CANINE
Age: >1Y
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: MIXED BREED DOG
Completed 70 of 70 Results

Accession Number: C3648-11
Requested: 05/03/2011 06:45pm
Received: 05/03/2011 07:10pm
Status: 05/04/2011 09:47am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT	UNITS	REF RANGE	RESULT DATETIME
WBC	**14.1**	K/ul	7 - 22	05/04/2011 09:21am
RBC	**6.60**	M/ul	4.3 - 8.77	05/04/2011 09:21am
Hgb	**14.7**	g/dl	11 - 19	05/04/2011 09:21am
HCT	**41.8**	%	34 - 60	05/04/2011 09:21am
MCV	**63.4**	fL	63 - 77	05/04/2011 09:21am
MCH	**22.3**	pg	15 - 29	05/04/2011 09:21am
MCHC	**35.1**	g/dl	32 - 37	05/04/2011 09:21am
RDW	**17.1**	%	No Ref Range	05/04/2011 09:21am
Platelets	**282**	K/ul	160 - 650	05/04/2011 09:21am
Plasma Protein	**6.1**	g/dl	6 - 8	05/04/2011 09:21am
Technologist: Instrument	**ELT - JAIMEE BUMGARNER**		No Ref Range	05/04/2011 09:21am
Segs %	**73**	%	No Ref Range	05/04/2011 09:21am
Segs	**10293**	/ul	3000 - 11500	05/04/2011 09:22am
Lymph %	**20**	%	12 - 30	05/04/2011 09:21am
Lymph	**2820**	/ul	1000 - 4800	05/04/2011 09:22am
Eosinophil %	**7**	%	2 - 10	05/04/2011 09:21am
Eosinophil	**987**	/ul	100 - 1250	05/04/2011 09:22am
Platelet Estimate	**APPEARS ADEQUATE**		No Ref Range	05/04/2011 09:21am
Platelet Estimate Number	**400**	K/ul	No Ref Range	05/04/2011 09:21am
Platelet Morphology	**OCCASIONAL PLATELET CLUMPING**		No Ref Range	05/04/2011 09:21am
RBC Morphology	**1+ POIKILOCYTOSIS, MODERATE CRENATED RBC**		No Ref Range	05/04/2011 09:21am
Technologist Diff	**H. PEAVY, MT(ASCP)**		No Ref Range	05/04/2011 09:21am

Container

Report Date
04/16/2012 12:19 PM

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Owner: RES-DEWBERRY, MIKE
MAILSTOP 9825

MISSISSIPPI STATE, MS 39762

Accession Number: C4349-11

Case Coordinator: Missy Bolin
Received: 05/27/2011 Finalized: 05/31/2011
Sampled: 05/27/2011

To: .
RES--RELAXIN REPRO (MCMILLAN)
PATHOBIOLOGY AND POPULATION MEDICINE
CVM 9825
MISSISSIPPI STATE UNIVERSITY, MS 39762

Phone # 662-325-1104

Case Summary			
Species	Animals	Tests	Completed
CANINE	1	1	1

Final Report

Animal ID: DOG 15
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex:
Weight: 0lb
Breed: TERR RAT
Completed 20 of 20 Results

Requested: 05/27/2011 05:42pm
Received: 05/27/2011 05:45pm
Status: 06/02/2011 09:29am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	11.1		K/ul	7 - 22	05/27/2011 10:57pm
RBC	3.68	L	M/ul	4.3 - 8.77	05/27/2011 10:57pm
Hgb	8.8	L	g/dl	11 - 19	05/27/2011 10:57pm
HCT	26.6	L	%	34 - 60	05/27/2011 10:57pm
MCV	72.2		fL	63 - 77	05/27/2011 10:57pm
MCH	23.8		pg	15 - 29	05/27/2011 10:57pm
MCHC	33.0		g/dl	32 - 37	05/27/2011 10:57pm
RDW	18.3		%	No Ref Range	05/27/2011 10:57pm
Platelets	947	H	K/ul	160 - 650	05/27/2011 10:57pm
Plasma Protein	9.1	H	g/dl	6 - 8	05/27/2011 10:57pm
Technologist: Instrument	ELT - LINDSEY TURBYFILL			No Ref Range	05/27/2011 10:58pm
Segs %	73		%	No Ref Range	05/27/2011 10:57pm
Lymph %	17		%	12 - 30	05/27/2011 10:57pm
Eosinophil %	10		%	2 - 10	05/27/2011 10:57pm
Nucleated RBC	1		/100 WBC	.5 - 1	05/27/2011 10:57pm
Platelet Estimate	APPEARS INCREASED			No Ref Range	05/27/2011 10:57pm
Platelet Estimate Number	1072		K/ul	No Ref Range	05/27/2011 10:57pm
Platelet Morphology	OCCASIONAL PLATELET CLUMPING			No Ref Range	05/27/2011 10:57pm
RBC Morphology	SLIGHT POLYCHROMASIA, 1+ ANISOCYTOSIS, SLIGHT POIKILOCYTOSIS, RARE TARGET CELLS, FEW MICROFILARIA SP			No Ref Range	05/31/2011 10:17am
Technologist Diff	ELT - LINDSEY TURBYFILL			No Ref Range	05/27/2011 10:58pm

Report Date
04/16/2012 12:19 PM

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Animal ID: DOG 15
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex:
Weight: 0lb
Breed: TERR RAT

Completed 20 of 20 Results

Accession Number: C4349-11

Requested: 05/27/2011 05:42pm
Received: 05/27/2011 05:45pm
Status: 06/02/2011 09:29am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

Comments:

RBC Morphology	Result value for test RBC Morphology previously reported as SLIGHT POLYCHROMASIA, 1+ ANISOCYTOSIS, SLIGHT POIKILOCYTOSIS, RARE TARGET CELLS on 05/27/2011 10:58 p.m.
RBC Morphology	updated 5/31/11 by nsm

Container

Report Date
04/16/2012 12:20 PM

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Owner: **RES-DEWBERRY, MIKE**
MAILSTOP 9825

MISSISSIPPI STATE, MS 39762

To: .
RES--RELAXIN REPRO (MCMILLAN)
PATHOBIOLOGY AND POPULATION MEDICINE
CVM 9825
MISSISSIPPI STATE UNIVERSITY, MS 39762

Accession Number: C4827-11

Case Coordinator: Missy Bolin
Received: 06/14/2011 Finalized: 06/15/2011
Sampled: 06/13/2011

Phone # 662-325-1104

Case Summary

Species	Animals	Tests	Completed
CANINE	1	1	1

Final Report

Animal ID: DOG 17
Species: CANINE
Age: >1Y
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: TERR RAT

Completed 25 of 25 Results

Requested: 06/14/2011 04:58pm
Received: 06/14/2011 05:01pm
Status: 06/15/2011 08:34am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Animal ID: DOG 17
Species: CANINE
Age: >1Y
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: TERR RAT

Completed 25 of 25 Results

Accession Number: C4827-11
Requested: 06/14/2011 04:58pm
Received: 06/14/2011 05:01pm
Status: 06/15/2011 08:34am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	14.7		K/ul	7 - 22	06/15/2011 08:32am
RBC	4.30		M/ul	4.3 - 8.77	06/15/2011 08:32am
Hgb	8.5	L	g/dl	11 - 19	06/15/2011 08:32am
HCT	28.4	L	%	34 - 60	06/15/2011 08:32am
MCV	66.1		fL	63 - 77	06/15/2011 08:32am
MCH	19.9		pg	15 - 29	06/15/2011 08:32am
MCHC	30.0	L	g/dl	32 - 37	06/15/2011 08:32am
RDW	17.8		%	No Ref Range	06/15/2011 08:32am
Platelets	137	L	K/ul	160 - 650	06/15/2011 08:32am
Plasma Protein	7.3		g/dl	6 - 8	06/15/2011 08:32am
Technologist: Instrument	ELT- DESIREE COOLEY			No Ref Range	06/15/2011 08:32am
Segs %	73		%	No Ref Range	06/15/2011 08:32am
Segs	10731		/ul	3000 - 11500	06/15/2011 08:33am
Lymph %	17		%	12 - 30	06/15/2011 08:32am
Lymph	2499		/ul	1000 - 4800	06/15/2011 08:33am
Mono %	7		%	3 - 10	06/15/2011 08:32am
Mono	1029		/ul	150 - 1350	06/15/2011 08:33am
Eosinophil %	3		%	2 - 10	06/15/2011 08:32am
Eosinophil	441		/ul	100 - 1250	06/15/2011 08:33am
Nucleated RBC	3	H	/100 WBC	.5 - 1	06/15/2011 08:32am
Platelet Estimate	APPEARS ADEQUATE			No Ref Range	06/15/2011 08:32am
Platelet Estimate Number	640		K/ul	No Ref Range	06/15/2011 08:32am
Platelet Morphology	OCCASIONAL PLATELET CLUMPING, MODERATE MEGA PLATELETS			No Ref Range	06/15/2011 08:32am
RBC Morphology	1+ ANISOCYTOSIS, 1+ POIKILOCYTOSIS, OCCASIONAL TARGET CELLS			No Ref Range	06/15/2011 08:32am
Technologist Diff	N. MCBRAYER, MT(ASCP)			No Ref Range	06/15/2011 08:32am

Container

Report Date
04/16/2012 12:19 PM

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Owner: **RES-DEWBERRY, MIKE**
MAILSTOP 9825

MISSISSIPPI STATE, MS 39762

To: .
RES--RELAXIN REPRO (MCMILLAN)
PATHOBIOLOGY AND POPULATION MEDICINE
CVM 9825
MISSISSIPPI STATE UNIVERSITY, MS 39762

Accession Number: C4975-11

Case Coordinator: Missy Bolin
Received: 06/20/2011 Finalized: 06/21/2011
Sampled: 06/20/2011

Phone # 662-325-1104

Case Summary			
Species	Animals	Tests	Completed
CANINE	2	2	2

Final Report

Animal ID: DOG 19
Species: CANINE
Age: >1Y
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: MIXED BREED DOG
Completed 47 of 47 Results

Requested: 06/20/2011 04:56pm
Received: 06/21/2011 09:33am
Status: 06/21/2011 10:35am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT	UNITS	REF RANGE	RESULT DATETIME
WBC	14.8	K/ul	7 - 22	06/21/2011 10:27am
RBC	5.30	M/ul	4.3 - 8.77	06/21/2011 10:27am
Hgb	12.9	g/dl	11 - 19	06/21/2011 10:27am
HCT	38.4	%	34 - 60	06/21/2011 10:27am
MCV	72.4	fL	63 - 77	06/21/2011 10:28am
MCH	24.4	pg	15 - 29	06/21/2011 10:28am
MCHC	33.7	g/dl	32 - 37	06/21/2011 10:28am
RDW	14.6	%	No Ref Range	06/21/2011 10:28am
Platelets	299	K/ul	160 - 650	06/21/2011 10:28am
Plasma Protein	6.4	g/dl	6 - 8	06/21/2011 10:27am
Technologist: Instrument	ELT - TINA BLOXSOM		No Ref Range	06/21/2011 10:29am
Segs %	71	%	No Ref Range	06/21/2011 10:28am
Segs	10508	/ul	3000 - 11500	06/21/2011 10:30am
Lymph %	25	%	12 - 30	06/21/2011 10:28am
Lymph	3700	/ul	1000 - 4800	06/21/2011 10:30am
Eosinophil %	4	%	2 - 10	06/21/2011 10:28am
Eosinophil	592	/ul	100 - 1250	06/21/2011 10:30am
Nucleated RBC	1	/100 WBC	.5 - 1	06/21/2011 10:28am
Platelet Estimate	APPEARS INCREASED		No Ref Range	06/21/2011 10:28am
Platelet Estimate Number	704	K/ul	No Ref Range	06/21/2011 10:28am
Platelet Morphology	OCCASIONAL PLATELET CLUMPING		No Ref Range	06/21/2011 10:28am
RBC Morphology	SLIGHT POLYCHROMASIA, SLIGHT POIKILOCYTOSIS		No Ref Range	06/21/2011 10:29am
Technologist Diff	ELT - TINA BLOXSOM		No Ref Range	06/21/2011 10:29am

Container

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Animal ID: DOG 20
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex: MALE
Weight: 0lb
Breed: TERR AMERICAN PIT BULL
Completed 47 of 47 Results

Accession Number: C4975-11
Requested: 06/20/2011 04:56pm
Received: 06/21/2011 09:33am
Status: 06/21/2011 10:35am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	18.0		K/ul	7 - 22	06/21/2011 10:32am
RBC	5.09		M/ul	4.3 - 8.77	06/21/2011 10:32am
Hgb	10.5	L	g/dl	11 - 19	06/21/2011 10:32am
HCT	31.9	L	%	34 - 60	06/21/2011 10:32am
MCV	62.7	L	fL	63 - 77	06/21/2011 10:32am
MCH	20.6		pg	15 - 29	06/21/2011 10:32am
MCHC	32.8		g/dl	32 - 37	06/21/2011 10:32am
RDW	23.1		%	No Ref Range	06/21/2011 10:32am
Platelets	416		K/ul	160 - 650	06/21/2011 10:32am
Plasma Protein	8.9	H	g/dl	6 - 8	06/21/2011 10:32am
Technologist: Instrument	ELT - TINA BLOXSOM			No Ref Range	06/21/2011 10:34am
Segs %	51		%	No Ref Range	06/21/2011 10:32am
Segs	9180		/ul	3000 - 11500	06/21/2011 10:35am
Lymph %	13		%	12 - 30	06/21/2011 10:32am
Lymph	2340		/ul	1000 - 4800	06/21/2011 10:35am
Mono %	9		%	3 - 10	06/21/2011 10:32am
Mono	1620	H	/ul	150 - 1350	06/21/2011 10:35am
Eosinophil %	27	H	%	2 - 10	06/21/2011 10:32am
Eosinophil	4860	H	/ul	100 - 1250	06/21/2011 10:35am
Platelet Estimate	APPEARS INCREASED			No Ref Range	06/21/2011 10:33am
Platelet Estimate Number	838		K/ul	No Ref Range	06/21/2011 10:33am
Platelet Morphology	OCCASIONAL MEGA PLATELETS, RARE PLATELET CLUMPING			No Ref Range	06/21/2011 10:33am
RBC Morphology	SLIGHT POLYCHROMASIA, SLIGHT ANISOCYTOSIS, 1+ POIKILOCYTOSIS			No Ref Range	06/21/2011 10:33am
Technologist Diff	ELT - TINA BLOXSOM			No Ref Range	06/21/2011 10:34am

Container

Report Date
04/16/2012 12:21 PM

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Owner: **RES-DEWBERRY, MIKE**
MAILSTOP 9825

MISSISSIPPI STATE, MS 39762

To: .
RES--RELAXIN REPRO (MCMILLAN)
PATHOBIOLOGY AND POPULATION MEDICINE
CVM 9825
MISSISSIPPI STATE UNIVERSITY, MS 39762

Accession Number: C5888-11

Case Coordinator: Missy Bolin
Received: 07/22/2011 Finalized: 07/25/2011
Sampled: 07/22/2011

Phone # 662-325-1104

Case Summary

Species	Animals	Tests	Completed
CANINE	1	1	1

Final Report

Animal ID: DOG 21
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: MIXED BREED DOG

Completed 26 of 26 Results

Requested: 07/22/2011 05:09pm
Received: 07/23/2011 10:09am
Status: 07/24/2011 11:29am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

HEMATOLOGY - CBC, SMALL ANIMAL

MISSISSIPPI STATE UNIVERSITY
MSU CVM DIAGNOSTIC LABORATORY
240 WISE CENTER DRIVE, P.O. BOX 6100

MISSISSIPPI STATE, MS 39762
Phone #: 662-325-1104 Fax #: 662-325-4548

Animal ID: DOG 21
Species: CANINE
Age:
Owner: RES-DEWBERRY, MIKE

Sex: FEMALE
Weight: 0lb
Breed: MIXED BREED DOG

Completed 26 of 26 Results

Accession Number: C5888-11

Requested: 07/22/2011 05:09pm
Received: 07/23/2011 10:09am
Status: 07/24/2011 11:29am

Item	Priority	Status
SA_CBC	ROUTINE	COMPLETE

TEST	RESULT		UNITS	REF RANGE	RESULT DATETIME
WBC	14.4		K/ul	7 - 22	07/24/2011 11:16am
RBC	4.67		M/ul	4.3 - 8.77	07/24/2011 11:16am
Hgb	10.8	L	g/dl	11 - 19	07/24/2011 11:16am
HCT	33.6	L	%	34 - 60	07/24/2011 11:16am
MCV	72.0		fL	63 - 77	07/24/2011 11:16am
MCH	23.1		pg	15 - 29	07/24/2011 11:16am
MCHC	32.0		g/dl	32 - 37	07/24/2011 11:16am
RDW	15.0		%	No Ref Range	07/24/2011 11:16am
Platelets	170		K/ul	160 - 650	07/24/2011 11:16am
Plasma Protein	6.8		g/dl	6 - 8	07/24/2011 11:16am
Technologist: Instrument	ELT - TINA BLOXSOM			No Ref Range	07/24/2011 11:17am
Segs %	65		%	No Ref Range	07/24/2011 11:16am
Segs	9360		/ul	3000 - 11500	07/24/2011 11:28am
Lymph %	27		%	12 - 30	07/24/2011 11:16am
Lymph	3888		/ul	1000 - 4800	07/24/2011 11:28am
Mono %	2	L	%	3 - 10	07/24/2011 11:16am
Mono	288		/ul	150 - 1350	07/24/2011 11:28am
Eosinophil %	6		%	2 - 10	07/24/2011 11:16am
Eosinophil	864		/ul	100 - 1250	07/24/2011 11:28am
Nucleated RBC	1		/100 WBC	.5 - 1	07/24/2011 11:16am
Platelet Estimate	APPEARS INCREASED			No Ref Range	07/24/2011 11:16am
Platelet Estimate Number	720		K/ul	No Ref Range	07/24/2011 11:16am
Platelet Morphology	MANY PLATELET CLUMPING, FEW MEGA PLATELETS			No Ref Range	07/24/2011 11:16am
WBC Morphology	OCCASIONAL REACTIVE LYMPHOCYTES			No Ref Range	07/24/2011 11:17am
RBC Morphology	SLIGHT POLYCHROMASIA, SLIGHT ANISOCYTOSIS, 3+ POIKILOCYTOSIS, MANY CRENATED RBC			No Ref Range	07/24/2011 11:17am
Technologist Diff	ELT- DESIREE COOLEY			No Ref Range	07/24/2011 11:17am

Container