PERINATAL
AND LATE NEONATAL MORTALITY
IN THE DOG

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DISCLAIMER

Apart from the assistance stated in the Acknowledgements and where due reference is made in the text, this thesis represents the original work of the author.

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DEDICATION

This thesis is dedicated to the breeders, without whose help none of this work could have been done.

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ABSTRACT

Pup mortality is reported to be a significant problem in the dog. The purpose of this thesis was to identify the extent and causes of the mortality and the risk factors. Mortality was classified according to the clinical condition of the pup at birth and the pathological investigation was designed to investigate the validity of this classification.

Total pup mortality, excluding elective euthanasia for show reasons, was 18.5%. Perinatal mortality, that is, stillbirths and deaths that occurred in the first week, accounted for 90.9% of these losses.

Each breed surveyed exhibited a specific mortality pattern and the results of one breed could not be used to anticipate the outcome in another breed. As a consequence of this, there was a marked difference in the predictor variables, or risk factors, identified for each breed. Birth weight and inter-pup whelping intervals were the most consistent variables that increased the odds of a pup dying.

The principal cause of pup mortality was attributed to foetal asphyxia, that is, apparently normal pups subjected to excessive hypoxia during the birth process and they were either still born or born in a distressed condition and subsequently died.

Death attributed to foetal asphyxia accounted for 7.8% of all pups born and 42.5% of the total mortality. The majority of these pups (82.2%) died during whelping or in the first 24 hours after birth.

The death of just over half of these pups could be directly attributed to dystocia. The remaining pups were compromised during what appeared to be a normal whelping.

Neonatal atelectasis, pulmonary congestion, inhalation of amniotic fluid and meconium, leptomeningeal and generalised systemic congestion were the principal pathological findings in these pups.

Average birth weights, inter-pup whelping intervals, parity, pup presentation and litter position were all significant predictors of mortality due to foetal asphyxia.

The abnormal pup was defined as a pup at birth that was mummified, had died prior to birth, was small for date or had gross congenital defects present. These accounted for the death of 4.9% of all pups born and 26.3% of all losses.

The only significant predictors of mortality due to the birth of an abnormal pup were the inter-pup interval and birth weight. Since the abnormality occurred *in utero* and was not related to the birth process this result had no bearing on the outcome.

The death of live born, apparently normal pups, in the neonatal period accounted for 5.7% of all pups born and 31.2% of the total mortality. Over half these losses were attributed to fading puppy syndrome. The remainder were due to mismothering / mismanagement and other miscellaneous causes.

The majority of fading pups examined were not normal at birth. Growth retardation and the consequent increased susceptibility to foetal hypoxia, lung pathology indicative of foetal asphyxia and intrauterine and/or very early neonatal infections were the principal causes of mortality attributed to fading puppy syndrome identified in this study.

The canine perinate is totally dependent on the bitch both in the uterus and in the immediate post partum period. The investigation of pup mortality can not be divorced from the assessment of maternal health, the influence of the whelping process and the post whelping care of the immature pups by the bitch. These factors must be correlated with gross and histological changes identified in dead pups to determine the sequence of events that contributed to the death of the whelp.

PREFACE

Lawler (1989) summarises our present understanding of neonatal mortality in the cat and dog. 'Suggested causes of neonatal morbidity and mortality include environmental factors (extremes of temperature and humidity, sudden weather changes and sanitation), nutrition, inadequate thermoregulation, trauma, cannibalism, anatomic birth defects, inborn errors of metabolism, dystocia or prolonged labour and the associated hypoxia or anoxia and infections. In particular, dystocia and prolonged labour are very significant causes of early death in the dog and cat'.

The broad aims of this thesis were to examine the epidemiological and pathological aspects of perinatal and late neonatal mortality in the dog. It is only with accurate information that both the veterinarian and breeder can formulate protocols for whelping management, clinical and pathological assessment of the foetus and neonate and treatment regimes necessary to reduce mortality in this species.

CHAPTER 1: A REVIEW OF THE LITERATURE

1.1 INTRODUCTION

Pup mortality, both during parturition and in the neonatal period, is a significant clinical problem that is poorly documented in the veterinary literature. Mortality rates of 10-30% by weaning (Lawler, 1989) and 15-40% in the first 12 weeks of life (Sturgess, 1998) are reported.

The majority of pup losses are stillbirths and deaths within the first week of life, that is, perinatal mortality. Lawler (1989) specified 65% of the losses occurred in the first week, with about half of these being stillbirths.

Published reports on neonatal mortality frequently cited in the literature are Andersen (1957), Potkay and Bacher (1977) and Blunden (1986). Comparison of these results is difficult. In the first two reports, there were no programs to observe and assist bitches during whelping and monitor pups in the immediate neonatal period. This was not specified in Blunden's study. Different breeds of dogs were investigated and Blunden targeted kennels where neonatal mortality was said to be a problem. There were no strict time frames as to what constituted the neonatal period or time of weaning, nor was there any consistency in the classification of pup deaths.

Pup mortality has been attributed to a wide variety of causes including dystocia, stillbirths, congenital defects, low birth weight or runting, trauma and fading puppy syndrome. Lawler (1989) specified dystocia and prolonged labour as significant causes of early death but cited no references. Fifty-five percent (55%) of deaths in Blunden's study were attributed to unknown causes and were ascribed to Fading Puppy Complex (FPC). Undetermined causes were also prominent findings in both Andersen's (1957) and Potkay and Bacher's (1977) reports. Infectious diseases accounted for only a small percentage of deaths (Lawler, 1989).

Because the whelping and immediate post partum period were unsupervised, there was no investigation of the influence of the parturition process on pup outcome. Further there were no detailed clinical histories available to correlate with the post mortem findings in the dead pups. This correlation of the post mortem findings with the clinical histories of both the mother and infant is central to the investigation of perinatal mortality in the human infant (Langley, 1971).

There are a number of fundamental observations and principles in human obstetrical and perinatal care that may be applicable to the canine perinate.

Perinatal mortality is defined in human medicine as stillbirths and deaths that occur in the first week of life (Farquhar *et al*., 1974). The concept of perinatal mortality depends on the fact that most infants that die do so as a direct or indirect consequence of factors that were present before or during parturition. Early neonatal deaths can therefore be added to stillbirths to give a measure of total foetal loss (Baird and Thomson, 1969).

Anoxia (foetal asphyxia) is responsible for the greatest loss of life in the foetal and neonatal period (Potter and Craig, 1976). Foetal asphyxia and its consequences may best be averted if two goals are achieved (Jacobs and Phibbs, 1989). First, the unhealthy foetus must be

identified prior to labour. Second, abnormal labour must be prevented because even a healthy foetus may eventually succumb to repetitive nonphysiologic stresses.

Part of the strategy for reducing perinatal mortality in the infant is risk scoring. This is a technique based on assessing the antenatal, intrapartum and neonatal risk factors that predict perinatal mortality and identifies the patient who requires specialised care (Depp, 1986).

A thorough investigation of perinatal and late neonatal mortality in the pup therefore requires observation and monitoring of the parturition process, clinical assessment and weighing the neonatal pup at birth and correlation of the post mortem findings with the clinical histories. This necessitates a close cooperation between the breeder and veterinarian and a simple method of classification of pup mortality that can be uniformly applied.

Further, risk scoring as per human obstetrical care may not be applicable to the canine patient. However, unless some form of risk assessment during pregnancy and whelping can be developed it may not be possible to reduce the current high levels of pup mortality reported. The results of this study were statistically analysed in an attempt to identify risk factors that occur in the dog.

1:2 PERINATAL AND LATE NEONATAL MORTALITY IN THE DOG.

The definition of the neonatal period within veterinary medicine is generally considered to be the transitional phase from foetal to adult life (Jones, 1987). During this interval changes in structure and function occur as the animal moves from the physically, chemically and microbiologically protected environment of the uterus to face the adaptive requirements for survival in a complex environment. Papich and Davis (1986) have defined the neonatal period, for the dog and cat, as approximately the first 30 - 45 days. After this time, the hepatic and renal drug elimination mechanisms can be expected to approach adult values.

The three principal studies of pup mortality published in the literature are those by Andersen (1957), Potkay and Bacher (1977) and Blunden (1986).

ANDERSEN (1957)

This study involved 234 consecutive litters and 1157 pups whelped in a Beagle breeding colony. Total mortality (stillborn and pups that died under six weeks of age) was 29.4%.

Dogs were observed several times on the day of whelping and lactating dams were inspected at least twice daily. The use of medical treatment on pups and dams was avoided to obtain basic information concerning the natural incidence of pup mortality.

The loss reported at birth was 10.9% of all pups born and 37.0% of the total mortality. The death of pups at birth included stillbirths, malformations, and undetermined causes as well as those that died as a result of dystocia, trauma and cannibalism. Early neonatal deaths accounted for 8.3% of all pups born and 28.2% of the total mortality. The total of these losses, that is perinatal mortality, was 19.1% and accounted for 65.3% of all pup deaths.

The principal cause of pup death was attributed to maternal factors and mismothering (trauma, excessive licking, lactational failure and cannibalism) and accounted for 6.7% of all pups born. Stillbirths, which included all pups that were non-viable at the time of birth, was the second highest cause of loss reported (4.6%), followed by exposure (4.4%), undetermined (2.4%), disease (2.5%), dystocia (2.3%), malformations (2.2%), accidents (1.7%) runts (1.4%) and parasites (0.8%).

POTKAY AND BACHER (1977)

This was a three-year study of total morbidity and mortality in a Foxhound breeding colony. Programs to observe and assist bitches during whelping and to monitor puppies continuously during the first two to three days were not in effect.

Three hundred and thirty-nine litters and 2872 pups were whelped. Total mortality before weaning at six weeks of age was 17.4% (501 pups) and 4% died between weaning and 30 weeks of age.

The stillborn loss was 2.2% and the early neonatal mortality was 9.7%. These losses combine to give a perinatal mortality of 11.9% which was 68.3% of all pup deaths.

The principal cause of pup mortality was attributed to undetermined reasons and accounted for 4.0% of all pups born, followed by stillbirths (2.2%), nutritional disease (2.0%), runting

(1.9%), trauma (1.7%), miscellaneous (1.7%), pneumonia (1.3%), congenital anomalies (1.0%) gastrointestinal disease (1.0%) and intussusception (0.8%).

BLUNDEN (1986)

This was a study of Fading Puppy Complex conducted at 30 kennels where neonatal mortality was said to be a problem. The data from 86 litters and 518 pups were analysed. There were 43 stillbirths (8.3%) and a further 152 pups (29.3%) died in the first three weeks of life. Total mortality was 37.7%.

The principal cause of pup mortality was attributed to fading puppy complex and accounted for 15.8% of all pups born. This was followed by stillbirths (8.3%), maternal/ management linked causes (trauma and lactational problems) (4.8%), infections (4.5%), runts (2.1%), congenital abnormalities (1.4%) prematurity (0.2%) and parasites (0.2%).

The evaluation and comparison of these results was difficult as there was no uniformity in what constituted stillbirths and the neonatal period nor the classification of mortality. Andersen (1957) for example, distinguishes between stillbirths and other causes of death at birth such as dystocia, trauma, etc., while the other authors did not. Similarly, Andersen cites disease as accounting for the loss of 2.5% of pups, Blunden refers to infections accounting for the loss of 4.5% of pups, while Potkay and Bacher specify gastrointestinal disease, pneumonia, intussusception and nutritional disease as specific entities accounting for 5.8% of losses.

There are, however, a number of important similarities that have not changed between Andersen's study in 1957 and Blunden's study in 1986. Perinatal mortality constitutes a significant loss in a canine breeding program with the birth of apparently normal but dead pups (stillborn), a principal component of this loss. Maternal influences, runting and congenital anomalies were all significant causes of mortality.

A large number of pups died of unknown causes. In Blunden's report, 15.8% of all pups born had no identifiable cause of death and the deaths were attributed to fading puppy complex. The comparable figure for Potkay and Bacher was 4.0% and for Andersen 2.4%.

The primary causes of late neonatal mortality in both Andersen's (1957) and Potkay and Bacher's (1977) reports were attributed to disease (infections and nutritional) and exposure (heat and cold).

1:3 THE AETIOLOGY OF PERINATAL AND LATE NEONATAL MORTALITY

Fox (1963) described the following clinical course in neonatal dogs that died between one and three days of age. 'There was a rapid decrease in weight after birth, with persistent hypothermia and slowing of the heart rate. Respiration also slowed and near the time of death prolonged spells of apnoea lasting as long as 60 seconds occurred. Spasticity and postural flexor dominance (foetal posture) were characteristic of the syndrome in early stages. Tetanic rigours with hyperextension of forelimbs and spine occurred shortly before death and often during or just prior to respiratory arrest. Other signs were similar to those observed in human infants and ascribed to cerebral anoxia; muscle flaccidity followed by tetanic rigours and tonic-clonic "walking" with the hind and forelimbs. Post mortem examination revealed various states of cardiac failure and circulatory arrest. This clinical syndrome occurred irrespective of the causal factor. It is a therapeutical irreversible syndrome of hypothermia and cardiopulmonary failure that develops, and regardless of the cause is characteristically fatal'.

This characteristic clinical course is the result of the immaturity on the neonatal pup.

1:3:1 NEONATAL IMMATURITY

The newborn puppy is an immature animal, dependent on its dam for survival in the first three weeks and as a consequence the aetiology of neonatal death is frequently complex and often undetermined (Chandler, 1990; Blunden, 1998).

Fox (1963 and 1965) recognised that immaturity of the homeostatic cardiovascular mechanism in newborn puppies could result in their death from a cardiopulmonary failure syndrome. Rather than being the primary factor, cardiopulmonary failure is frequently the final common pathway for a puppy that is compromised by the other factors with or without the complications of infections (Chandler, 1990).

Prior to two weeks of age the heart is not responsive to atropine indicating the immaturity of the vagus nerve. The baroreceptors are not functional until four days after birth (Fox, 1965). The pulmonary response is also minimal in newborn puppies (Bright and Holmberg, 1990). This immaturity of the homeostatic cardiovascular mechanism can cause a vicious cycle of collapse and inadequate response that becomes irreversible (Chandler, 1990).

An important factor affecting the mean arterial blood pressure (MABP) in the perinatal period is blood glucose concentration. One study demonstrated an approximately 50% decrease in MABP in pups with severe hypoglycaemia (Hernandez *et al.*, 1980). This was believed to be related to the inhibition of contractile processes in the myocardium and/or smooth muscle in the peripheral vasculature.

The newborn puppy is particularly vulnerable because of four major factors. These are, their thermoregulatory mechanism is poorly developed, there is a risk of dehydration, a risk of hypoglycaemia and immunological immaturity (Blunden, 1998).

Neonatal Thermoregulation

In all systems of whelping management, the newborn pup is introduced at birth into a cooling environment, ie. an ambient temperature of less than 80 degrees F (26.2 Deg C). Cooling is enhanced because the animal is wet. Deep body temperature falls during the first 20 minutes. The degree of hypothermia that develops depends on the speed at which the dam cleans and dries the puppy and on the environmental temperature in which the dam has whelped. The pup gradually regains a higher body temperature by staying in close contact with the mammary glands of the dam. Once whelping starts the surface temperature of the mammae is only a degree or so less than the deep rectal temperature of the mother (Crighton, 1968).

In the face of cooling, or even the threat of cooling, the puppy seeks shelter. If this fails to arrest the cooling it then searches for a source of external heat which it is able to absorb to compensate for heat loss. Thus, provided a source of heat is immediately available, it can maintain a relatively stable rectal temperature despite a cooling environment. It is also suggested that the newborn puppy is a true homeotherm that substitutes compensatory thermal conduction for its lack of compensatory thermogenesis. The shivering reflex and vasoconstriction mechanism are not operant in the newborn (Crighton, 1968).

Poor maternal instinct may result in neglect of puppies at the time of birth so that the initial hypothermia is not corrected. Weak or premature pups may be unable to establish the necessary physical contact with the dam to achieve normothermia. If the periods of contact are intermittent, moderate hypothermia may persist (Crighton, 1968).

Neonatal Glycogen Reserves

The newborn relies almost exclusively on hepatic glycogen for energy for the first 24 hours. Hepatic glycogen stores may be low at birth owing to intrauterine malnutrition associated with excessive multiple pregnancy or maternal malnutrition. Within 8-12 hours after birth most hepatic glycogen has undergone glycogenolysis and the newborn is forced to rely on nutritional intake to maintain euglycaemia. It is during this interval that the newborn is exquisitely susceptible to the development of hypoglycaemia (Center *et al.*, 1990). Failure to suck results in rapid depletion of the liver reserve of glycogen and the development of hypoglycaemia by the second day.

Neonatal Ontogeny

The endothelio-chorial placenta in dogs is advanced in intimacy and allows for *in-utero* transfer to the foetus of from 1 - 20% of total newborn serum antibody concentration (Greene, 1984a). Since IgA is large and is not transported across the placenta most of the immunoglobulin transferred *in utero* is IgG. In the absence of ingestion of colostrum the puppy is probably protected for at least one week due to a small amount of *in utero* transfer of immunoglobulins (Appel and Gillespie, 1972).

There is little information available in regard to the period during which the pup is able to absorb immunoglobulins intact across the intestinal wall. Work by Gillette and Filkins (1966) indicated that absorption appeared optimal if antibody was fed to the puppies eight hours after birth, was almost complete 15 hours after feeding and was not detectable if fed 24 hours after birth.

Secretory IgA present in colostrum and milk is important because of the protection that it provides against mucosal pathogens in the newborn animal. Secretory IgA provides

continuous protection against gastrointestinal infection as long as nursing continues (Greene, 1984a).

Neonatal puppies (like neonates of other species) are born germ free into a contaminated environment. They lack the indigenous population of microorganisms on skin and mucosal surfaces that is characteristic of the older neonate and adult animals. They acquire their own population from contact with their dam and other animals, from milk, food and other contaminated fomites (Greene, 1984a).

Within the first week of life newborn animals are more susceptible to infection than older animals because they lack a well-developed microflora and because they have a non-selective protein transport mechanism that facilitates the absorption of immunoglobulins. This protein transport mechanism may also transport pathogenic microorganisms across the mucosal barrier. Maternal immunity is essential in providing protection during this critical period (Greene, 1984a).

The normal microflora is important in protecting body surfaces from colonisation by foreign bacteria through several established mechanisms. They produce antibacterial substances (bacteriocins) and toxic metabolites, compete for nutrients and sites of adherence and degrade ingested and locally produced toxins (Greene, 1984a). Indirectly their presence enhances the function of existing immunologic mechanisms.

Neonatal Renal Function

The kidney of the newborn contains an outer zone of non-differentiated tissue in the cortex which requires 2-3 weeks to undergo nephrogenesis and become functional (Mosier, 1978).

Functional tests of normal puppies have established that the glomerular filtration rate at birth ranges from 21-50% that of the adult and the tubular secretion rate at eight weeks of age ranges from 12-15% that of the adult. This leads to a slow clearance of fluids, increased sodium loss and inability to conserve fluids. There is a positive correlation of glomerular filtration rate and mean arterial blood pressure (Robinson, 1983).

Neonates have a greater extracellular water and total water compartment than adults (Jones, 1987). This difference is due mostly to extracellular fluid. As 82% of bodyweight is water and kidney function is immature, the neonate is particularly susceptible to dehydration (Blunden, 1998).

Water turnover rate is twice that of the adult (Mosier, 1978). Neonatal puppy fluid maintenance requirements are approximately 132 - 220 mls/kg/day.

What this means to the veterinarian is that nearly all sick pups clinically look the same. When presented, they are usually hypothermic, hypoglycaemic and dehydrated. It is then difficult to do a detailed clinical examination, without first correcting this potentially fatal cycle.

In practice, supportive therapy is often given too late to alter survival outcome, and there is often insufficient investigation of neonatal mortalities to assess the cause of breeding loss accurately (Blunden, 1998).

1:3:2 MATERNAL INFLUENCES

The outcome of pregnancy depends, largely, on the overall health of the dam. Nutrition and management of the dam during pregnancy, whelping and the neonatal period greatly influence the outcome of the breeding program. Many factors have predictable effects on pregnancy and neonatal survival. Selection of animals with metabolic abnormalities or the genetic predisposition to develop them should be avoided (Johnson *et al*., 1987). This broad statement is inadequately documented in the veterinary literature. Similarly, maternal cardiac and renal disease and diabetes mellitus are all reported to affect the health of the foetus but again is poorly documented.

Maternal Age

Age has a profound influence on the reproductive performance. Andersen (1965) studied a colony of 65 Beagle bitches. The number of pups weaned was greatest (4.28 pups) and puppy mortality was lowest (18.5%) when dams were two years old. In another group of 17 Beagles the number of pups weaned was highest (4.19) and neonatal mortality was lowest (15%) when dams were 3.5 years old. By five years of age conception failure was more than 50%. When these same bitches were seven years old, an average of 1.22 pups were weaned and puppy mortality was 38.9%. By nine years of age, only 0.6 pups were weaned per litter and mortality was 76.3%.

Maternal Infections

Maternal infections are a well known cause of perinatal morbidity and mortality (Johnson et al., 1987). The dam may show no clinical signs and yet the infectious agent may have profound effects on the foetus or neonate. The type of disease that results from *in utero* infections depends more on the stage of gestation at which infection occurs than on the aetiological agent (Greene, 1984a).

Maternal infections with Canine Parvovirus, Herpes Virus, Distemper, *Brucella Canis* and toxoplasmosis are all reported to cause foetal and neonatal mortality (Percy *et al*., 1971; Carmichael, 1976; Krakowka *et al*., 1977; Robinson *et al*., 1979; Higgins *et al*., 1981; Lenghaus and Studdert, 1982; Hashimoto *et al*., 1983; Greene and Kakuk, 1984; Greene, 1984c).

Mycoplasmas are common inhabitants of the canine vagina and prepuce but may cause reproductive disease if inoculated into the uterus (metritis) or vas deferens (orchitis). Their role in spontaneous embryonic death, resorption, abortion and stillbirth is unknown (Johnston and Raksil, 1987).

Campylobacter jejuni infection has been reported in association with premature labour and abortion. *C. jejuni* was cultured from the foetal liver and stomach (Bulgin *et al*., 1984).

Escherichia coli is a common aerobic bacteria present in the vagina of the normal bitch. It is commonly cultured from the uterus of bitches with pyometra and from vaginal discharges following foetal death and abortion (Greene and Kakuk, 1984).

Salmonella bacteria have been isolated as a cause of abortion and foetal death in a Boxer. Their role in foetal death like that of *E. coli*, may be that of a primary pathogen or as a secondary ascending invader (Redwood and Bell, 1983).

Beta-haemolytic streptococci are common inhabitants of the vagina of the normal bitch. These bacteria have been isolated from the uterus of some bitches with pyometron and from some puppies with neonatal septicaemia. Mantovani *et al*. (1961) reported the presence of *beta-haemolytic streptococci* (type 1) from the vagina and lymph node aspirate of five collie bitches with a history of abortion, infertility and neonatal death.

Maternal Behaviour

The maternal behaviour post whelping can significantly affect pup mortality. In Andersen's study (1957), maternal factors and mismothering, which included trauma, excessive licking, lactational failure and cannibalism, caused the death of 6.8% of all pups born and was the principal cause of pup mortality. Maternal/management causes (trauma and lactation problems) accounted for the loss of 4.8% of pups in Blunden's study (1986).

The lack of effective maternal care allows environmental influences, particularly the environmental temperature, to affect the puppies (Crighton, 1968). Certain bitches refuse to relax and adopt lateral recumbency. They may sit or move away from the puppy. This creates a situation where an otherwise healthy puppy has an unstable rectal temperature (Crighton, 1968).

Excessive maternal activity is displayed by other bitches. They spend too much time cleaning a litter and certain pups may have great difficulty in keeping in contact with the dam for lengthy periods. The widely recognised phenomenon where a bitch selects one or two puppies and then rejects them will result in hypothermia even if the rejects are healthy. Poor mammary gland development at the time of whelping may be one further factor in postpartum hypothermia in a litter (Crighton, 1968).

1:3:3 ENVIRONMENTAL INFLUENCES

A low ambient temperature not only tends to produce hypothermia in a puppy but it may also cause a dam to adopt a posture of ventral recumbency so that radiant heat loss from the engorged mammary gland is minimised. Thus a puppy, already hypothermic, may be denied access to a source of heat (Crighton, 1968).

In a neonatal mortality study by Andersen (1957), monthly climatic variations did not coincide with fluctuations in puppy mortality. However, puppy deaths did coincide with sudden diurnal changes in climatic conditions. The highest losses were observed in the autumn which was characterised by rapid changes in temperature where a warm day may be followed by a cold night. Fatalities in puppies between eight and 21 days of age occurred in summer when the temperature rose to 108 degrees F. Evidently they attempted to seek a cooler area and were unable to return to their litter nest after direct exposure to the sun.

1:3:4 NEONATAL DISEASES

Fading Puppy Complex (Syndrome)

The fading puppy syndrome has been a recurring problem for breeders and veterinary surgeons for decades. Various theories have been formulated to account for this syndrome and some have doubted that it is a genuine disease syndrome (Blunden, 1998). One cause of confusion has been to incorporate all conditions leading to poor weight gain and ill-thrift in the first months of life under this one syndrome. A whole range of aetiological possibilities have been considered including maternally related factors such as poor mothering, inadequate lactation, trauma and inadequate nutrition of the dam in pregnancy, congenital anomalies, neonatal isoerythrolysis, low birth weight, thymic atrophy and infectious agents including bacteria, viruses, protozoa and parasites. Abnormal lung surfactant has also been suggested (Blunden, 1998)

Fading puppies are defined by Blunden (1986) as those puppies where no obvious cause of death is found after careful post mortem examination, including bacteriology and histopathology and in the context of good knowledge of the clinical history and management practices of the kennel. These puppies are apparently born healthy but fail to thrive and usually die in the first seven days. In Blunden's study of 114 puppies, examination of the growth plate at the costochondral junction revealed that marked growth arrest had occurred in 75% of fading puppies that had died by day three. This suggested that at least a number of puppies were not thriving from a very early stage in neonatal life and it was possible that growth retardation had commenced in late pregnancy. Certain clinical and pathological findings were common in this group. Signs of illness included general lassitude and weak sucking responses from day one or two and restlessness, plaintive and persistent crying, lateral recumbency with limb paddling, inability to stay on the teat and occasionally rigours with progression to signs of generalised weakness and death.

Sturgess (1998) defined fading puppy syndrome as a clinical description rather than a diagnosis and covered a multitude of infectious and non-infectious conditions of the neonate which cause animals born apparently healthy to gradually become inactive, lose their suckle reflex and die in the first two weeks of life.

Blunden (1998) identified a tendency for certain dams within particular kennels to have successive fading litters.

Neonatal Infections

Infectious diseases accounts for only a relative small percentage of deaths (Sturgess, 1998) and the majority of these deaths occurred in the late neonatal period. This time frame for neonatal infections may be related to the fact that in the absence of the ingestion of colostrum the puppy is probably protected for at least one week by the *in utero* transfer of immunoglobulins.

Many of the attempts to reproduce bacterial diseases in puppies have been unsuccessful which may mean that, although the bacteria are involved in the aetiology of neonatal mortality, some triggering or precipitating factor is also needed (Evans, 1978).

Possible routes of infection are oral, via the umbilicus, by the ingestion of vaginal discharge during the process of parturition or from the infected environment, by inhalation and across the placenta (Evans, 1978). There is reasonable evidence to suggest that at least three types of bacteria are involved in neonatal mortality (Evans, 1978). These are *Haemolytic streptococci*, *Escherichia coli* and *Brucella* species (Jones, 1987).

Clostridial perfringens Type A was recovered in greater frequency from vaginal swabs of bitches with a history of neonatal losses than from normal breeding bitches (Blunden, 1983). *Cl. perfringens* can appear in the faeces of apparently normal puppies at 24-28 hours after birth. The possibility remains that some factors such as incorrect feeding, hypothermia or the use of antibiotic could affect the natural gut flora while it is becoming established, allowing the organism to become established with an ensuing enterotoxaemia (Blunden, 1983)

Protozoal Infections

Postnatal infections with *Toxoplasma Gondii* may occur as acute or chronic clinical entities (Greene and Prestwood, 1984).

Acute postnatal infection is rapidly progressive and disseminated toxoplasmosis may occur when large numbers of oocysts or bradyzoites are ingested.

Viral Infections

Infectious canine hepatitis (ICH) is caused by canine adenovirus 1 (Greene, 1984b). The young pup infected with CAV-1 may present in acute collapse after a short illness. Clinical signs are usually vague and may include dullness, inactivity, reluctance to nurse and coma. Some puppies proceed to coma and death within just a few hours (Center *et al.*, 1990).

Newborn puppies can acquire canine herpes virus infection (CHV) infection from passage through the birth canal or from contact with litter mates, oronasal secretions of the dam, or fomites. *In utero* infection has been documented. CHV in neonatal puppies is associated with an acute fatal illness occurring between 1-3 weeks of age (Greene, 1984b). The narrow range of susceptibility of neonates may relate to *in utero* exposure or may be attributed to exposure to the virus during or soon after birth. Additional predisposing factors include the lack of thermoregulatory mechanisms during the first week of life and an incubation period of four to six days.

Myocardial disease associated with canine parvovirus infection develops following *in utero* or early neonatal infection of puppies (less than eight weeks) from non-immune bitches. Virus can persist in cardiac fibres in a latent form until myocytes multiply into multi nucleated cell types between three and eight weeks of age.

Primary Immunodeficiency Diseases

Primary immunodeficiency is a congenital failure of the immune system that has a proven or suspect genetic basis and that predisposes an animal to disease (Greene, 1984a). Primary immunodeficiency diseases should be considered in the neonatal pup when non-colostrum deprived animals have recurrent, prolonged infection that responds poorly to treatment and neonatal deaths in litters of common parentage (Guilford, 1987).

Parasites

Andersen (1957) found that parasites caused a few mortalities. These occurred at 4-5 weeks of age. It is considered possible that puppy deaths after the fourth day can be due to worm infestations and that the migrating ascarid *Toxocara canis* in particular is involved (Evans, 1978). As many as 90% of puppies are affected prenatally with *T. canis*. Infestation may also occur via the colostrum (Evans, 1978).

In contrast to the situation for *T. canis*, intrauterine infection with the canine hookworm, *Ancylostoma caninum*, is of minor significance. Suckling pups acquire *A. caninum* larvae from colostrum and milk as well transcutaneously and by ingestion of other contaminated material (Evans, 1978).

Congenital Defects

Congenital defects are defined as abnormalities of a structure or function present at birth. Many, but not all, congenital defects are genetically caused (Leipold, 1978).

Congenital disorders may be lethal to the neonate at birth or shortly after, or result in euthanasia of the animal. Many of the defects present at birth are not identifiable without clinical or pathological identification (such as ocular) and do not contribute to neonatal mortality. Many structural abnormalities develop as clinical entities later in life, e.g. portosystemic shunts or only become evident when the animal becomes ambulatory (Leipold, 1978).

Reported pup losses associated with grossly recognised congenital defects varies. Potkay and Bacher (1977) recorded a 1.0% loss, Blunden (1986) 1.4% and Andersen (1957) 2.2%. Functional defects may lead to neonatal death and are being diagnosed with increasing frequency. Inherited coagulation disorders and metabolic defects are two such entities.

Some inherited coagulation disorders reported to cause neonatal death are Factor II, Factor VIII and Factor X deficiencies (Fogh and Fogh, 1988).

Metabolic diseases of the dog and cat were investigated by Jezyk (1983). A variety of disorders were identified in a relatively small population and concluded that if it is even a rough estimate of the prevalence of such disorders in the general population then these diseases may represent a significant cause of death. That such a disorder might be present must therefore be suspected by the clinician. Often the basis for this suspicion is epidemiologic in nature, such as repeated losses from successive litters by the same bitch or unusual patterns of puppy mortality in related bitches (Lawler, 1989).

Low Birth Weight (Runting)

Pup mortality attributed to runting varied from 1.4% (Andersen, 1957) to 2.1% (Blunden, 1986). There were no criteria, e.g. specific birth weight, by which runts or "small for date" pups were recognised.

Puppies which are more than 25% below the average birth weight are reported to have a higher mortality rate (Mosier, 1978). Weight is a reflection of the relative maturity of the organism. Low birth weight puppies are physiologically immature when compared to

littermates of average birth weights. They are also at greater risk from hypothermia and can not compete well for milk against their larger litter mates.

In human medicine the term Intrauterine Growth Retardation (IUGR) is used and this describes the clinical entity, that is, low birth weight for gestational age. The magnitude of the problem of IUGR is second only to prematurity as a cause of perinatal mortality in the infant. While prematurity causes primarily an increased neonatal mortality, IUGR causes a vastly increased intrauterine foetal death rate. Death from intrapartum asphyxia alone is 10 times higher than for appropriately grown infants with 14% of all stillbirths and 6% of all neonatal deaths occurring in infants whose birth weights are less than the third percentile for gestational age (Renfield, 1975).

In the vast majority of small-for-date infants there is no identifiable cause to explain their small size. Two major factors influence foetal growth. These are the inherent growth potential of the foetus and the growth support it receives by way of the `supply line' - the placenta and maternal organism (Renfield, 1975).

Acute perinatal distress, while not affecting foetal growth per se, is characteristic of IUGR. Hypoxic insults to the deprived infant may occur during any stage of gestation. The limited nutritional and circulatory reserves of such a foetus make even normal labour and delivery, in itself a hypoxic stimulus, a more stressful event for the infant. The higher rate of antepartum and intrapartum stillbirths and low one minute Apgar score in this group attests to this concept (Renfield, 1975).

The extent of growth retardation and its contribution to perinatal loss in the dog requires further investigation. For this, normal birth weight and the standard deviation from the mean must be identified for each breed of dog and mortality relative to the birth weight variation investigated.

1:3:5 DYSTOCIA

Dystocia or prolonged labour and the associated hypoxia or anoxia are very significant causes of early death in the dog (Lawler, 1989). Dystocia is defined as a difficult birth or inability to expel the foetus(es) from the birth canal at the time of parturition (Macintire, 1994).

There are, however, limited reports in the literature on the extent of the contribution of dystocia to pup mortality. Andersen (1957) reported the loss of 2.3% of all pups born due to dystocia.

Linde-Forsberg and Eneroth (1998) consider the true incidence of dystocia in the bitch to be around 5% overall, but it may amount to almost 100% in some breeds of dog, especially of the achondroplastic type and those selected for large heads.

Traditionally, dystocia is classified as being either of maternal or foetal origin, or a combination of both. Maternal causes are reported to account for 75.3% of dystocias occurring in the dog, with 48.9% due to primary complete inertia (maternal primary inertia) and 23.1% due to primary partial inertia (maternal secondary inertia). Foetal causes accounted for 24.7% of dystocias encountered, with malpresentations (15.4%) and foetal oversize (6.6%) the predominant forms occurring (Linde-Forsberg and Eneroth, 1998).

The diagnosis of dystocia and acceptable time constraints have been reported by Johnston (1988), Jones and Joshua (1982) and Macintire (1994).

The diagnosis of dystocia is dependent on demonstrating failure to start labour on due date (use knowledge of ovulation date, rectal temperature drop), failure to progress normally in labour (more than four to five hours in stage two labour before delivery of the first pup, or more than two to three hours between puppies), foetal membranes in vulva for greater than 15 minutes, strong contractions lasting more than 30 minutes with no foetus. Jones and Joshua (1982) state that the first pup is usually born within one hour of the onset of meaningful straining, but often appears within as little as 20 minutes; up to 2 hours need not cause anxiety. By six hours placental separation is occurring and the life of the presenting puppy may be in jeopardy, thus six hours should be regarded as the maximal period permissible without investigation; even after two hours some separation may exist. The interval between birth comprises two parts - resting and straining. Rest periods vary from 5 minutes to 3 hours and occasionally even longer, although it is arguable if 4 hours rest falls within normal limits. Provided vigorous straining without progress does not occur, the longer intervals do not call for urgent attention. A common pattern is for a bitch to produce two or three puppies at short intervals e.g. ten to 30 minutes and then go into a rest phase of one to three hours before repeating the process. The birth of a large litter may occupy 24 hours or so but if there is no excessive effort needed for each birth the dam should not become unduly tired.

From a veterinary standpoint one of the most difficult clinical decisions is the distinction between the end of normal labour and the beginning of abnormal labour. The above time constraints may be excessive and require review. Further, the specific contribution of dystocia to pup mortality and the post mortem and histopathological findings in these pups requires detailed investigation.

1:4 PERINATAL ASPHYXIA

Asphyxia refers to a condition of hypoxemia, hypercarbia and acidosis (James and Adamsons, 1964). Tissue acidosis and dysfunction concurrently occur, and will, if not rapidly corrected, lead to irreversible organ damage and death (Jacobs and Phibbs, 1989). The direct cause of death from asphyxia is an insufficiency of oxygen supplied to the tissues by the blood (Potter & Craig, 1976).

In the past, perinatal asphyxia in the infant was thought to have a clinical course that primarily indicated central nervous system involvement. However, the clinical course can be quite varied (Brann and Dykes, 1977). This variation was documented by Sexson *et al.* (1976). The organ systems affected, in order of decreasing frequency, were pulmonary, cardiovascular, central nervous, gastrointestinal and renal. No one system appeared to be the primary target of asphyxia.

In humans, anoxia is responsible for greater loss of life in the foetal and neonatal period than is any other known agent (Potter and Craig, 1976). In the pig, perinatal deaths associated with anoxia and hypoxia are considered to account for a high percentage of losses before 4 days of age (Sprecher *et al*., 1974). With the exception of the work by Drake and McCarthy (1964), there are no published reports on perinatal losses and the pathological changes associated with anoxia in the dog.

1:4:1 THE PHYSIOLOGY OF THE BIRTH PROCESS

The dog has been used as extensively as an experimental animal in medical research in the development of our understanding of the tolerance and response of the foetus and neonate to anoxia (Fazekas *et al*., 1941; Himwich *et al*., 1941; Glass *et al*., 1944; Mott, 1961; Shelly, 1961; Arango and Rowe, 1971; Duffy *et al*., 1981; Monheit *et al*., 1988).

During labour, with each uterine contraction there is a decrease in uterine blood flow, placental perfusion and gas exchange, thus the foetus/foetuses are exposed to intermittent periods of relative hypoxia. The fit mature foetus, with abundant stores of glycogen and brown fat, can withstand such periods of hypoxia for prolonged periods without developing serious oxygen debt and lactic acidosis. However, foetuses exposed to chronic placental malnutrition/hypoxia are already on the brink of asphyxia and have little reserve after the onset of parturition. The ability of the foetus to withstand the stress of parturition thus depends on both the condition of the foetus at the onset of parturition and on the parturition itself (duration, number of contractions etc.) (Best, 1988).

The foetus is well adapted to this environment and has several compensatory mechanisms to lessen the effects of both hypoxia and asphyxia. These adaptations and compensatory mechanisms have been loosely termed foetal reserve. The determinants of foetal reserve are maternal, foetal and placental. The foetal adaptations to a hypoxic *in utero* environment are increased oxygen affinity of foetal haemoglobin, increased ability of tissues to extract oxygen and greater tissue resistance to acidosis. The foetal compensatory mechanisms to asphyxia are bradycardia, redistribution of blood flow with increased flow to the brain, adrenals and heart and decreased flow to the lungs, carcass, gut, liver, spleen and kidneys. There is also decreased oxygen consumption and aerobic glycolysis (Jacobs and Phibbs, 1989).

Blood studies reveal that newborn dogs survive despite the fact that their arterial blood contains practically no oxygen after the first few minutes of anoxia (Fazekas *et al*., 1941).

Mature animals succumb after breathing undiluted nitrogen after three minutes, while the average values for the newborn dog is 23 minutes (Himwich *et al*., 1941).

During total anoxia some vital activities continue long after oxygen depletion of the blood and it is evident that energy for these activities must be obtained by anaerobic processes (Mott, 1961). In puppies subjected to total anoxia breathing always ceases before circulatory collapse (Mott, 1961). The brain is the limiting factor to the tolerance to anoxia (Himwich *et al.*, 1941).

There is close correlation between cardiac carbohydrate concentration and the survival time during anoxia. Cardiac carbohydrate is rapidly depleted during anoxia. When the stores of carbohydrate in the animal are reduced by fasting or anoxia, the reduction in survival time is directly proportional to the drop in initial cardiac carbohydrates (Mott, 1961).

1:4:2 PATHOPHYSIOLOGY OF ANOXIA

The foetal response to hypoxia and asphyxia is very similar to the mammalian diving response which occurs in response to facial or nasopharyngeal immersion. It is characterised by hypertension, bradycardia, selective vasoconstriction (preserving blood flow to the heart, brain and placenta), decreased total oxygen consumption, anaerobic glycolysis with peripheral accumulation of lactic acid, reduced cardiac output, and when severe results in decreased cardiac performance (Best, 1988).

Monheit *et al*. (1988) experimentally induced anoxia in the canine foetus *in utero* by occlusion of the maternal descending aorta. The gestational age of the pups varied from seven to nine weeks. The pathophysiological sequence of events occurring in the canine foetus with impaired oxygen delivery are a latency period with no changes followed by a drop in pO_2 which stabilised later at a lower pressure. There was then a late foetal heart rate deceleration, the pattern of which was not related to the progressively deteriorating condition, a progressive increase in pCO_2 and a progressive decrease in pH.

Data on the normal blood gas concentrations, acid-base levels and haemodynamics in the canine neonate have been published by Arango and Rowe (1971).

Table 1:1 Summary of foetal and neonatal data (Monheit *et al*., 1988* and Arango and Rowe 1971**)

Mean	**Before Experiment***	**tpH 7.05***	**Normal 0-1 days of age****
Heart Rate	145 ± 4	104 ± 9	208 ± 31.24
pH	7.33 ± 0.02	7.18 ± 0.05	7.41 ± 0.05
pO_2	35 ± 2	19 ± 3	47.3 ± 6.7
pCO_2	41 ± 3	72 ± 5	46.6 ± 8.2
(tpH : tissue pH)			

Foetal heart rate late deceleration is a relatively rapid and early response to hypoxia and is not associated initially with acidosis. The drop in pH beyond that seen during normal labour is an advanced sign of foetal distress and is the final component of the previously described sequence (Monheit *et al*., 1988).

In the human infant a pH of 7.20 is indicative of foetal distress (Yuen *et al.*, 1977). The data of Monheit *et. al.* (1988) demonstrates that a pH of 7.18 ± 0.05 is accompanied by a foetal heart rate of 104 ± 9 and this is a recoverable level of anoxia.

Utilising ultrasonography, normal foetal heart rates are reported to be greater than twice that of the maternal heart rate. Foetal heart rates less than this are considered to be suggestive of foetal distress (G. R. Johnston, personal communications).

1:4:3 RECOGNITION OF PERINATAL ASPHYXIA IN THE INFANT

The principal symptom of intrauterine anoxia is an irregularity or slowing of the foetal heart rate that is unrelated to uterine contractions. Even in normal infants the heart rate is often slow during a contraction but returns to normal between contractions. With anoxia, a temporary increase in the heart rate may precede the slowing or irregularity and may be associated with a brief period of increased foetal activity. Intestinal peristalsis is increased and the anal sphincter is relaxed when the anoxia is severe. Consequently, when the head is the presenting part, the presence of meconium in the amniotic fluid is a grave sign. In breech presentation, relaxation of the anal sphincter is common and meconium has no pathological significance (Potter & Craig, 1976).

During anoxia there is also stimulation of the foetal respiratory centre so that intrauterine respiratory movements become deeper and more sustained. The effect of this is to draw amniotic fluid into the lungs. Amniotic fluid contains a considerable amount of debris including squamous cells. This debris is not absorbed and collects in the potential air spaces of the lungs (Drake and McCarthy, 1964).

In humans normal foetal capillary pH tends to show a slight decline towards the end of first stage labour. During the second stage there is a strong tendency for foetal acidosis. This is most pronounced just prior to delivery. Normally foetal pH should be above 7.25. It tends to be slightly lower towards the end of the second stage of labour. A pH between 7.20 and 7.25 is equivocal and bears repeating. A pH less than 7.20 is indicative of foetal acidosis (Yuen *et al.*, 1977).

A number of factors influence the rate of recovery from birth asphyxia (James, 1965). The two most important factors are analgesia and anaesthetic drugs prior to delivery and prematurity. Delayed recovery is also seen in the most asphyxiated infants probably on the basis of circulatory impairment and central nervous system depression. This group includes infants that have aspirated meconium prior to delivery.

At birth the presence of asphyxia (foetal distress) is recognised by observation of respiratory activity and heart rate, muscle tone, skin colour and reflex activity. These may be evaluated by the Apgar Scoring System (Meadow and Smithells, 1983).

Table 1:2 Apgar Score (Adapted from Meadow and Smithells, 1983).

Sign	**0**	**1**	**2**
Heart Rate	Absent	<100/min	>100/min
Respiratory Effort	Absent	Slow, irregular weak cry.	Regular, with cry.
Muscle Tone	Limp	Some tone in limbs.	Active movements.
Reflex Irritability	Nil	Grimace only.	Cry.
Colour	Blue-Pale	Body pink, extremities blue.	Pink all over.

Much work has gone into the developing more accurate methods of determining the length and severity of an intrauterine asphyxial episode. Currently the Apgar score is still the most widely available indicator of the asphyxiated baby when other causes such as drugs, trauma, congenital abnormalities and sepsis have been ruled out (Brann and Dykes, 1977).

The scoring system is only valid if accurately assessed, with close attention being given to the exact time at which the score is recorded. An accurate five minute score appears to correlate very well with the degree of asphyxia as assessed by excess lactate accumulation and acid base studies. A very low 10 minute score indicates severe asphyxia and a considerably less optimistic prognosis (Tooley and Phibbs, 1975).

The loss of tone in the infant is one of the first components of the Apgar score to disappear in the severely asphyxiated infant and it is the last of the components to return. For this reason it is one of the best clinical indicators of the severity of intrauterine insult (Brann and Dykes, 1977).

It is probable that the same parameters used to recognise asphyxia (distress) at birth in the infant can be adopted for the clinical evaluation of the neonatal pup. A modified Apgar Score may be appropriate. Until more definitive work is done, the degree of bradycardia acceptable in the neonatal pup is unknown. The data of Monheit *et al.* (1988) suggests that the lowest acceptable heart rate may be 104 ± 9.

1:4:4 THE CLINICAL COURSE OF THE ASPHYXIATED INFANT

The infant's course during the first few days after birth also indicates the severity of asphyxia. There will be at least transient dysfunction of organ systems that were ischaemic and hypoxic. Because the redistribution of blood flow that occurs during asphyxia tends to preserve the flow and oxygen delivery to the brain and myocardium, these are the last organs to be affected. Accordingly, after mild or moderate asphyxia there will be renal dysfunction but no evidence of myocardial or cerebral dysfunction. After asphyxia severe enough to affect the brain there is usually evidence that several other organ systems have been affected. With profound asphyxia and massive tissue breakdown creatinine will be elevated more than can be explained by renal failure (Jacobs and Phibbs, 1989).

The full-term infant, following a prolonged period of intrauterine asphyxia (defined as foetal hypoxia, hypercarbia and mixed acidosis associated with late decelerations of the foetal heart rate and/or passage of meconium) can have a varied clinical course after presenting in the delivery room with an Apgar score of five or less at one and five minutes, with or without evidence of shock. The varied clinical course following intrauterine asphyxia results from the multiplicity of organ systems affected (Brann and Dykes, 1977).

The organ systems involved in a study of 35 affected infants with low Apgar scores, in order of decreasing frequency were:- pulmonary system, cardiovascular system, central nervous system, gastrointestinal system and renal system. No infant demonstrated a problem with the coagulation system. No one system appeared to be the primary target of asphyxia (Brann and Dykes, 1977).

When the central nervous system is the principal organ affected, the following clinical course may be seen. After a difficult but successful resuscitation the infant, if not closely observed, may appear to be moderately well for the first few hours. However, if examined closely, the

infant may have some mild hypotonia and poor sucking response during the transitional period. At approximately eight to ten hours of age episodes of lip smacking and/or eye blinking may be noted with the appearance of a tonic-clonic seizure at 12 to 16 hours (Brann and Dykes, 1977).

The clinical course of the asphyxiated canine neonate has not been reported. The ability of the pup to survive after birth is dependent on its ability to nurse from the bitch, maintain body heat and compete with other pups in the litter. It is probable that most of these pups die from starvation and hypothermia and death is often attributed to this rather than the primary cause related to asphyxia.

1:4:5 THE PATHOLOGY OF ANOXIA IN THE INFANT

The pathological changes associated with anoxia in the canine neonate are poorly documented. The post mortem findings in pups which died from anoxia occurring after rupture of the foetal membranes may be negative (Drake and McCarthy, 1964). More chronic anoxia tends to produce a considerable number of haemorrhages in the lungs, parietal pleura and thymus gland. With stimulation of the foetal respiratory centre and intrauterine respiration, inhalation of amniotic fluid, epithelial cells and meconium may occur.

In contrast, the pathological changes associated with anoxia in the infant are well documented. These changes vary with the severity and duration of anoxia and to some extent with the maturity of the infant (Potter & Craig, 1976).

Anoxia of the foetus is not a specific lesion. Usually the recognition of the conditions producing anoxia must depend more on the clinical history than on positive autopsy findings. Most of the intrapartum stillbirths will show the relatively nonspecific lesions of anoxia and it is difficult to suggest any other mechanism for their relatively sudden death during labour (Morison, 1970).

Severe anoxia reduces the blood pressure and at the same time injures capillary endothelium. Although no changes are visible if death follows immediately when there is a period of survival and the blood pressure is restored to normal, blood cells and plasma escape through the injured capillary walls. At this time, haemorrhage, oedema or cellular necrosis may appear (Potter & Craig, 1976).

Oedema of the loose tissue is another general manifestation of anoxia. It is little in evidence in anoxia of short duration, such as may result from some accident during delivery but, especially in continued anoxia during intrauterine life, it may be severe.

The Respiratory System

Post asphyxial lung disease in the newborn infant with severe perinatal acidosis was investigated by Thibeault *et al*., (1984). Perinatal asphyxia, severe acidosis and hypoxia are known to cause increased pulmonary vascular resistance with right to left shunting through foetal channels and depression of surfactant metabolism. Asphyxia in the pre-term and term infants, in the absence of respiratory distress syndrome or meconium aspiration syndrome, was associated with a transient respiratory insufficiency requiring assisted ventilation. These infants had right to left shunting and may well have had temporary pulmonary hypertension. Diffuse pulmonary haemorrhage may develop and may in part be related to ischaemic myocardial dysfunction or a bleeding diathesis as well as pulmonary vascular damage from profound acidosis. Ineffective neonatal resuscitation allowing for the development of meconium aspiration syndrome and persistent respiratory acidosis contributed to the severity of the illness (Thibeault *et al*., 1984).

Infants with asphyxia complicated by meconium aspiration syndrome develop profound lung disease including pulmonary haemorrhage and persistence of the foetal circulation (Thibeault *et al*., 1984).

Hyaline membrane disease (HMD) occurs in infants who are born before term but susceptibility depends more on the stage of lung maturation at the time of delivery than on precise gestational age (Stahlman, 1975).

The presence of meconium in the amniotic fluid is regarded as abnormal. Although many infants whose amniotic fluid has contained meconium at delivery are completely normal at the time of birth one can be confident that some episode of asphyxia, however brief, has occurred, inducing the passage of meconium.

The passage of meconium, either in utero or intrapartum, always presents the opportunity for its inspiration into the tracheo - bronchial tree, especially with repeated episodes of severe asphyxia which induce forceful `terminal - type' gasping.

The infant who has suffered from chronic in utero asphyxia and malnutrition has the severest form of massive aspiration syndrome or meconium aspiration pneumonia and may die intrapartum or in the neonatal period.

Infants who aspirate meconium are usually full term or post mature and frequently have a history of foetal distress, low Apgar scores and meconium stained amniotic fluid. The respiratory symptoms usually begin shortly after birth with tachypnoea, intercostal retractions and occasionally cyanosis (Bancalari and Berlin, 1978).

The complications recorded are moderate to severe respiratory failure requiring oxygen therapy, some of which require mechanical ventilation and pneumomediastinus which may progress to pneumothorax. Some infants have clinical evidence of a large right to left shunt with progressive hypoxemia and massive pulmonary and cerebral haemorrhage (Bancalari and Berlin, 1978).

The Central Nervous System

Damage to the neuronal cells and white matter of the brain secondary to anoxia or hypoxia can be recognised only if the infant survives for at least several hours after an acute or prolonged insult. Since most infants with intrauterine anoxia have no recovery period and the majority die in the first 24 hours, brains with easily recognised alterations are not commonly found (Potter & Craig, 1976).

Apart from petechial haemorrhages and especially subependymal and intra-ventricular haemorrhages, anoxic lesions of the central nervous system are not easily recognised (Morison, 1970). In stillborn infants and in those dying within a few days of birth changes in nerve cells are, at best, equivocal. Gross bleeding into the substance of the brain is rare (Potter & Craig, 1976).

When prolonged labour or difficult delivery, especially of the shoulders, causes anoxia the thoracic and abdominal viscera are usually normal except for severe congestion, but the leptomeninges are often extremely congested and infiltrated by red blood cells. The cells are most numerous in the subarachnoid space: they may also be found in semi-localised areas or may form a uniform thin layer of blood over the cerebral hemispheres and around the cerebellum (Potter & Craig, 1976).

Hypoxic-ischaemic brain injury produced by perinatal asphyxia is the most important neurological problem of the perinatal period (Kreusser and Volpe, 1984). It is the most frequent cause of neurological morbidity in the full-term infant and, in conjunction with intraventricular haemorrhage, it results in most of the major neurological sequelae in the premature infant (Volpe, 1975).

The neuropathological features of neonatal hypoxic-ischaemic encephalopathy are brain swelling, selective neuronal necrosis, status marmoratus, parasagittal cerebral injury, periventricular leukomalacia and focal and multifocal ischaemic brain necrosis. The neuropathological features vary considerably with the gestational age of the infant (Kreusser and Volpe, 1984). The damage to the full-term infant is located peripherally in the cerebral cortex and underlying hemisphere white matter.

Necrotising Enterocolitis

Necrotising enterocolitis probably results from an ischaemic insult to the intestines. The association between prematurity, hypoxia and respiratory distress has occurred with sufficient frequency to postulate interlinked cause and effect (Randolph, 1975).

Progression to the florid syndrome of necrotising enterocolitis is often gradual and insidious. Ultimately the intestine may undergo ischaemic necrosis with perforation and peritonitis.

Pathological findings may be those of diffuse involvement of the gastrointestinal tract including either the stomach or large bowel, or the disease can be segmental in distribution and restricted to only a short segment of the small intestine (Randolph, 1975).

Renal Cortical and Medullary Necrosis

Renal cortical and medullary necrosis may occur rarely in infants with the same background of shock, sepsis, dehydration and asphyxia. Haematuria and renal insufficiency are the chief clinical manifestations. Disseminated intravascular coagulation has been postulated as a cause of some cases of cortical necrosis in the newborn (Potter & Craig, 1976).

Ischaemic Myocardial Necrosis

Infants with perinatal asphyxia may occasionally present transient cardiac dilatation and heart failure and may show evidence of myocardial ischaemia on an electrocardiogram. On occasion they also present with tricuspid insufficiency due to necrosis of the anterior papillary muscles of the tricuspid valves (Bancalari and Berlin, 1978).

The asphyxiated perinate is particularly susceptible to ischaemic myocardial necrosis (IMN). Ischaemic myocardial necrosis occurs in either ventricle with equal frequency, most commonly in the anterior papillary muscles and less commonly in the posterior papillary muscles. Severely asphyxiated perinates often have multifocal ischaemic injuries throughout the heart. They may have a 'geographical' injury pattern of multiple infarcts in the middle and outer thirds of the ventricular walls. The largest septal infarcts occur near the AV ring and may involve the AV node (Rosenberg and Donnelly, 1991).

Coagulation necrosis is the predominant ischaemic injury found in perinates. Wavy fibres, said to be an early sign of ischaemic injury are rarely identified with certainty in perinates

because myocardial fibre separation is so common. Coagulation necrosis appears in infants within 12 hours of the onset of ischaemia and may be the only finding after 24 - 30 hours. The earliest changes include swelling of myocardial cells and crenation of their nuclear membranes. Thereafter cell cytoplasm becomes increasingly granular, then glassy and increasingly eosinophilic. Eventually, nuclei become pyknotic or lysed. Aside from occasional thrombo-emboli within necrotic capillaries, thrombi are rare in coronary vessels.

Hypoxic-ischaemic encephalopathy, necrotising enterocolitis, renal cortical and medullary necrosis and ischaemic myocardial necrosis have not been reported in the dog.

Hypoxic-ischaemic encephalopathy may not have been identified in the canine neonate for a number of reasons. Rapid autolysis of neonatal nervous tissue often renders histopathology useless and as most pups die within the first few days of birth changes in nerve cells would be, at best, equivocal as reported in the infant. Further, the response of the canine brain to asphyxia may differ to that of the infant. In infants themselves, the neuropathological features of neonatal hypoxic-ischaemic encephalopathy vary considerably with the gestational age of the infant (Kreusser and Volpe, 1984).

Neuro-respiratory syndromes in the newborn of several species have many phenomena in common, although the overall clinical picture they present may be quite different. Considerable species difference in the degree of maturation of the central nervous system at birth probably accounts for variations in the clinical sequel of asphyxial damage to the central nervous system (Johnson and Rossdale, 1975).

This species variation in the central nervous system response has been documented in sheep (starvation - mismothering -exposure complex) and horses (convulsive foal syndrome).

Birth injury to the lamb foetal central nervous system is present in virtually all parturient deaths. In most post-parturient deaths the lesions occur exclusively in a large proportion of lambs classified as the `starvation - mismothering - exposure' complex. Gross pathological haemorrhages range from small to massive extravasations in single or multiple sites in and around the cranial and spinal meninges. The haemorrhages have been interpreted as a sensitivity index of presumed damage to the basic neural elements of the foetal central nervous system resulting from varying degrees of asphyxia and vascular trauma during birth. Severe injury caused death during or shortly after birth from the effect of asphyxia. Less severe injury impairs the feeding, locomotor and possibly metabolic activity of neonates and therefore dying from the `starvation - mismothering - exposure' complex (Haughey,1981).

In the horse, despite there being no obvious clinical distinction, the brains from the convulsive foals show two patterns of neuropathologic change, namely necrosis and haemorrhage (sometimes with oedema). In the necrosis group severe cortical, diencephalic and brain-stem necrosis was recorded with postnatal apnoea, one period lasting for 10 minutes. In many of the cases in the haemorrhagic type of lesion this change occurred during life because of associated brain swelling or oedema. The question arises as to whether the pathogenesis of the necrosis type is the same as that for the haemorrhagic lesion. Most of the foals suffering from haemorrhage only (with or without oedema) died earlier than those affected with ischaemia but this may be due to the time required for the ischaemic changes to become morphologically recognisable. On the other hand it may indicate that animals with haemorrhage suffered greater damage than that required to produce a gradual ischaemia. Both types of lesions are presumably related to events that occur just before, during or after birth.

The one contradictory feature is that so many of the affected animals appear to have experienced an easy birth (Palmer and Rossdale, 1975).

It is probable that a hypoxic-ischaemic encephalopathy equivalent exists in the canine neonate, but both the clinical course and histopathological features have yet to be defined.

1:5 RISK FACTORS / RISK SCORING

Human obstetrical and parturient care revolves around "risk scoring" and foetal monitoring, with the ultimate aim of maintaining maternal health and delivery of healthy neonate. The scoring techniques and foetal evaluation used in human obstetrical care (Depp, 1986) are not adaptable to the canine. However, unless some accurate form of risk assessment during pregnancy and whelping can be developed it may not be possible to reduce the current high levels of pup mortality reported.

The influence of parturition on pup mortality has been inadequately documented. An increased risk of pup death with increasing maternal age has been documented by Andersen (1957). Pup mortality is also reported to vary between breeds (Lawler 1989). Other maternal risk factors such as parity and litter size are not documented. In contrast, the pig, which is also polyviviparous, has been extensively studied and is the only other domestic species that is of any comparative value (the data on the cat are as sparse as that published on the dog). Also, the mortality problem in the pig is apparently similar to that recorded in the dog. Glastonbury (1976) reported that 74% of all preweaning mortalities occurred before the fourth day of life and Sprecher *et al*. (1974) considered that a high percentage of these deaths were associated with anoxia and hypoxia.

The risk factors identified in the pig that are associated with parturition are litter size, position in the birth order, parturition time and umbilical cord detachment (Dzuik and Harmon, 1969: Wrathall, 1971: Svendsen and Bille, 1981).

While the losses from intrapartum stillbirths associated with inefficient parturition were considerable, the same factors which caused the losses also reduced the viability of some liveborn piglets (English and Morrison, 1983). Randall (1971) has demonstrated a relationship between indices of anoxia in live piglets at birth, such as blood pH (caused by higher blood lactate levels following anoxia *in utero*), and the pCO_2 of arterial blood with a composite piglet viability score.

The most promising means for reducing stillbirths in pigs is through chemical control of parturition induction and duration. By inducing parturition at a convenient time, efforts can be concentrated on reviving some of the apparently stillborn pigs and to save `weak' pigs. As many as 25% of pigs born `apparently dead' were revived by artificial respiration. By reducing the duration of parturition anoxic pigs have an increased opportunity to survive (Sprecher *et al*., 1974).

There is a need for detailed evaluation and statistical analysis of whelping records to identify those factors that increase the risk of perinatal death in the dog. Because of the great variability between breeds in relation to adult weight and litter size a large number of breeds or breed groups would be required.

1:6 SUMMARY

While pup mortality is considered to be a significant clinical problem, the investigation of losses has been poorly documented in the veterinary literature. There is no uniformity in the definition of the neonatal period nor in the classification of pup mortality. While the concept of perinatal mortality in human medicine depends on the fact that most infants that die do so

as a direct or indirect consequence of factors that were present before or during parturition, this association has not been substantiated in the dog.

Dystocia and prolonged labour are considered to be significant causes of pup mortality (Lawler, 1989). While the incidence of dystocia in the bitch has been reported (Linde-Forsberg and Eneroth, 1998), the extent of pup losses attributed to foetal asphyxia is again poorly documented. To accurately identify losses due to foetal asphyxia whelpings must be supervised and the clinical identification, gross and histological findings of foetal distress in the pup defined. Acceptable normal whelping intervals and the accurate identification of the growth-retarded pup needs to be examined.

A substantial number of pup losses are attributed to fading puppy syndrome or undetermined causes. This finding again emphasises the necessity of accurate clinical histories. In human medicine the post mortem examination itself does not usually give more than the immediate cause of death and quite often this may not be obvious. It is necessary to correlate naked eye, histological and microbiological findings with the clinical observations on both the mother and the baby before the sequence of events leading to the death of the infant can be traced (Langley, 1971). In addition to this the physiological immaturity of the pup's thermoregulatory, cardiopulmonary, immunological, and renal systems and the risk of hypoglycaemia complicate the clinical and post mortem picture, regardless of the primary cause of illness.

To formulate management and treatment protocols to reduce pup mortality we need to know the true extent of pup mortality, not only across the spectrum for all breeds, but for individual breeds as well. The classification of pup mortality needs to be simplistic and based on the history of the whelping and clinical evaluation of the pup at birth. This classification then becomes the basis of the pathological evaluation of post mortem material.

Risk factors such as bitch breed, age, parity, whelping time and litter size must be identified. The influence, if any, of pup birth weight, sex, presentation, placental attachment and litter position must be investigated. Such an investigation would necessitate a veterinarian to be present at the whelpings. Because of the large number of litters and different breeds required this becomes a practical and economical impossibility. Alternatively, a system that requires breeders to observe and record whelping data and clinically assess and monitor the pup and bitch is the best alternative available. The accuracy of the breeder's observations may be subject to error. With education and clear guidelines this error can be minimised and a large amount of relevant clinical information then becomes available for the investigation of pup mortality.

CHAPTER 2: EPIDEMIOLOGY STUDY

2:1:1 INTRODUCTION

Depending upon the breed, methods of breeding, stock selection, husbandry and sanitation practices, puppy and kitten losses by weaning may vary from 10-30% or more of all full-term neonates. Typically, about 65% of puppy and kitten losses occur during the first week of life, and about one half of these losses are stillbirths. By contrast, post-weaning losses should not normally exceed one to 1.5%. In the neonatal period, most losses are probably due to non-infectious causes Lawler (1989).

Losses have been attributed to a variety of aetiological agents (Andersen, 1957, Potkay and Bacher, 1977, Blunden, 1986 and Lawler, 1989). However, fundamental deficiencies in these reports and the physiological immaturity of the neonatal pup, combine to confuse our understanding of the aetiology of pup mortality.

The purpose of this epidemiology study was to define the gross mortality problem that exists and to investigate the influence of maternal factors, such as breed, age, parity, litter size and whelping times on the mortality distribution. Similarly, the influence of pup factors such as, birth-weight, sex, birth presentation, placental attachment and litter position were examined. These results were then statistically analysed to identify those factors that increase the risk of perinatal death in the dog.

The study utilised detailed, breeder-supervised whelping histories and a simple form of mortality classification based on the clinical description of the pup at birth. This classification then provided the basis for the pathological study in Chapter Three.

2:1:2 MATERIALS AND METHODS

A survey (Table 2:1) of registered breeders of pure-bred dogs was conducted. There were no selection criteria established other than that required by the constraints of the survey itself. There was no distinction made between experienced and inexperienced breeders, nor any selection of specific breeds or lines within a breed. All terminology and requirements of the survey were clearly explained to the breeders in advance by myself on either a one to one basis in the clinic or at breed club lectures. Most of the breeders who returned these surveys were clients and this meant that, for the majority of records, I was able to talk to the breeder individually, both before and after the whelping. Respondents who were not clients were contacted where clarification of information was required.

I designed the survey to include the most likely risk factors that may be associated with parturition. The survey required the breeder to be present at the whelping and record the following information: the name of the dam and sire, bitch breed, age, parity and whelping date, the time that each pup was born, the pup's sex, birth presentation (anterior or posterior), whether the placenta was attached to the pup or detached and passed separately, birth weight and daily weight changes.

The breeder was asked to record if any assistance was given during the whelping by either themselves or by a veterinarian (manual, chemical or caesarean) and the reason why the bitch required help. Manual assistance means that the pup required physical assistance for delivery

because it was either too big or malpositioned. Both chemical assistance, that is oxytocin, and caesarean sections were performed by a veterinarian. When this occurred, the breeder was required to clarify with the veterinarian the reason why the bitch needed help.

If a pup was born dead or died before six weeks of age, the breeder was requested to give the age and cause of death, if known or suspected. Most of the breeder observations were self-explanatory, for example, "pup wedged and had to be pulled out by veterinarian" or "pup born gasping and could not be revived".

The survey required breeders to record the start of first and second stage labour. Many breeders reported that they had difficulty in determining exactly when these started. Consequently, in the analysis of whelping times, total whelping time and inter-pup whelping intervals were determined from the birth of the first pup (the inter-pup whelping interval is defined as the time between the delivery of two consecutive pups).

Breeders were required to identify individual pups at birth and weigh them daily until seven days of age. The method of identification used varied. For some breeds individual coat patterns were used. In breeds where this was not possible, breeders were instructed to clip the pup's coat, e.g. first male: clipped right shoulder; second male clipped: left shoulder, etc. These forms of identification maintained accuracy of records for birth order and weight gains. Pups were weighed at home by the breeder. The majority of breeders weighed their pups on kitchen scales. Most of these scales were metric, though three breeders still used imperial scales and reported weights had to be converted to metric values.

Some survey returns were incomplete in regard to birth weight, whelping times, presentation or placental attachments. The results specify the number of observations, for example, the sex ratio of pups was recorded for 2540 whelps. Incomplete survey results were acceptable if all bitch parameters and all pup mortalities and birth order were included.

2:1:3 DEFINITIONS

In previous studies there has been no consistency in the definition of what constitutes a stillbirth or the neonatal period, nor in the classification of mortality.

The following definitions were used in this study:

1. **Stillborn**. A stillborn pup is defined as any pup born dead and included mummified pups and fully developed pups that had died prior to birth (autolysis present indicating death prior to the commencement of whelping).

2. **Neonatal Mortality**. Neonatal Mortality applies to those liveborn pups dying within the first six weeks after birth. A pup was judged liveborn if either respiratory effort or cardiac activity could be detected.

3. **Early Neonatal Mortality**. Early Neonatal Mortality applies to those liveborn pups dying in the first seven days after birth.

4. **Late Neonatal Mortality**. Late Neonatal Mortality applies to those liveborn pups dying between eight and 42 days of age.

5. **Total Mortality**. Total Mortality is defined as the combined loss due to Stillbirths and Neonatal Mortality.

6. **Perinatal Mortality**. Perinatal Mortality is defined as the combined loss due to Stillbirths and Early Neonatal Deaths.

Classification of Mortality

I have classified pup mortality relative to the whelping history and clinical observation provided by the breeder or attending veterinarian. Post mortem and histopathological findings were not used for this classification except for those pups found to have gross congenital defects present and miscellaneous causes of mortality that could be confirmed, for example herpes virus infection and trauma. There are three gross classifications. These are the abnormal pup, foetal asphyxia and the live-born normal pup which subsequently dies.

1. **The Abnormal Pup**: A pup was classified as abnormal if it was mummified, had died prior to birth, was premature, small for date or had gross congenital defects.

Foetal mummification was identified by the birth of dry foetal mass surrounded by shrivelled, dried foetal membranes (Arthur, 1973).

Pups were classified as having died prior to birth if they were fully developed but had evidence of autolysis indicating that they had died within days before the commencement of labour.

The identification of prematurity is complicated by the fact that pregnancy is 63 days +/- 1 from the day of ovulation. Therefore bitches mated as late as five days after ovulation may whelp at 58 days post-mating (Johnston, 1988). Some bitches habitually whelp early and produce viable whelps after gestation periods of little over 56 days (Jones and Joshua, 1982). I have defined prematurity as live pups whelped at less than 56 days post-mating.

As in the previous reports, there were no weight ranges specified as to what constituted a small for date pup and their identification left to the breeder. Birth weights were investigated later in the thesis and small for date, or more correctly, growth retarded pups identified as being below one standard deviation lower than the mean for their breed. Gruenwald (1969) used this form of scoring system for infant birth weights. Conversely pups with birth weights greater than one standard deviation above the mean, were considered large pups.

All pups with gross congenital defects which either caused the death or euthanasia of a pup, including those identified on post mortem, are included in this category.

2. **Foetal Asphyxia**: The death of an apparently normal full-term pup that was either stillborn or born in a clinically distressed condition and subsequently died was attributed to foetal asphyxia. It was assumed that these pups were subjected to excessive hypoxia during the birth process. At birth, the presence of asphyxia (or neonatal distress) was clinically recognised by the observation of gasping respiration, slow heart rate, hypotonia and/or cyanotic or white tongue and/or pads. There were a number of occasions where breeders recorded no respiration following birth but were able to detect a slow heart beat. A pup showing any evidence of viability at birth, regardless of how long it lived, was judged

liveborn. This category included unexplained stillborn and live distressed pups (that is, deaths with no obstetrical explanation) and deaths associated with dystocia.

3. **The Live Normal Pup**: The live normal pup was one considered both physically and clinically normal following birth and which subsequently died. This group was further categorised according to historical evidence defining the clinical course of illness prior to death.

- Fading Puppy Syndrome (FPS). The "Fading Puppy" in this study was defined as a pup considered at birth to be normal, but failed to thrive.

- Mismothering/Maternal Ill-health. Pup mortality due to mismothering/maternal ill-health was only diagnosed when the breeder could present clear evidence that this was the primary problem.

- Miscellaneous. This included accidents, trauma, infections and other miscellaneous causes of mortality.

Dystocia

Dystocia was classified as either maternal or foetal in origin (Arthur, 1973).

Maternal Primary Uterine Inertia: The clinical history indicated that the bitch failed to initiate stage two labour. The usual history indicated that the bitch was either due or overdue to last mating, and clinically was restless, nesting and sometimes a green vulval discharge was present. However, the anticipated whelping date was usually based on mating dates rather than ovulation date and it is probable that some of these bitches were simply not at full term.

Maternal Secondary Uterine inertia: This was diagnosed when the bitch was in stage two labour and failed to progress. The history indicated bitch exhaustion and prolonged inter-pup intervals associated with ineffective or no contractions.

Foetal Disproportion: This was defined as the foetus being too large for the maternal birth canal. There was no distinction between foetal oversize and small maternal birth canal. The history indicated simply that the pup was too big to fit and either had to be assisted manually or required caesarean section for delivery.

Foetal Presentation: This was defined as abnormalities in the presentation of the foetus at the pelvic inlet.

Undoubtedly there will be errors in the classification assigned to a pup. The validity of this classification of both foetal distress and fading puppy syndrome is investigated in the pathology study (Chapter 3).

2:1:4 STATISTICAL ANALYSIS

The statistical analyses were performed with the assistance of the Biometrical Consulting Service of the University of Sydney. The data were analysed by either the P^2 test or logistic regression.

Statistical Method

The P^2 test was used to analyse the significance of the influence of Seasons on pup mortality and the possible relationship of some of the causes of mortality.

Logistic regression was used to analyse two data sets, one that records the characteristics of the litter, and the other that contains information on each individual pup.

1. **Litter Data**

An analysis was undertaken that included all litters (500), regardless of breed or breed group in an attempt to gain important predictors of mortality for dogs in general. In addition, analyses were undertaken for each breed group (Toy, Small, Medium, Large and Giant Breeds) and the two most commonly surveyed breeds (Shetland Sheepdog and Chihuahua).

The analysis method in all cases for the litter data was binary logistic regression (Dobson, 1990). This models the proportion of pups in a litter that died. The data is binary since for each pup in a litter there are two outcomes, the pup either died or it did not. The computer analysis software used for the litter analyses was Genstat 5, Release 4.1.

The stepwise regression facility in Genstat was used to build a model. Stepwise regression finds the combination of explanatory variables that best explains the observed variation in the data.

It was assumed that breed and breed group were correlated with all other X variables (predictor or explanatory variables). Therefore they should not be included in the same model. The deviance test (Appendix 1) indicated that breedgroup.breed could be dropped from the model (since the F pr. value for the change was 0.2000 > 0.05), that is, there was no additional variability between breeds that had not been taken into account by the different breed groupings.

After conducting a stepwise regression (Appendix 2), it was found that the most important predictors for pup mortality in litters were average inter-pup interval ($\$_1 avgipi$), parity ($\$_2 parity$), average birth weight ($\$_3 avgbirth$), litter size ($\$_4 littersize$) and type of assistance required: caesarean section ($\$_5 assistcode_7$), chemical ($\$_6 assistcode_c$)and manual ($\$_7 assistcode_m$). $= regression coefficient for each variable.

The optimal model was:

$$\text{logit}\left\{\frac{\text{Total mortality}}{\text{litter size}}\right\} = \$E + \$_1 avgipi + \$_2 parity + \$_3 avgbirth + \$_4 littersize + \$_5 assistcode_7 + \$_6 assistcode_c + \$_7 assistcode_m$$

The optimum models and output of the litter data for the Toy, Small, Medium, Large and Giant Breed Groups and the Chihuahua and Shetland Sheepdog breeds are presented in Appendices 3 through to 9. The model and output for the Giant Breed Group and the Great Dane (Appendix 7) are the same.

2. **Pup Data**

As for the litter data, the analysis undertaken was all pups regardless of breed or breed group. The pup data set was culled so there were no missing variables (Appendix 10). The statistical analysis was done on 1566 pups. In addition, analyses were undertaken for each breed group (Toy, Small, Medium, Large and Giant Breeds) and the three most commonly sampled pups (German Shepherd, Great Dane and Shetland Sheepdog).

Using nominal logistic regression the response variable is the type or cause of mortality (Classification of Mortality). These are 0 : pup survived; 1 : abnormal pup (died); 2 : foetal asphyxia (died); 3 : fading puppy syndrome (died); and 4 : miscellaneous (died) and a number of biological explanatory variables were examined to see if they were significant predictors of the type of mortality.

This data set has a nominal categorial response (Agresti, 1996). The analysis was conducted using Minitab Release 13.1. The method of forward selection was used to determine the optimal model for the data subset or group of interest. This method builds a model from scratch. It adds the variable that explains the most variation in mortality first, then the variable that explains the second greatest amount of variation next (given that the first variable is included in the model) and so on.

The list of explanatory variables included in these models are bitch age, parity, litter size, birth order (litter position), birth weight, pup sex, presentation, placental attachment, inter-pup whelping interval and assistance (none, caesarean, manual, chemical).

The optimal model for all pup data was:

$$\log\left\{\frac{B_{ik}}{B_{io}}\right\} = \$_{ok} + \$_{1k}interpup\ interval_{i1} + \$_{2k}birthweight_{i2} + \$_{3k}parity_{i3} + \$_{4k}presentation + \$_{5k}littersize_{i5}$$

$$* \left\{\frac{B_{ik}}{B_{io}}\right\} = \frac{\text{Odds of outcome category (pups which died)}}{\text{The reference category (pups which lived)}}$$

* $ = regression coefficient
* k = outcome category

The optimum models and output of the pup data for the Toy, Small, Medium, Large and Giant Breed Groups and the German Shepherd, Great Dane and Shetland Sheepdog breeds are in Appendices 11 through to 17. The model and output for the Giant Breed Group and the Great Dane (Appendix 15) are the same.

2.2 RESULTS

2.2.1 OVERVIEW OF REPRODUCTIVE PERFORMANCE

Five hundred surveys from 125 breeders were returned, which included 44 breeds of dogs, and 2574 pups. The data were collected over a seven year period from March 1991 to January 1998. The summarised data are presented in Table 2:2

Table 2:2 Overview of mortality.

Number of Litters	**500**	
Number of pups	**2574**	
Stillborn	180	7.0%
Early neonatal mortality	296	11.5%
Perinatal mortality	476	18.5%
Late neonatal mortality	43	1.7%
Total neonatal mortality	399	13.2%
Total mortality	519	20.2%
Euthanasia for show reasons*	44	1.7%
Early neonatal mortality*	252	9.8%
Perinatal mortality*	432	16.8%
Neonatal mortality*	295	11.6%
Total mortality*	475	18.5%

* Forty-four (44) early neonatal deaths were due to euthanasia of otherwise healthy pups for show reasons. These losses are noted within individual breeds but otherwise removed from further statistical consideration. These data have been included in early neonatal, perinatal and total mortality on the assumption that these pups would have reached 6 weeks of age.

Twenty-five Rhodesian Ridgebacks were euthanased for lack of ridges (19), double or triple crowns (2) and black coat colour (4). The Dorsal Sinus, which occurs as an inherited defect in this breed, was considered to be a congenital abnormality for the purpose of this study. Four Great Danes were euthanased for blue coat colour and 15 Boxers were euthanased for white coat colour.

Total mortality was 18.5% and 90.9% of these losses occurred in the perinatal period. Early neonatal mortality of 9.8% exceeded stillbirth losses of 7.0%.

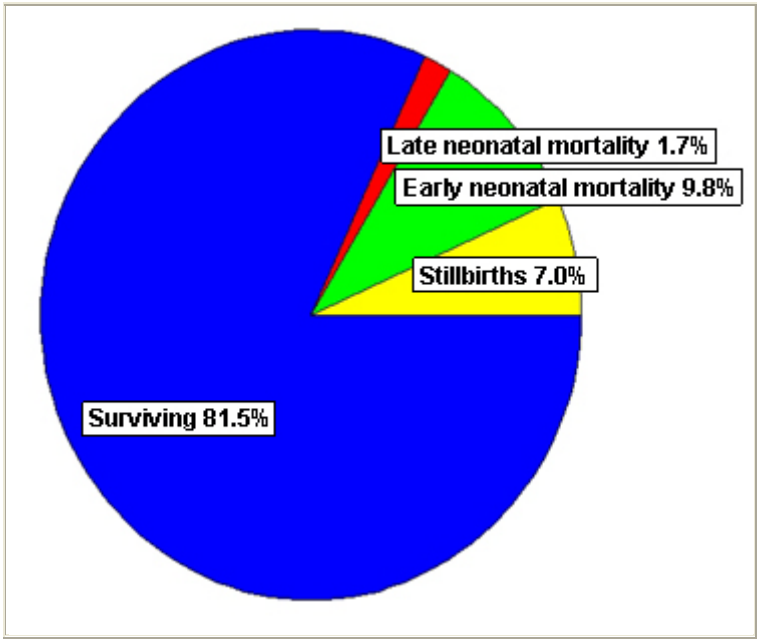

Figure 2:1 Mortality distribution.

Late neonatal losses were 1.7% of all pups born and 9.1% of total mortality. Of these, 14 pups were clinically ill during the early neonatal period, and only survived this period because of breeder assistance. Removing them from the data set reduces the incidence of illness in the late neonatal period to 29 pups (1.1% of all pups born and 6.1% of the total mortality).

The age distribution of perinatal mortality is graphed in Figure 2:2. The majority of pups (61.5%) were either born dead or died in the first 24 hours after birth. The losses on Day 1 included euthanased pups with congenital defects.

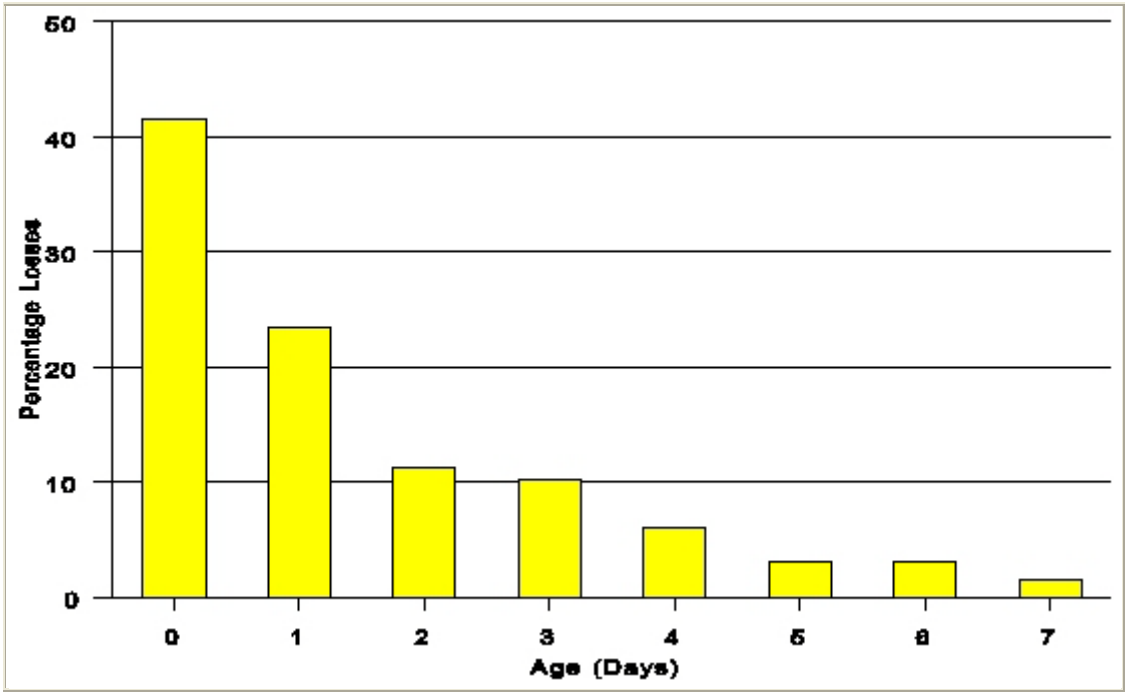

Figure 2:2 Age distribution of perinatal losses**.**

PUP PARAMETERS

The sex of pups was recorded for 2540 whelps. The Male : Female ratio was 1318:1222 (51.9%:48.1%).

The birth presentation of the pups was observed for 1654 whelps. Pups born by caesarean section were not included. The Anterior:Posterior presentation ratio was 1163:491 (70.3%:29.7%).

Breeders recorded whether the pup was born with the placenta attached to the pup or passed separately from the pup for 1912 whelps. Pups born by caesarean section were not included. The Placenta attached : Placenta detached ratio was 1479:415 (78.3%:21.7%).

WHELPING HISTORY

A total of 318 whelpings (64.4%) required no assistance for the delivery of the litter. Conversely, this meant that dystocia occurred in 35.6% of the whelpings (plus six elective caesarean sections). The breeder or veterinarian manually assisted the delivery of a pup(s) because of foetal disproportion or presentation in 40 whelpings (8.0%). In a further 44 whelpings (8.3%) veterinary intervention in the form of chemical assistance (oxytocin with or without calcium) was required for the delivery of a pup(s) within a litter.

There were 98 litters delivered by caesarean section (19.5%), six of which were elective. Of these 92 non-elective caesareans 62 (67.3%) were for the delivery of the entire litter while the remaining 30 (32.6%) were performed because of problems that developed during the whelping.

In 243 (48.6%) litters no mortalities was recorded and a further 74 (14.8%) had stillbirths only. In 33 litters (6.6%) the entire litter died. It should be noted, however, that 16 of these were singleton pup litters.

MATERNAL MORTALITY AND MORBIDITY

With the exception of one bitch, no maternal mortality was recorded. This was a Saint Bernard which was diagnosed during pregnancy as having an osteosarcoma of the humerus. An elective caesarean section was performed and the bitch euthanased for humane reasons immediately following the caesarean section.

Maternal illness or behavioural problems occurred in 29 bitches, that was 5.8% of whelpings (Table 2:3). Eclampsia was the most frequent problem encountered and was seen only in the toy breeds.

In those bitches requiring caesarean section maternal illness or behavioural problems occurred in 9.2% of whelpings. Mismothering or poor acceptance of pups and agalactia were seen more frequently in bitches requiring caesarean section than those delivering naturally.

Table 2:3 Maternal morbidity

Clinical condition	Total number of bitches affected	Number of bitches affected following caesarian section
Mastitis	5	0
Agalactia	4	3
Eclampsia	6	0
Maternal aggression	3	0
Mismothering/ Poor Acceptance	5	4
Uterine rupture	1	1
Uterine prolapse	1	0
Hypoglycaemia	1	1
Uterine cramping *	3	1

* Uterine cramping is considered to cause the bitch to be very restless after whelping and she fails to nurse the pups properly. The condition is relieved by the use of Buscopan Compositum®.(Hyoscine-N-butyl bromide 4mg/ml, dipyrone 500mg/ml)

WHELPING PERFORMANCE RELATIVE TO SEASON

The whelping performance relative to season is presented in Table 2:4. The greatest number of litters were whelped during Autumn (March to May): 143 (28.5%), followed by Summer (December to February): 121 (24.2%), Winter (June to August): 119 (23.8%) and Spring (September to November): 117 (23.4%). Bitches were generally not bred from every season, so whelping distribution reflects not only the cyclic nature of the bitch but also breeder selection.

Table 2:4 Whelping performance and mortality relative to season

Season	Survived	Stillborn	Early Neonatal Mortality	Late Neonatal Mortality	Total Pups
Summer	517*	42	57	11	627
	510.68	43.31	61.55	10.46	
Autumn	587	62	70	6	725
	591.44	50.16	71.29	12.12	
Winter	468	40	77	8	593
	483.76	41.02	58.31	9.91	
Spring	527	36	48	18	629
	513.13	43.51	61.85	10.51	
Total	2099	180	252	43	2574

* Expected counts are printed below observed counts.

Chi-Sq = 0.078 + 0.123 + 0.337 + 0.028
0.033 + 2.797 + 0.023 + 3.087
0.513 + 0.026 + 5.991 + 0.368
0.375 + 1.666 + 2.669 + 5.334 = 23.450
DF = 9, P-Value = 0.005

The P^2analysis indicated there was a seasonal influence on when pups died. Close inspection of the breakdown of the Chi-square components reveals that early neonatal deaths in Winter was the most important contributor, followed closely by late neonatal deaths in Spring. These two alone represent just less than half of the significant relationship indicated by the P-value.

2:2:2 MORTALITY

18.5% of all pups were either born dead or died in the first six weeks. Pup losses relative to the classification of mortality are summarised in Table 2:5.

Table 2:5 Pup losses relative to the classification of mortality

Cause of death	**Losses (% of total number of pups born**
Mummified	13 (0.5%)
Death prior to birth	18 (0.7%)
Small for date	23 (0.9%)
Gross congenital defects	71 (2.8%)
Premature	0 (0%)
Total abnormal pups	**125 (4.9%)**
Dead normal	134 (5.2%)
Live distressed	68 (2.6%)
Total loss due to foetal asphyxia	**202 (7.9%)**
Fading puppy syndrome	88 (3.4%)
Mismothering/mismanagement	20 (0.8%)
Miscellaneous	40 (1.6%)
Total loss of live-born normal pups	**148 (4.9%)**

* Premature defined as pups born before 56 days pregnancy.

THE ABNORMAL PUP

The death of 4.9% of all pups born and 26.3% of the total mortality was attributed to abnormal pups.

Mummified Pups

Mummified pups (13) accounted for 0.5% of all pups born and 2.7% of all losses.

Mummified pups occurred in 12 litters or 2.6% of all litters whelped. The number of pups in these litters was 66 with a total mortality of 50%. Losses were attributed to mummification (13), small for date (6), foetal asphyxia (10) and fading pups (3)). Four of the 12 litters recorded no other losses other than mummification.

Compared to the occurrence of small for date pups in all litters (23/2574), the occurrence of small for date pups in litters containing mummified foetuses (6/66) was significantly higher ($P^2 = 51.4$, d.f. = 1, $p < 0.001$).

Similarly, compared to the occurrence of pups which died as a consequence of foetal asphyxia in all litters (202/2574), the occurrence of death due to foetal asphyxia in litters containing mummified foetuses (10/66) was also significantly higher ($P^2 = 5$, d.f.= 1, $p < 0.05$).

Death Prior to Birth

Pups which were born fully developed but exhibited varying degrees of autolysis (death prior to birth), accounted for 0.7% (18) of all pups born and 3.8% of total losses.

Fourteen litters (2.8% of all litters whelped) had pups which had died prior to birth. The number of pups in these litters was 75 with a total mortality of 56%. Losses were attributed to death prior to birth (18), fading pups (16) and small for date pups (1). Seven of the 14 litters had no other losses other than death prior to birth.

Compared to the occurrence of fading pups in all litters (88/2574), the occurrence of fading pups in litters containing foetuses which had died prior to birth (16/75) was significantly higher ($P^2 = 75.08$, d.f. = 1, $p < 0.001$).

Gross Congenital Defects

A total of 71 pups with grossly recognised congenital defects occurred in 57 litters, that is, 11.4% of litters (Appendix 18). This represents 2.8% of all pups born and 15.0% of all losses. The majority of these pups (92.9%) were born alive and either died or were euthanased in the first 48 hours after birth. Those surviving past two days of age did so only because the defect was not recognised earlier by the breeder. Sixty-two percent (62.0%) of congenital abnormalities (44) occurred as a single affected pup within a normal litter.

The most commonly recorded congenital defect was a cleft palate which accounted for 38.0% of identified gross abnormalities. Dorsal sinuses and omphalocoels were the second most commonly identified abnormalities. The dorsal sinus is a distinct entity occurring in the Rhodesian Ridgeback. The omphalocoel occurred sporadically in different breeds and in one litter of Labradors two of eight pups were affected.

The total mortality for litters where congenital defects occurred was 28.0% with 67.6% of the losses due to congenital defects.

Twelve litters had multiple pups with congenital abnormalities. Three litters had more than one pup affected by cleft palate and three other litters had other abnormalities occurring when a cleft palate was recorded in the litter.

The Small for Date Pup

The identification of a pup being small for date was made by the breeder. Twenty-three small for date pups were either born dead (10) or died in the neonatal period (13). This accounted

for 0.9% of all pups born and 4.8% of the total mortality. Small for date pups occurred in 21 litters or 4.2% of all litters whelped.

The number of pups in these litters was 125 with a total mortality of 36%. Losses were attributed to small for date pups (23), foetal asphyxia (9), fading puppy syndrome (5), congenital defects (3) and mummification (3).

Compared to the occurrence of foetal asphyxia in all litters (202/2574), the occurrence of foetal asphyxia in litters containing small for date pups (9/125) was not significant ($P^2 = 2.9$, d.f. = 1).

Similarly, compared to the occurrence of fading pups in all litters (88/2574), the occurrence of death due to fading pups in litters containing small for date pups (5/125) was also not significant ($P^2=0.7$, d.f.=1).

However the occurrence of mummified pups in litters with small for date pups was highly significant. The occurrence of mummified pups in all litters was 13/2574 and the occurrence of mummified pups in litters with small for date pups was 3/125 ($P^2=34.6$, d.f.= 1, $p < 0.001$).

FOETAL ASPHYXIA

Pups were classified as having died as a result of foetal asphyxia if the breeder considered them to be normal full-term pups at birth and were either stillborn or born in a clinically distressed condition and subsequently died. The death of 202 apparently normal pups was attributed to foetal asphyxia. This accounted for 42.5% of the total mortality and 7.9% of all pups born. The majority of these pups (82.2%) were either stillborn or died in the first 24 hours after birth (Figure 2:3). All but three pups died in the first week, and these three required supplementary feeding and care to reach this age.

Figure 2:3 Perinatal mortality distribution of foetal asphyxia.

Based on the breeder's observation and assessment, 86 or 42.6% of those pups considered to have died as a consequence of foetal asphyxia were compromised during an apparently normal whelping with no evidence of dystocia (that is, deaths with no obstetrical explanation).

The death of 116 pups was attributed to clinically recognised dystocia (4.5% of all pups born and 24.4% of total mortality). The predominant cause of dystocia resulting in pup mortality was maternal secondary uterine inertia. This accounted for 52 deaths (2.0% of all pups born) and 44.8% of losses associated with dystocia.

Foetal disproportion was the second most common cause of pup mortality due to dystocia. This accounted for 32 deaths (1.2% of all pups born) and 27.5% of losses due to dystocia.

Maternal primary uterine inertia accounted for the death of 22 pups (0.9% of all pups born) and 18.9% of mortality associated with dystocia.

Foetal presentation accounted for the death of ten pups, or 0.4% of all pups born and 2.1% of losses associated with dystocia.

BORN LIVE NORMAL

One hundred and forty-eight pups (148) were considered by the breeder to be normal at birth and died during the neonatal period. This was 31.2% of all pup deaths and 5.7% of all pups born. Table 2:6 summarises the causes of these losses.

Table 2:6 Causes of mortality of the live normal pup.

Cause of Death	Losses (% of total number of pups born)
Fading puppy syndrome	88 (3.4%)
Mismothering/Maternal ill-health	20 (0.8%)
Miscellaneous	40 (1.6%)

Fading Puppy Syndrome

3.4% (88) of all pups born died from what appeared clinically to be fading puppy syndrome. This accounts for 18.5% of all losses. Pups that were originally considered to be fading pups but were found on post mortem to have gross congenital defects present have been classified as abnormal pups.

Forty-six litters (9.2%) consisting of 241 pups recorded losses from fading pups. The total mortality in these litters was 48.5% with 75.3% due to fading pups.

Fifteen single pups "faded" in a otherwise normal litter. A further 73 pups were lost from 18 litters. Within these litters total mortality was 55.1%. In seven litters all pups died and included two bitches which lost all pups in two consecutive litters.

Figure 2:4 Perinatal mortality distribution of fading puppy syndrome

90.9% of deaths attributed to fading puppy syndrome occurred in the early neonatal period, with losses peaking at three days of age (Figure 2:4).

Maternal Illness / Mismanagement

In five litters with the loss of 20 pups maternal ill-health and/or mismanagement was considered the major cause of pup losses. Two of these litters were delivered by elective caesarean sections. This represents 4.2% of all losses. All of these pups died in the neonatal period with 55% of deaths occurred at two days of age.

Miscellaneous

Thirty-six (36) pups were classified as miscellaneous deaths (Table 2:7). Eight of these (22.2%) died in the first week and their death attributed to trauma. They were either laid on, trodden on, or bitten by the bitch.

Table 2:7 Miscellaneous losses of pups categorised by confirmed or presumed causes of death.

Cause	Number of pups	Confirmed*	Unconfirmed**
Trauma	16	12	4
Herpes Virus	5	2	3
Drowned	1	0	1
Dwarfism	2	2	0
Swimmers	2	2	0
Viral Gastroenteritis	9	0	9
Pneumonia	4	2	2

* Confirmed: Pups were available for post mortem and if necessary histopathology.
** Unconfirmed: Either not available for post mortem or histology not performed.

2:2:3 MATERNAL FACTORS

The influence of maternal factors on pup mortality was investigated. These factors were bitch breed group, breed, age, parity, litter size and whelping times.

BREED GROUPS

Forty-four breeds were surveyed and varied from the Chihuahua through to the Great Dane. The number of litters in each breed varied from one to 64. Since there are no selection criteria other than that required by the survey, the breeds surveyed reflected breeder cooperation only.

Breeds were categorised into breed groups according to their adult body weight.

TOY < 5 Kg
SMALL 5 - 9 Kg
MEDIUM 10 - 19 Kg
LARGE 20 - 44 Kg
GIANT > 45 Kg

Table 2:8 summarises the number of litters, pups, average litter size and birth weights for the various breed groups. As expected, average litter size and birth weight increased with increasing adult weight.

Table 2:8 Average litter sizes and birth weights of the different breed groups

Breed Group	Number of breeds	Number of Litters	Number of pups	Average litter size	Average birth weight (grams)
Toy	10	183	664	3.6	162
Small	8	85	340	4.0	210
Medium	10	56	339	6.1	324
Large	14	152	1030	6.8	431
Giant	2	24	201	8.4	541

Comparative Mortality in Breed Groups

The comparative mortality of breed groups is summarised in Table 2:9.

The large breeds recorded the most efficient whelping performance results. They had the lowest total and early neonatal mortality (15.0% and 7.7% respectively). The stillborn loss (6.1%) was the second lowest of the breed groups.

The giant breeds recorded the most inefficient whelping performance results. They had the highest total mortality, stillborn and perinatal losses of all the breed groups (24.4%, 12.9% and 22.4% respectively). They also had the highest caesarean section rate (29.4%).

Table 2:9 Comparative mortality in the breed groups

Mortality*	**Average**	**Toy**	**Small**	**Medium**	**Large**	**Giant**
Number of pups	2574	664	340	339	1030	201
Total Mortality	18.5%	20.5%	21.5%	18.3%	15.0%	24.4%
Stillborn	7.0%	6.3%	8.5%	5.9%	6.1%	12.9%
Early Neonatal Mortality	9.8%	12.5%	12.6%	8.3%	7.7%	9.5%
Perinatal Mortality	16.8%	18.8%	21.2%	14.2%	13.8%	22.4%
Late Neonatal Mortality	1.7%	1.7%	0.3%	4.1%	1.2%	2.0%
Abnormal Pups	4.9%	3.8%	7.1%	4.1%	5.2%	4.0%
Foetal Asphyxia	7.9%	8.0%	9.1%	6.5%	6.7%	13.4%
Live Normal Pups	5.7%	8.7%	5.3%	7.7%	3.1%	7.0%
Caesarean Section	19.5%	24.6%	11.8%	17.9%	17.1%	29.2%

* Mortality relative to total number of pups born in each breed group and excluding elective euthanasia.

The principal cause of mortality varied between breed groups. For the giant, large and small breeds foetal asphyxia was the primary cause of pup mortality. In the toy and medium breeds the primary cause was the death of apparently normal pups after birth.

In the breed group statistical analysis (Appendix 1) the odds of mortality was not significantly different for any of the breed groups when compared to the giant breed.

There were two models developed for the statistical analysis of each of the breed groups. The first model was based on the litter data and the second on the pup data.

The Toy Breed Group

The optimum models and output of the litter data and pup data for the toy breed group are presented in Appendices 3 and 11 respectively.

Average birth weight, total whelping time, parity and whelpings which required assistance were significant predictors of mortality. For each ten gram increase in the average birth weight the odds of a pup dying decreased by 0.99x or 1% ($p = 0.03$). For each ten minute increase in total whelping time the odds of a pup dying increased by 1.004x ($p = 0.03$) and for each increase in parity the odds of a pup dying increased by 1.28x ($p = 0.04$). Dystocia occurring in a whelping and requiring either manual or caesarean delivery increased the odds of mortality by 4.55x and 3.73x respectively ($p = 0.03$ and $p = 0.05$).

Both birth weight and placental attachment were significant predictors of mortality due to the birth of abnormal pups in the toy breeds. For each ten gram increase in birth weight the odds of mortality decreased by 0.96x ($p < 0.001$). This is a result of the abnormality rather than a cause.

Litter size, placental attachment, inter-pup interval and presentation were significant predictors of mortality due to foetal asphyxia in the toy breeds. The odds of mortality decreased by 0.34x ($p = 0.01$) when the pup is born with the placenta attached. The odds of mortality increased by 3.90x ($p = 0.001$) when the pup's birth presentation was posterior rather than anterior. As litter size increased by one pup the odds of mortality decreased by 0.49x ($p < 0.001$). For each 10 minute increase in the inter-pup interval the odds of mortality due to foetal asphyxia increased by 1.01x ($p < 0.001$).

Litter size and birth weight were significant predictors of mortality due to fading puppy syndrome in the toy breeds. For each ten gram increase in average birth weight the odds of mortality due to fading puppy syndrome decreased by 0.98x ($p = 0.004$). For each increase in litter size the odds increased by 3.4x ($p < 0.001$).

Birth weight was a significant predictor of losses due to miscellaneous deaths. For each ten gram increase in average birth weight the odds of losses due to miscellaneous causes decreased by 0.96x ($p = 0.01$).

The Small Breed Group

The Shetland Sheepdog was the principal breed surveyed in the small breed group. Sixty-four of the 85 litters were Shetland Sheepdogs. For this reason the model and the output for the analysis of the litter data for the small breed group (Appendix 4) and the Shetland Sheepdog (Appendix 9) are the same.

The average inter-pup interval and birth weight were significant predictors of pup mortality. The odds of a pup dying increased by 1.02x ($p = 0.003$) for an increased average inter-pup interval of ten minutes. The odds of a pup dying increased by 0.99x ($p = 0.02$) for every ten gram decrease in average birth weight.

The model and the output for the analysis of the pup data for the small breed is presented in Appendix 12. Birth weight was a significant predictor of losses due to fading puppy syndrome. For every ten gram increase in average birth weight the odds of mortality due to fading puppy syndrome decreased by 0.96x ($p = 0.001$).

The Medium Breed Group

The model and the output for the analysis of the litter data in the medium breed group is presented in Appendix 5. There are no significant predictor variables.

The model and the output for the analysis of the pup data in the medium breed group is presented in Appendix 13. Maternal age was a significant predictor of mortality due to fading puppy syndrome with the odds increased by 5.83x ($p = 0.02$) for each increase in age by one year. The inter-pup interval was a significant predictor of mortality due to foetal asphyxia. For each ten minute increase in the inter-pup interval the odds of mortality decreased by 0.95x ($p = 0.03$).

The Large Breed Group

The model and the output for the analysis of the litter data in the large breed group is presented in Appendix 6. There were no significant predictor variables.

The model and the output for the analysis of the pup data in the large breed group is presented in Appendix 14. Average birth weight was a significant predictor of mortality due to the birth of abnormal pups with the odds increased by 0.99x ($p < 0.001$) for every ten gram decrease in average birth weight. Birth order was a significant predictor of mortality due to foetal asphyxia. For every increase in birth order the odds of mortality due to foetal asphyxia increased by 9.99x ($p = 0.003$).

The Giant Breed Group

All the litter and pup data analyses in the giant breed group were Great Danes. The model and the output for the analysis of both the litter and pup data in giant breed group are presented in Appendices 7 and 15 respectively.

Total whelping time was a significant predictor of mortality. The odds of a pup dying increased by 1.003x ($p = 0.04$) for each ten minute increase in the total whelping time.

Birth order and inter-pup whelping intervals were significant predictors of mortality due to foetal asphyxia. For every increase in the birth order the odds of mortality increased by 142.53x ($p = 0.01$). For every ten minute increase in inter-pup whelping intervals the odds of mortality increased by 1.01x ($p = 0.01$).

Birth weight and litter size were significant predictors of mortality due to the birth of abnormal pups. For each ten gram increase in the birth weight the odds of mortality decreased by 0.96x ($p = 0.03$). For every 1 pup increase in the litter size, the odds decreased by 0.40x ($p = 0.02$).s

BREEDS

The results of the whelping performance and mortality distribution for individual breeds are summarised in Appendix 19.

There is no obvious correlation between the overall mortality results of a breed group (when the definition of the group is based on adult body weight) and individual breeds within that group. For example, the mortality results for one toy breed can not be used to predict the outcome in another toy breed.

There were 15 breeds in the survey represented by more than ten litters. The mortality, caesarean section rate and principal causes of pup death for each of these breeds are summarised in Table 2:10. These results demonstrated that there was a marked variation in the pattern of mortality encountered, as well as the principal cause of loss, between the breeds of dogs surveyed. For example, in the Shetland Sheepdog and the German Shepherd it was the birth of abnormal pups that was the principal cause of mortality. In the Miniature Dachshund and Australian Silky Terrier early neonatal mortality noticeably exceeded stillbirths and fading puppy syndrome was the principal cause of mortality. In the Pekingese

and Chihuahua foetal distress associated with foetal disproportion was the primary cause of loss.

The litter data models and output of the statistical analysis for the two most commonly surveyed breeds (Chihuahua and Shetland Sheepdog) are presented in Appendices 8 and 9 respectively.

The two significant predictors of mortality in the Chihuahua were the average inter-pup interval and litter size. The odds of a pup dying increased by 1.01x ($p = 0.03$) for an increase in the average inter-pup interval by 10 minutes. The odds of a pup dying increased by 0.53x ($p = 0.02$) for every one pup increase in litter size. The average inter-pup interval and birth weight were the significant predictors of pup mortality in the Shetland Sheepdog. The odds of a pup dying increased by 1.02x ($p = 0.003$) for an increased average inter-pup interval of ten minutes. The odds of a pup dying increased by 0.99x ($p = 0.02$) for every ten gram decrease in average birth weight.

The pup data models and output for the statistical analysis of the three most commonly sampled pups (Great Dane, German Shepherd and Shetland Sheepdog) are presented in Appendices 15, 16 and 17 respectively.

In the Great Dane birth order and inter-pup whelping intervals were significant predictors of mortality due to foetal asphyxia. For every increase in the birth order the odds of mortality increased by 142.5x ($p = 0.01$). For every ten minute increase in inter-pup whelping intervals the odds of mortality increased by 1.01x ($p = 0.01$). Birth weight and litter size were significant predictors of mortality due to the birth of abnormal pups. For each ten gram increase in the birth weight the odds of mortality decreased by 0.96x ($p = 0.03$). For every one pup increase in the litter size, the odds decreased by 0.40x ($p = 0.02$).

In the German Shepherd the odds of mortality due to foetal asphyxia increased by 51.01x ($p = 0.01$) for every increase in birth order by one pup. The average birth weight and litter position were significant predictors of mortality due to the birth of abnormal pups. The odds of mortality due to the birth of abnormal pups increased by 9.59x ($p = 0.04$) for each increase in birth order and every ten gram increase in average birth weight the odds decreased by 0.99x ($p < 0.001$).

In the Shetland Sheepdog birth weight was the only significant predictor of mortality. For every ten gram increase in average birth weight the odds of mortality due to the birth of abnormal pups decreased by 0.97x ($p < 0.001$).

MATERNAL AGE

The whelping performance relative to the age of the bitch and regardless of breed is summarised in Table 2:11.

Table 2:11 Average parity, litter size and inter-pup interval relative to age of bitch.

Age (years)	Number of Litters	Number of Pups	Average Parity	Average Litter Size	Average Inter-pup Interval (min)
<12 mths	2	7	1	4.5	30.5
1	84	380	1.1	4.5	57.2
2	108	630	1.4	5.8	60.1
3	91	474	2.0	5.2	52.3
4	72	355	2.5	4.9	58.3
5	65	355	3.4	5.5	70.4
6	38	206	4.2	5.4	62.1
7	25	108	4.8	4.3	65.9
8	11	44	4.7	4.0	79.9
9	4	15	4.8	3.8	119.5

* Average Inter-pup Interval. The inter-pup interval is the time between delivery of two consecutive pups.

It must be remembered that breeders will cull poor performing bitches and therefore those in the older age groups are generally bitches that the breeder considers to be a good breeding proposition according to health and previous history.

Two bitches whelped their first litter aged less than 12 months. Both of these were mismatings where the breeder elected to allow the bitch to whelp rather than to abort the pups.

The average parity indicated that breeders elected to breed their bitches approximately five times by seven 7 years of age, with the oldest bitches bred at 9 years.

Peak fertility occurred at two years with the highest average litter size of 5.8 pups. Thereafter average litter size tended to decrease with increasing age.

Three-year-old bitches whelping their second litter had the shortest inter-pup interval. Thereafter inter-pup interval tends to increases with increasing age of the bitch.

Table 2:12 compares average litter size and average parity with increasing maternal age in the toy and large breeds. (There were insufficient numbers of litters in the other breed groups for valid comparisons). These results differed from those reported in Table 2:11.

The toy breeds were bred younger and more frequently than the large breeds. The average number of litters for an eight- year-old toy breed was 6.6 and for the large breed was 3.6.

The average litter size appeared to be unrelated to maternal age. This was seen in both the toy and large breeds.

Table 2:12 Average parity and litter size in the toy and large breeds relative to maternal age

Maternal Age	**Toy**			**Large**		
	Number of litters	Average parity	Average litter size	Number of litters	Average parity	Average litter size
1	42	1.1	3.7	19	1.0	6.9
2	32	1.7	3.8	36	1.1	7.5
3	28	2.4	3.8	34	1.7	6.3
4	25	3.2	3.7	20	2.1	5.7
5	24	3.9	3.4	22	3.3	7.3
6	12	5.5	3.5	12	3.5	6.9
7	15	5.4	3.1	5	4.6	7.4
8	4	6.4	3.8	8	3.6	5.0
9				1	3.0	6.0

Mortality Relative to Age

Mortality relative to the age of the bitch is summarised in Table 2:13 and Figure 2:5. Bitches aged one year had the lowest perinatal losses and with a few exceptions losses increased with increasing maternal age. However, in the statistical analyses of the litter data for all litters and breed groups, maternal age was not a significant predictor of mortality (Appendices 2 to 7).

There was a very high mortality in pups whelped from bitches less than 12 months of age, primarily due to fading puppy syndrome. This result was based on only two litters and therefore should not be considered a reliable result.

Table 2:13 Mortality relative to the age of the bitch

Age (years)	Number of dead pups	Perinatal mortality	Abnormal pups	Foetal asphyxia	Fading pups

<12 mths	4	57.1%	0.0%	0.0%	57.1%
1	44	11.1%	3.4%	6.6%	0.5%
2	91	13.3%	3.3%	7.6%	1.9%
3	95	16.7%	4.6%	5.7%	6.5%
4	74	19.2%	6.5%	7.6%	5.4%
5	75	20.0%	5.9%	11.6%	2.8%
6	56	23.8%	5.8%	13.1%	6.3%
7	18	15.8%	7.4%	4.6%	4.6%
8	13	29.6%	9.1%	0.0%	18.2%
9	5	33.3%	6.7%	0.0%	26.7%

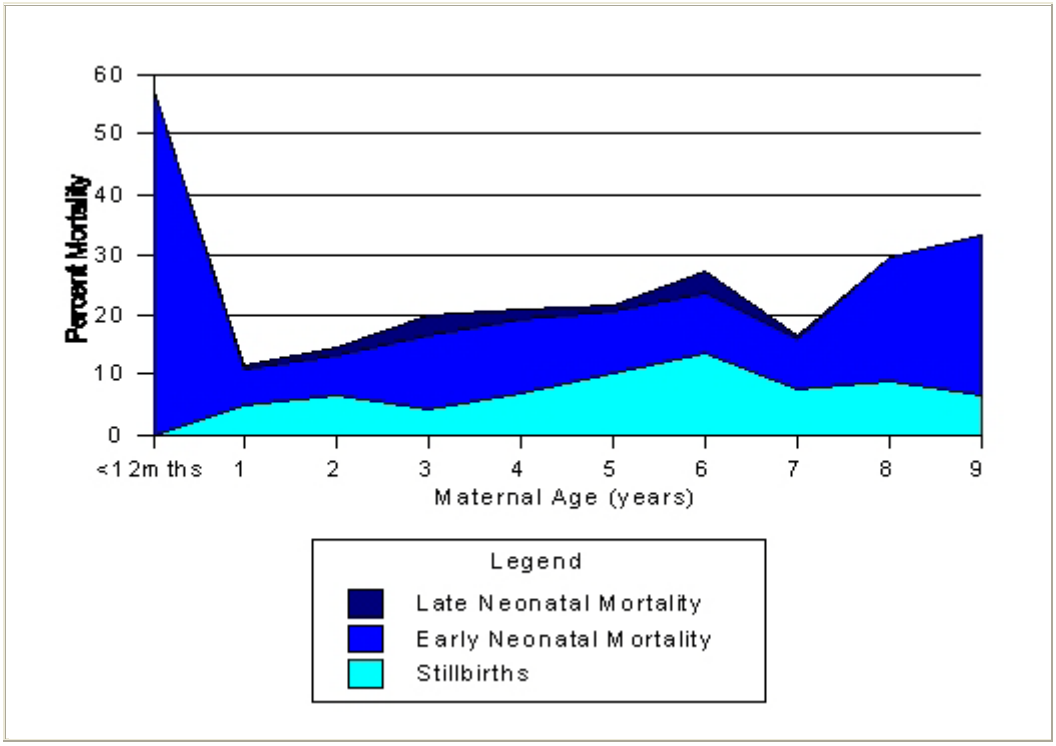

Figure 2:5 Total mortality relative to maternal age.

The causes of pup mortality are graphed in Figure 2:6. Fading puppy syndrome was the principal component of losses at the extremes of bitch age, that is, bitches less than 12 months and older than seven years. In the statistical analyses of the pup data for all pups and breed groups, maternal age was not a significant predictor of mortality due to fading puppy syndrome (Appendices 10 to 15) with the exception of the medium breed group. In this group the odds of mortality due to fading puppy increased by 5.83x ($p = 0.02$) for each increase in age by one year (Appendix 13).

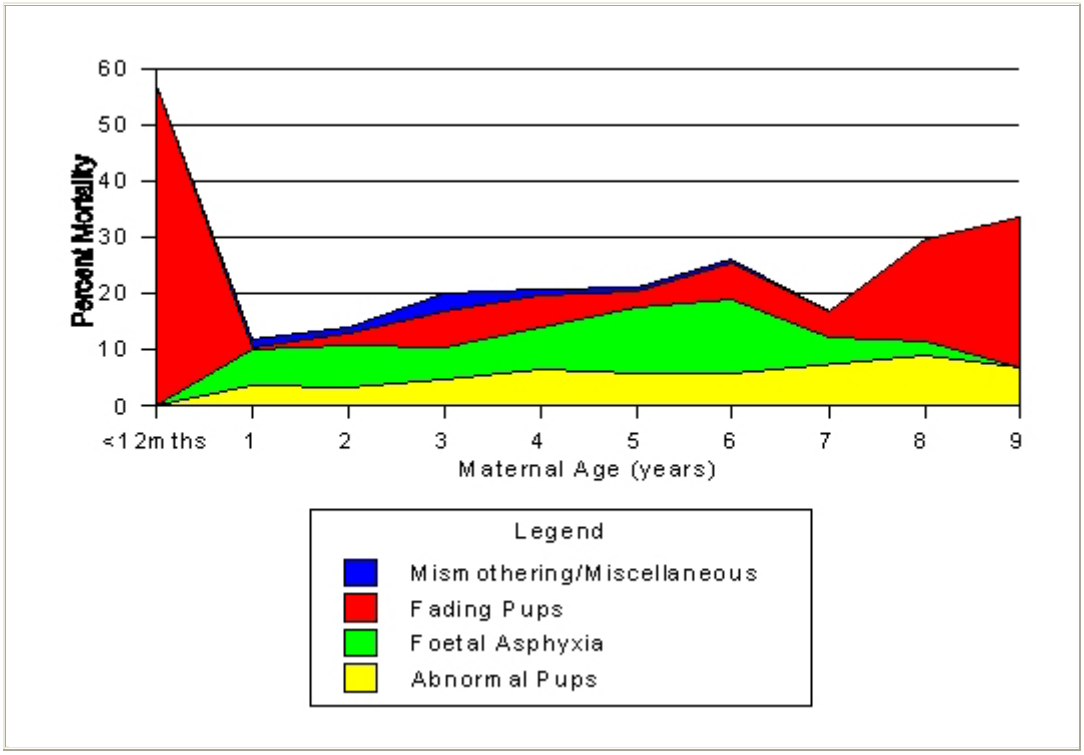

Figure 2:6 Classification of mortality relative to maternal age

The percentage of abnormal pups whelped tended to increase with increasing maternal age. However, maternal age was not a significant predictor of mortality due to the birth of abnormal pups (Appendices 10 to 15).

Pups dying as a result of foetal asphyxia also tended to occur more frequently with increasing maternal age until six years (Table 2:14). However, again maternal age was not a significant predictor of mortality due to foetal asphyxia (Appendices 10 to 15). The pattern of mortality due to foetal asphyxia may have been modified by the performance of caesarean section, breeder or veterinary assistance during the whelping and breeder selection.

There was no obvious association between the occurrence of dystocia and maternal age. Bitches aged one or two years required less breeder or veterinary assistance for the delivery of the litter, with the exception of eight-year-olds. This pattern may reflect breeder selection, that is, only breeding from bitches with good whelping histories.

The inter-pup whelping interval tended to increase with increasing maternal age (Table 2:11). This did not appear to influence either pup mortality or the occurrence of dystocia.

Table 2:14 Mortality due to foetal asphyxia and the frequency of dystocia relative to maternal age

Maternal Age (Years)	Number of dead pups	Mortality due to foetal	Total dystocia	Manual assistance	Chemical assistance	Caesarean section*

		asphyxia				
<12 mths	0	0.0%	0.0%	0.0%	0.0%	0.0%
1	25	6.6%	27.4%	7.1%	3.6%	16.7%
2	48	7.6%	29.6%	7.4%	7.4%	14.8%
3	27	5.7%	40.7%	12.1%	8.8%	19.8%
4	27	7.6%	33.3%	4.2%	9.7%	19.4%
5	41	11.6%	49.2%	10.8%	16.9%	21.5%
6	27	13.1%	42.1%	7.9%	13.1%	21.1%
7	5	4.6%	36.0%	8.0%	8.0%	20.9%
8	1	2.3%	9.1%	0.0%	0.0%	9.1%
9	0	0.0%	50.0%	0.0%	0.0%	50.0%

Mismothering and miscellaneous causes of mortality and death during the late neonatal period contributed to only a small percentage of losses over all age groups.

MATERNAL PARITY

The whelping performance relative to the parity of the bitch and regardless of breed is summarised in Table 2:15.

Table 2:15 Average age, litter size and inter-pup interval relative to maternal parity.

Parity	Number of litters	Number of pups	Average age	Average litter size	Average inter-pup interval (min)
1	194	1050	2.9	5.4	42.3
2	133	681	3.4	5.1	38.6
3	76	419	4.5	5.5	64.9
4	42	203	5.1	4.8	59.1
5	32	155	6.1	4.8	58.3
6	12	41	6.6	3.5	58.6
7	7	16	7.1	2.3	84.0
8	1	2	6.0	2.0	20.0
9	2	5	6.5	2.5	103.5

* Average inter-pup interval. The inter-pup interval is the time between delivery of two consecutive pups.

It must be remembered that breeders will cull poor performing bitches. Therefore those in the higher parity groups are generally bitches that the breeder considers to be a good breeding proposition according to health and previous history. The average age for the first litter was 2.9 years. The results suggested that there was decreasing average litter size with increasing parity. These results were, however, modified by the frequency at which bitches are bred in the different breed groups.

Bitches whelping their second litter at an average age of 3.4 years recorded the shortest inter-pup interval of 38.6 minutes, followed by parity one bitches. After that, there appeared to be no relationship between increasing parity and inter-pup whelping intervals.

Table 2:16 compares the average litter size and age for the toy and large breed groups at different parity.

The toy breeds were bred from more frequently and at an earlier age than the large breeds. The average age for a toy breed whelping her first litter was 1.5 years and two bitches had 9 litters by an average age of 6.5 years. The average age for the first litter in the large breeds was 2.1 years, and the maximum parity recorded was six litters.

Table 2:16 Average parity and litter size in the toy and large breeds relative to maternal parity.

Maternal Parity	Toy			Large		
	Number of Litters	Average Age	Average litter size	Number of Litters	Average Age	Average litter size
1	58	1.5	3.7	69	2.1	7.2
2	42	3.0	3.7	39	3.7	6.1
3	29	3.9	3.8	21	4.8	7.1
4	20	4.7	3.5	14	5.5	6.5
5	18	5.8	4.0	8	6.3	5.8
6	7	6.1	2.7	1	7.0	10.0
7	5	6.4	2.4			
8	1	6.0	2.0			
9	2	6.5	2.5			

In the toy breeds parity six and higher had smaller litters than the lower parity groups. In the larger breeds, litter size appeared to be unaffected by parity, however, the maximum parity recorded was six litters.

Mortality Relative to Parity

Mortality relative to the parity of the bitch is summarised in Table 2:17 and Figure 2:7.

Parities one and two had the lowest perinatal mortality (15.3%). There was a general pattern of increasing perinatal mortality with increasing parity. The drop at parity seven may reflect breeder selection. Stillborn losses appeared relatively constant between parity one and four: after that there was increasing stillborn loss with increasing parity. Bitch parity was a significant predictor of mortality with the odds of a pup dying increasing by 1.14x ($p = 0.03$) for each subsequent parity (Appendix 2). In the breed group analyses (Appendices 3 to 7)

parity was only a significant predictor in the toy breed group with the odds of a pup dying increasing by 1.28x (p = 0.04) for each subsequent parity (Appendix 3).

Table 2:17 Mortality relative to maternal parity

Maternal Parity	Number of dead pups	Perinatal Mortality	Abnormal Pups	Foetal Asphyxia	Fading pups
1	171	15.3%	4.3%	7.9%	2.6%
2	115	15.3%	6.2%	7.5%	3.4%
3	93	18.4%	5.0%	7.9%	6.2%
4	40	17.7%	5.4%	6.4%	6.4%
5	38	23.2%	5.2%	9.0%	6.7%
6	13	31.7%	14.6%	9.8%	7.3%
7	3	18.9%	6.3%	6.3%	6.3%
8	1	50.0%	0.0%	50.0%	0.0%
9	1	20.0%	20.0%	0.0%	0.0%

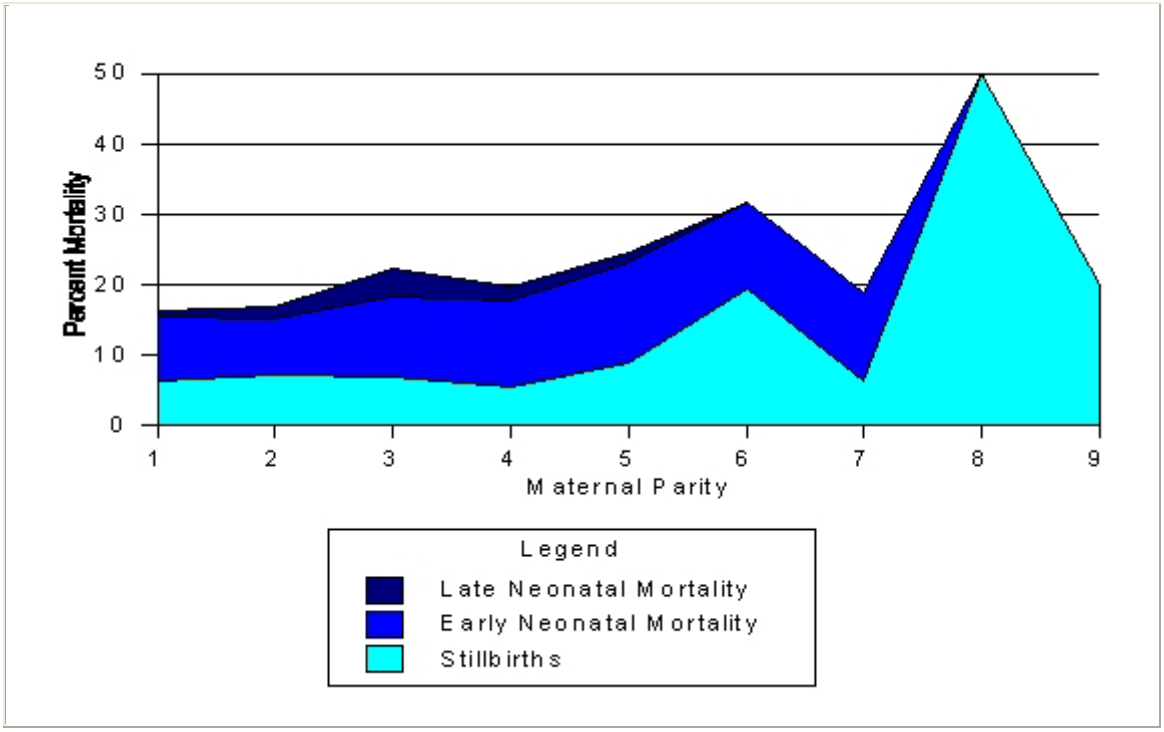

Figure 2:7 Total mortality relative to maternal parity

The causes of pup mortality are graphed in Figure 2:8. There appeared to be no definitive relationship between the cause of pup mortality and maternal parity.

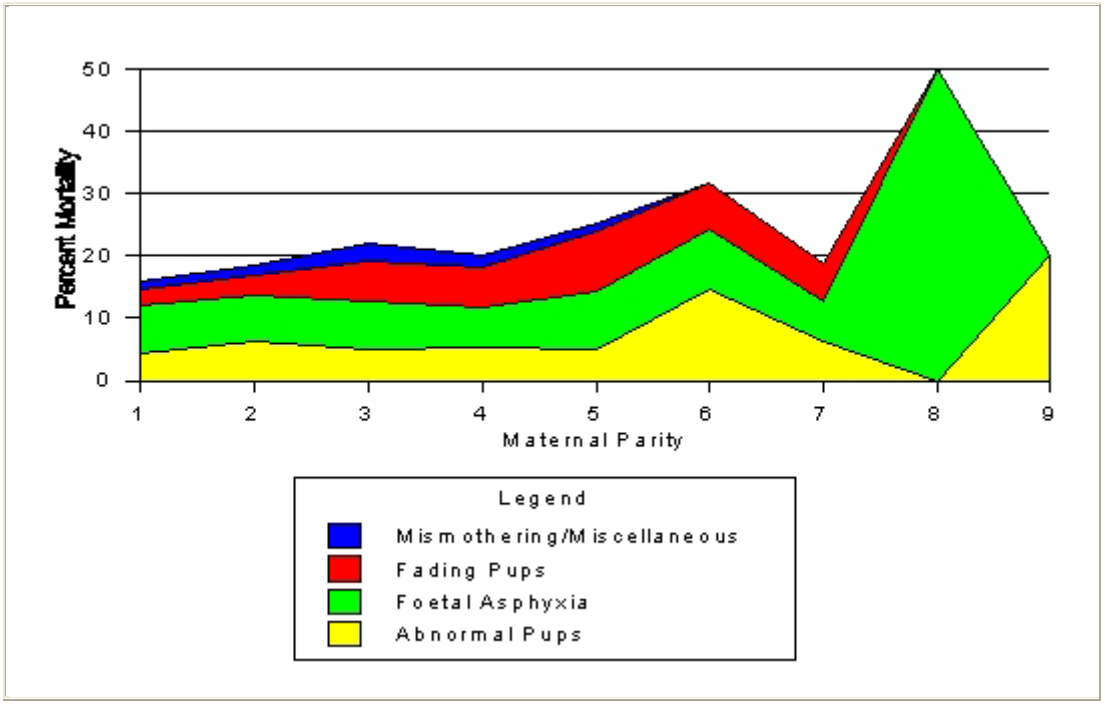

Figure 2:8 Classification of pup mortality relative to maternal parity

Parity was a significant predictor of mortality due to fading puppy syndrome. For each subsequent parity, mortality due to fading puppy syndrome increased by 1.47x ($p < 0.001$; Appendix 10). Therefore, the risk of mortality attributed to fading puppy syndrome occurred in the higher parity groups.

There appeared to be no relationship between the occurrence of abnormal pups and maternal parity. In the statistical analysis of all pup and breed group data, maternal parity was not a predictor of mortality due to the birth of abnormal pups (Appendix 10 to 15). Similarly maternal parity was not a predictor of mortality due to foetal asphyxia.

Mortalities due to foetal asphyxia and the occurrence of dystocia relative to maternal parity are summarised in Table 2:18. There was no obvious association with the occurrence of dystocia, death due to foetal asphyxia and maternal parity. There was a tendency for increasing chemical assistance (that is, oxytocin) required with increasing parity.

The pattern of mortality due to foetal asphyxia may have been modified by the performance of caesarean section, breeder or veterinary assistance during the whelping and breeder selection.

Table 2:18 Mortality due to foetal asphyxia and the frequency of dystocia relative to maternal parity.

Maternal Parity	Foetal Asphyxia	Total Dystocia	Manual Assistance	Chemical Assistance	Caesarean Section*
1	7.9%	30.4%	8.3%	3.6%	17.0%
2	7.5%	39.1%	8.3%	7.5%	21.8%
3	7.9%	43.4%	7.9%	15.8%	17.1%
4	6.4%	42.7%	7.1%	33.3%	26.2%
5	9.0%	31.5%	9.4%	12.5%	12.5%
6	9.8%	16.7%	8.3%	25.0%	0.0%
7	6.3%	28.6%	0.0%	14.3%	28.6%
8	50.0%	0.0%	0.0%	0.0%	0.0%
9	0.0%	0.0%	0.0%	0.0%	0.0%

Mismothering and miscellaneous causes of mortality and death during the late neonatal period contributed to only a small percentage of losses over most parity groups.

PARITY:AGE RELATIONSHIP

Table 2:19 and Figure 2:9 analysed the relationship of parity and age to perinatal mortality for parity one, two and three bitches.

Table 2:19 Mortality relative to age:parity relationship

Maternal Parity:Age	Number of Litters	Number of Pups	Average Litter Size	Perinatal Mortality	Percentage of Caesareans
1:1	78	357	4.6	11.5%	15.4%
1:2	74	465	6.3	14.2%	15.4%
1:3	27	147	5.4	21.1%	33.3%

1:4	12	64	5.3	26.6%	25.0%
1:5	1	5	5	20.0%	0.0%
2:1	6	23	3.8	4.4%	16.7%
2:2	30	148	4.9	10.8%	16.7%
2:3	46	229	5	12.2%	21.7%
2:4	28	156	5.5	16.3%	25.0%
2:5	15	89	5.9	31.5%	26.7%
2:6	4	21	5.3	5.0%	25.0%
3:2	4	17	4.2	11.8%	25.0%
3:3	14	81	5.8	22.2%	21.4%
3:4	22	100	4.5	12.0%	18.2%
3:5	21	114	5.4	18.4%	14.3%
3:6	12	89	7.4	24.8%	16.7%

Perinatal mortality for parity one bitches increased with maternal age. To reduce perinatal mortality bitches should whelp their first litter between one and two years of age.

Similarly, parity two bitches had increasing perinatal mortality with increasing maternal age. The results showed that they should have their second litter before four years of age.

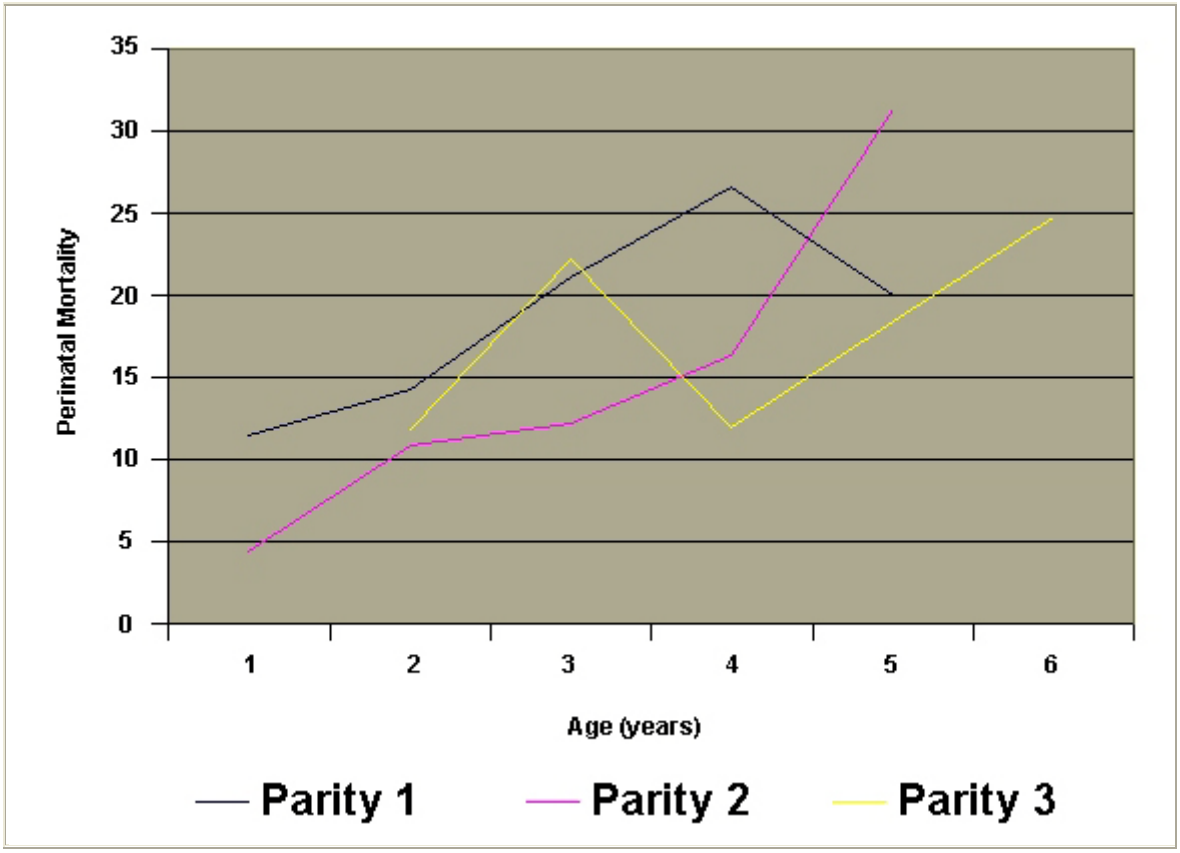

Figure 2:9 Mortality relative to the age:parity relationship

Bitches whelping their second litter had a lower perinatal mortality than parity one bitches. The performance of caesarean sections have modified these mortality outcomes.

Parity three bitches had the lowest mortality and caesarean section rate at four and five years of age. Breeding a bitch too frequently appeared to increase either perinatal mortality or caesarean section rates.

The parity:age correlation to perinatal mortality showed possibly significant results, but more litters are needed to confirm the findings and to investigate the reasons why this occurs.

LITTER SIZE

The effect of litter size on perinatal mortality irrespective of breed is summarised in Table 2:20.

Table 2:20 Mortality relative to litter size

Litter size	Number of Litters	Perinatal Mortality	Total Mortality
1	32	43.6%	50.0%
2	51	29.4%	32.3%
3	65	16.4%	16.9%
4	85	15.6%	17.4%
5	69	18.6%	19.7%
6	67	14.7%	15.4%
7	39	17.6%	19.8%
8	24	17.2%	18.2%
9	30	14.1%	14.4%
10	17	12.4%	13.5%
11	10	13.6%	13.6%
12	5	16.7%	38.3%
13	3	25.6%	25.6%
14	1	14.3%	14.3%
15	2	10.0%	10.0%

These results indicated that perinatal mortality was highest at litter sizes one and two and thereafter appeared to show no relationship to litter size. In the statistical analysis of all litter data, litter size was not a predictor of mortality (Appendix 2).

Because this evaluation did not take into consideration the different ranges of litter sizes that occur in the different breed groups, the effect of litter size on mortality for the two most commonly surveyed breed groups was evaluated (toy and large breed groups) as follows.

Toy Breed Group

There were 183 toy breed litters, consisting of 664 pups and litter size varied from one to eight, with an average of 3.6 pups (Table 2:21).

Table 2:21 Average age and birth weight relative to litter size in the toy breed group.

Litter size	Number of litters	Average age of dam (years)	Average birth weight (grams)
1	15	3.3	176.2
2	32	4.2	166.2
3	37	5.8	175.1
4	46	3.2	162.5
5	33	3.3	161.9
6	16	2.8	216.1
7	3	3.0	105.8
8	1	5.0	117.5

The litter size did not appear to be influenced by the age of the bitch. Similarly, average pup birth weight did not appear affected by litter size until litter size seven and eight.

Mortality relative to litter size is graphed in Figures 2: 10 and 2: 11. These results demonstrated that mortality in the toy breeds was highest at the extremes of litter size, with the greatest loss occurring at litter size seven. Minimum mortality was recorded between litter size three and four, that is, at the average litter size for the breed group. In the statistical analysis of the litter data of the toy breed group, litter size was not a predictor of mortality (Appendix 3).

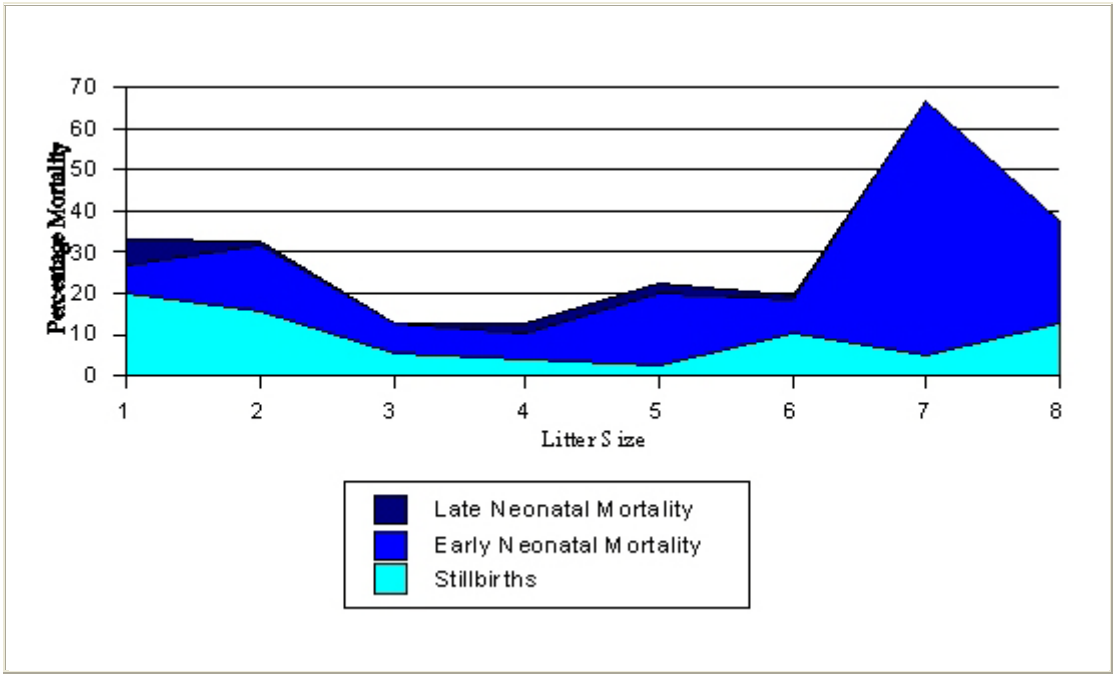

Figure 2:10 Total mortality in toy breed group relative to litter size.

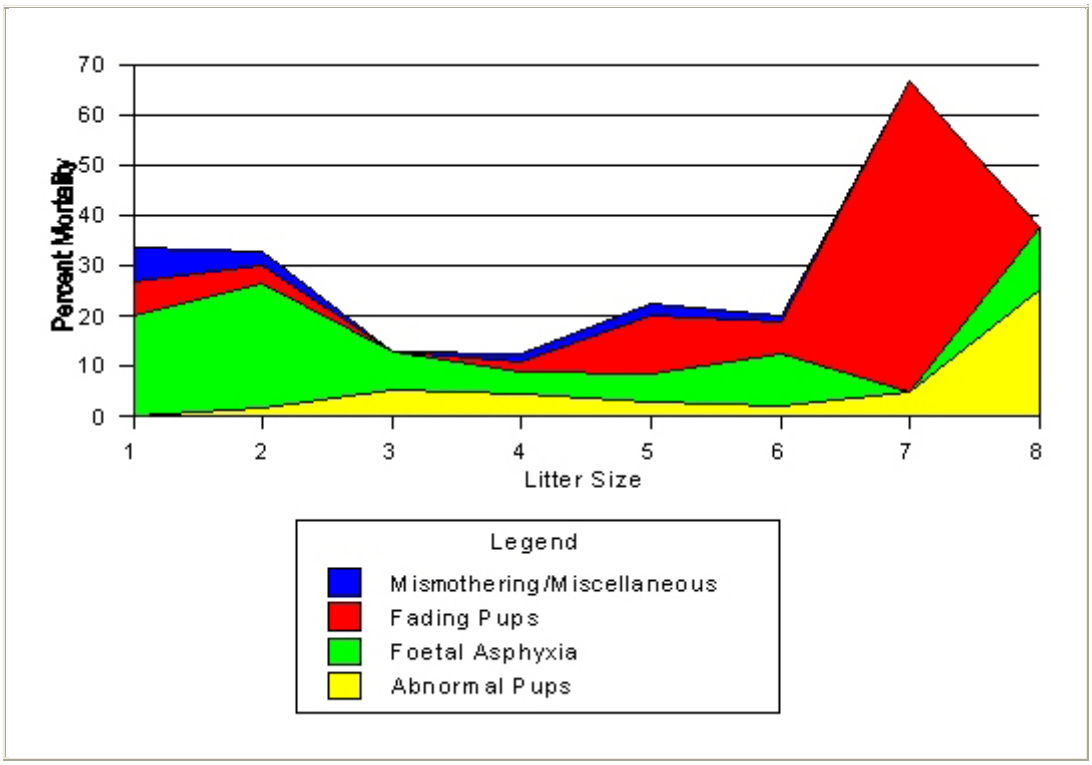

Figure 2:11 Classification of mortality in toy breed group relative to litter size

The causes of pup mortality varied at the litter size extremes. At litter sizes one and two the principal cause of mortality was foetal asphyxia. Foetal asphyxia as a result of both foetal disproportion and presentation was the greatest problem encountered. At litter seven, the principal cause of mortality was fading puppy syndrome. It was at litter size seven that the lowest average birth weight was also recorded.

In the statistical analysis of the pup data (Appendix 11) litter size was a significant predictor of mortality due to foetal asphyxia and fading puppy syndrome as suggested in the mortality distribution. As litter size increased by one pup the odds of mortality due to foetal asphyxia decreased by 0.49x ($p < 0.001$). For each increase in litter size the odds the odds of mortality due to fading puppy syndrome increased by 3.4x ($p < 0.001$).

Large Breeds

There were 152 large breed litters, consisting of 1030 pups and litter size varied from one to 14 pups, with an average of 6.8 pups. The data for this group is presented in table 2:22.

Table 2:22 Average age and birth weight relative to litter size in large breed group

Litter Size	Number of litters	Average Age of dam (years)	Average birth weight (grams)
1	8	3.9	604.7
2	8	3.5	464.7
3	6	3.5	400.3
4	13	3.5	481.4
5	12	3.5	440.5
6	21	3.7	527.5
7	19	3.5	458.1
8	17	2.5	354.1
9	21	3.5	413.0
10	14	3.9	350.0
11	8	4.1	482.4
12	2	2.0	490.3
13	2	1.5	355.2
14	1	3.0	432.8

In the large breed group, again litter size did not appear to be influenced by the age of the bitch. Similarly, the average pup birth weight did not appear to be influenced by litter size, with the exception of litter size one which recorded the highest average birth weight of 604.7 grams. Mortality relative to litter size is graphed in Figures 2:12 and 2:13. Litter size one recorded the highest mortality and thereafter fluctuated with increasing litter size. Litter size was not a significant predictor of mortality in the large breed group (Appendix 6).

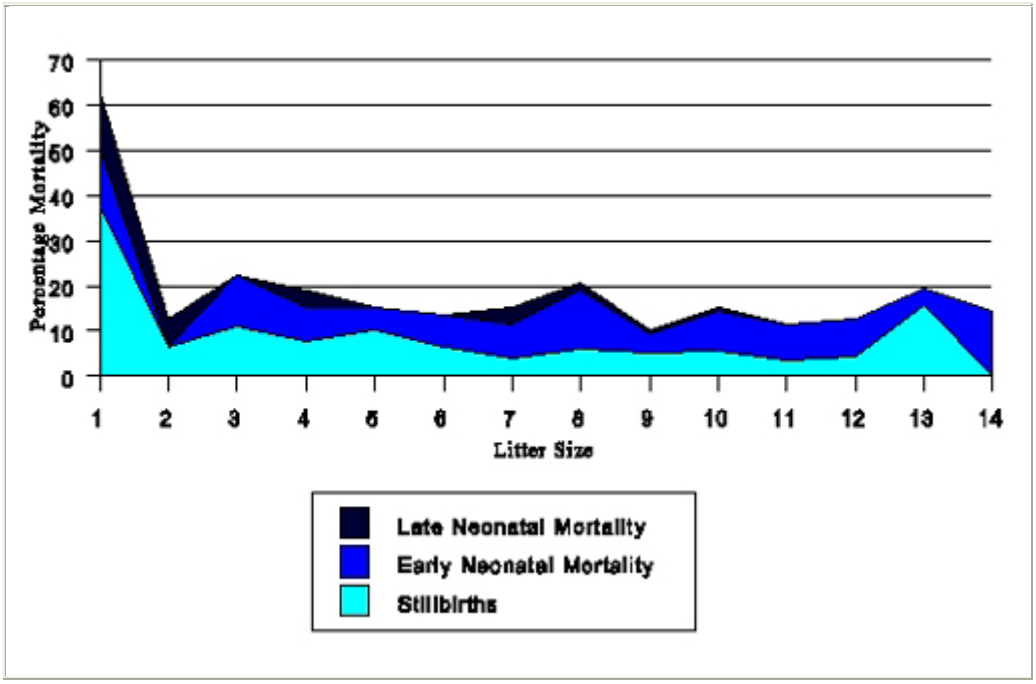

Figure 2:12 Mortality in large breeds group relative to litter size.

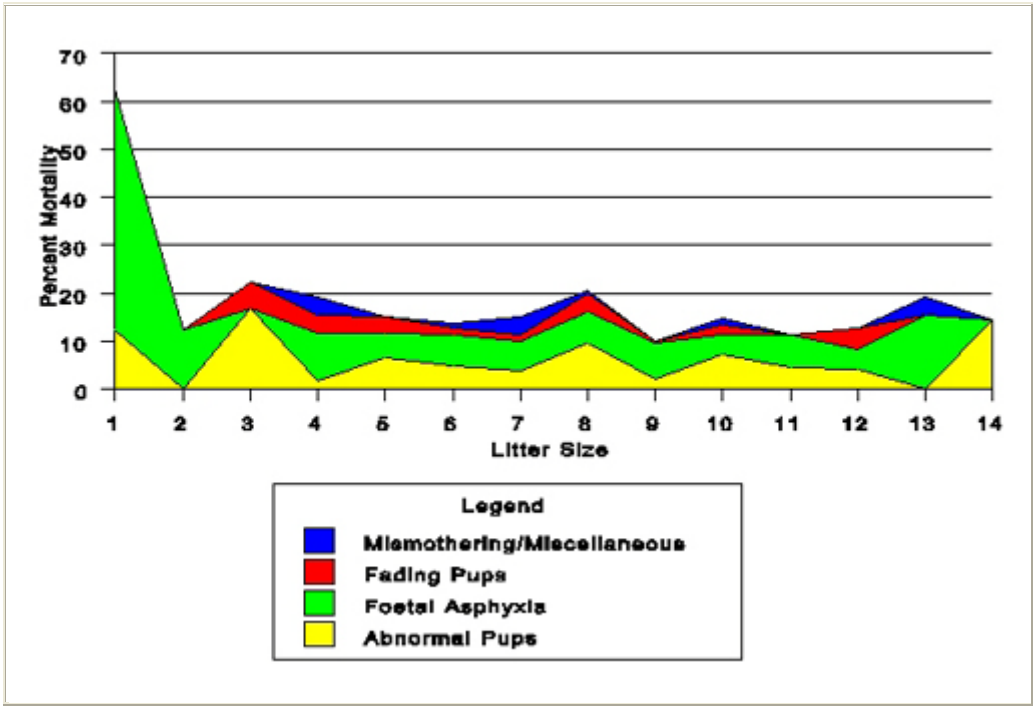

Figure 2:13 Classification of mortality in large breed group relative to litter size.

The majority of losses at litter size one were stillbirths, associated with foetal distress due to maternal primary uterine inertia. Mortality peaked again at litter size three, principally due to the birth of abnormal pups, equally represented by small for date pups, death prior to birth and congenital defects. Mortality was not increased in the large litters seen in the toy breeds. Litter size was not a predictor of mortality in the pup data analysis (Appendix 14), but litter position or birth order was significant with the odds of mortality due to foetal asphyxia increasing by 9.99x ($p = 0.003$) for every increase in the birth order. Similarly in the giant breed the odds of mortality due to foetal asphyxia increased by 142.53x ($p = 0.005$) for each increase in litter position.

In the litter and pup data analysis of the small, medium and giant breed groups (Appendices 4, 5, 7, 12, 13 and 15) litter size was not a predictor of mortality with one exception in the giant breed group (Appendix 15) where for every one pup increase in the litter size, the odds of the birth of an abnormal pup decreased by 0.40x ($p = 0.02$).

WHELPING TIMES

Because of the difficulty encountered by many breeders to accurately determine the start of both first and second stage labour, the duration of labour recorded commences from the time of birth of the first pup. The whelping time and average inter-pup intervals for the various litter sizes, regardless of breed, manual or chemical assistance and outcome are summarised in Table 2:23. These inter-pup intervals did not distinguish between rest periods and active straining.

Table 2:23 Average whelping times and inter-pup intervals relative to litter size for all breeds and outcomes.

Litter Size	Number of litters	Average whelping time (min)	Average inter-pup interval (min)
2	31	82.1	82.1
3	48	126.6	63.2
4	63	168.8	56.3
5	54	210.0	52.5
6	48	279.7	56.0
7	30	348.1	58.0
8	20	414.9	59.3
9	25	446.3	55.8
10	14	576.1	64.0
11	8	622.1	62.2
12	4	789.3	71.8
13	3	931.7	77.6
14	1	702.0	54.0
15	2	612.0	43.7

Litter size varied from one to 15. The whelping time increased with increasing litter size to litter size 13.

There was a decreasing inter-pup interval with increasing litter size to litter size five. Thereafter, the inter-pup interval generally increased with increasing litter size. Litter size five had the shortest inter-pup interval of 52.5 minutes and litter size two the longest inter-pup interval of 82.1 minutes.

There were 153 naturally whelped litters between litter size two and nine which required no assistance and recorded no mortalities. The mean whelping times and average inter-pup intervals for these litters are summarised in Table 2:24. The average inter-pup interval for these litters are all below 60 minutes and demonstrated no relationship to litter size. These results are graphed in Figure 2:14.

Table 2:24. Whelping time and inter-pup interval for litters requiring no whelping assistance and having no mortalities.

Litter Size	Number of litters	Mean Whelping Time	+/- 1 s.d.	Average Inter-pup Interval
2	15	37.8	18.1	37.8
3	21	111.1	54.6	55.5
4	39	131.7	57.3	43.9
5	29	174.0	91.6	43.5
6	25	234.6	121.0	46.9
7	11	263.0	101.0	43.8
8	5	298.6	96.6	42.5
9	10	394.6	112	49.3

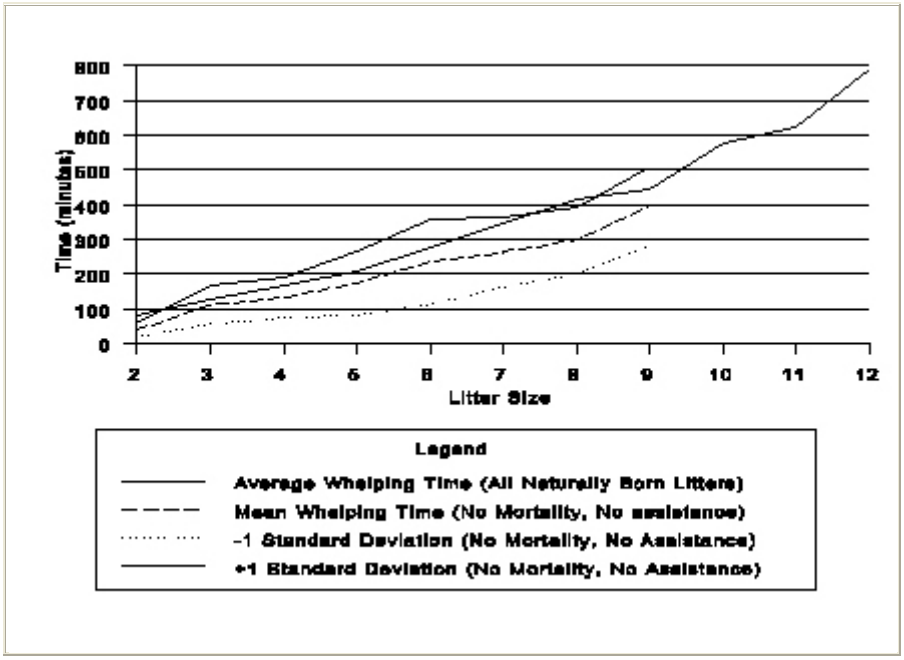

Figure 2:14 Whelping times and range for litters with no mortality and requiring no assistance.

Table 2:25 summarises the inter-pup intervals based on birth order in those litters which were unassisted and where no mortalities were recorded.

Table 2:25 Inter-pup interval relative to birth order in litters recording no assistance and no mortalities.

Birth order	Mean inter-pup interval (min)	-1 s.d.	+1 s.d.	Number of observations
2	44.5 +/- 40.7	3.8	85.2	159
3	44.6 +/- 32.1	12.5	76.7	142
4	41.3 +/- 28.8	12.5	70.1	121
5	44.8 +/- 36.4	8.4	81.2	82
6	53.1 +/- 40.2	12.9	93.3	53
7	42.6 +/- 25.7	16.9	68.3	28
8	66.4 +/- 35.3	31.1	101.7	17
9	72.9 +/- 55.3	17.6	127.9	12

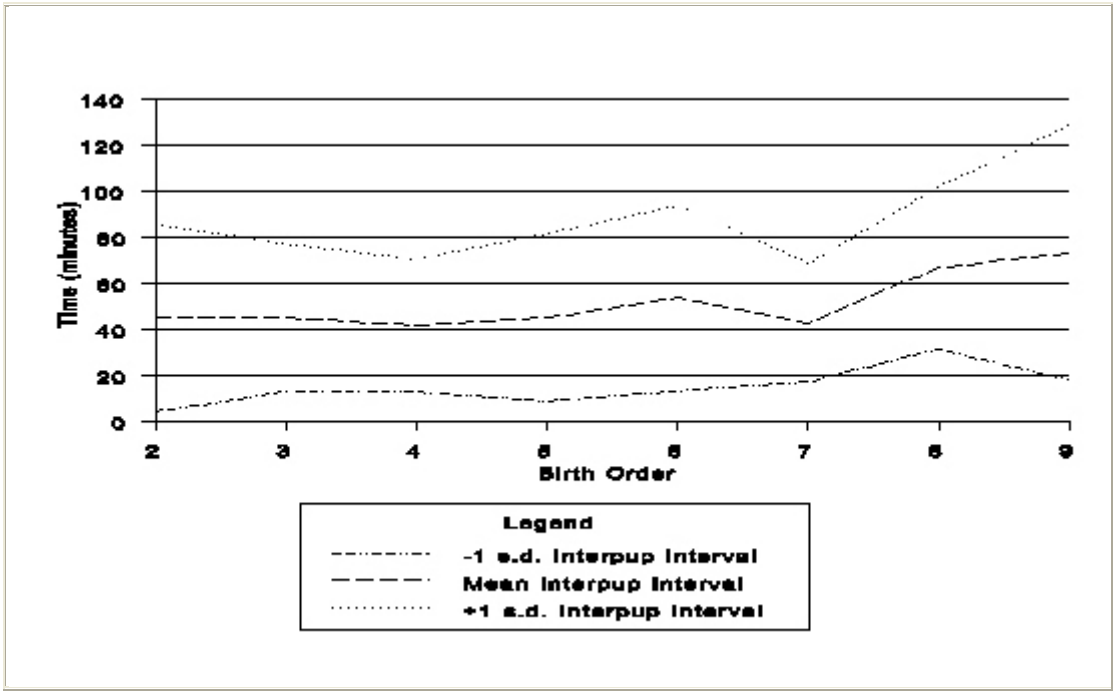

Figure 2:15 Inter-pup interval at the various birth orders.

These results differed from those in Table 2:25 and indicated that the inter-pup interval relative to litter position increased at the end of a large litter. Pups at birth order eight and nine were whelped at an average inter-pup interval greater than one hour.

Mortality relative to whelping time is summarised in Table 2:26 and excludes pups delivered by caesarean section. Total mortality tended to increase with increasing inter-pup whelping times with perinatal mortality lowest for pups whelped within 30 minutes or less. After a inter-pup interval of four hours all perinatal losses were stillbirths.

Table 2:26 Mortality relative to inter-pup whelping intervals.

Time (min)	Number of Pups	Stillbirths	Early Neonatal Mortality	Total Mortality
>31	647	3.7%	6.4%	11.3%
31-60	497	4.0%	8.1%	13.7%
61-90	210	3.3%	7.6%	11.4%
91-120	132	7.6%	10.6%	18.9%
121-150	55	7.3%	14.5%	23.6%

151-180	30	30.0%	3.3%	33.3%
181-210	16	6.3%	28.6%	31.3%
211-240	19	21.1%	10.5%	31.6%
241-270	8	25.0%	0.0%	25.0%
271-300	3	66.6%	0.0%	66.6%
301-330	3	66.6%	0.0%	66.6%
331-360	5	60.0%	0.0%	60.0%
>360	11	63.6%	0.0%	72.7%

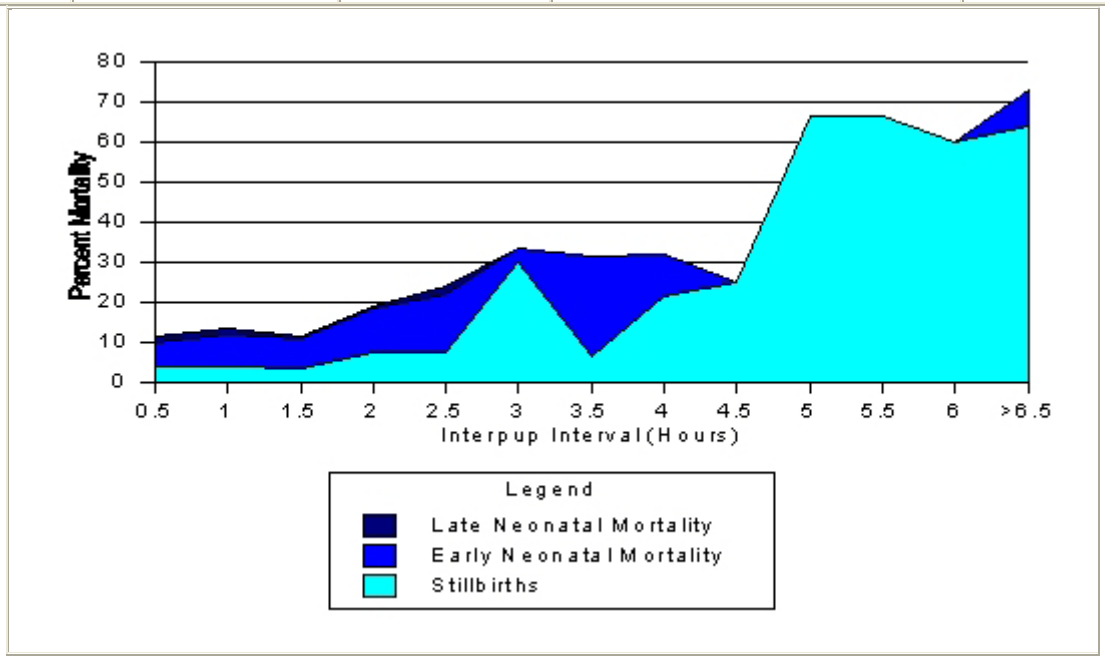

Figure 2:16 Total mortality relative to the inter-pup whelping intervals

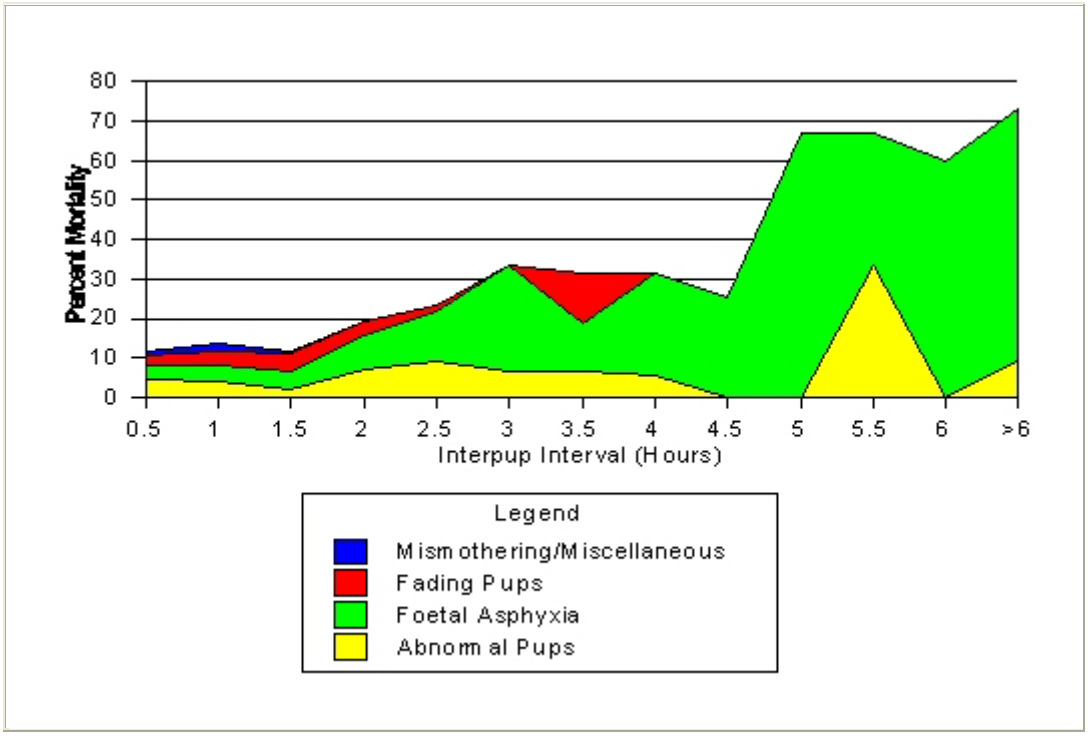

Figure 2:17 Classification of mortality relative to the inter-pup whelping intervals.

The results identified a marked increase in mortality after an inter-pup interval of one and a half hours and the principal cause of mortality after this time interval was foetal distress. Pups either born dead or distressed during what the breeder perceived as a normal whelping with no evidence of dystocia were born at intervals between 90 and 180 minutes. This highlights the variable time frames acceptable to the breeder as normal.

Pup death associated with clinically recognised dystocia occurred at all intervals but was the principal cause of mortality after 180 minutes. A small number of deaths associated with dystocia are seen in the time range of less than two hours. These losses were associated with foetal disproportion, foetal presentation and in the case of two pups, it was the pups preceding them that had a prolonged inter-pup interval.

Fading pups tended to occur at inter-pup intervals of less than two hours. The cluster of deaths attributed to fading puppy syndrome at three and a half hours may indicate an association of prolonged inter-pup intervals with some fading pups.

In nearly all the statistical analyses performed either total whelping time or the inter-pup interval are significant predictors of mortality. These results do not, however, identify what are the acceptable time frames. In the litter data (Appendix 2) the odds of a pup dying increased by 1.01x ($p < 0.007$) for an increase in average inter-pup interval of ten minutes. In the toy breed group (Appendix 3) for each ten minute increase in total whelping time the odds of a pup dying increased by 1.004x ($p = 0.03$). In the small breed group (Appendix 4) the odds of a pup dying increased by 1.02x ($p = 0.03$) for an increased average inter-pup interval of ten minutes. In the giant breed group (Appendix 7) the odds of a pup dying increased by 1.003x ($p = 0.04$) for each ten minute increase in the total whelping time.

In the toy, medium and giant breed groups inter-pup intervals are significant predictors of mortality due to foetal asphyxia. In the toy breed group (Appendix 11) the odds of mortality due to foetal asphyxia increases by 1.01x ($p < 0.001$) for each 10 minute increase in the inter-pup interval. In the medium breed group (Appendix 13) the odds of mortality decreased by 0.95x ($p = 0.03$) and in the giant breed group (Appendix 15) the odds of mortality increased by 1.01x ($p = 0.01$) for every ten minute increase in inter-pup whelping interval.

The inter-pup interval is not a significant predictor of mortality due to fading puppy syndrome in any of the statistical analyses performed.

In a number of the statistical results the inter-pup interval was significant predictor of mortality due to abnormal pups. This does not affect the outcome of mortality in this category.

2:2:4 PUP FACTORS

The influence of pup factors on mortality was investigated. These factors were birth weight, birth order or litter position, sex, presentation and placental detachment.

Birth Weight

In this study, 0.9% of all pups born and 4.8% of total mortality was attributed to pups being "small for date". There were no specified weight ranges to describe what constituted a small for date pup and their identification was left to the breeder.

There were 15 breeds represented in the survey by more than ten litters and the mean birth weight and one standard deviation from the mean has been calculated for each breed (Table 2:27).

There were 2143 pups in these 15 breeds. The birth weight was not recorded for 112 of these pups. 14.6% of pups (313) were below one standard deviation of the mean for birth weight, 15.1% (324) were greater than one standard deviation above the mean and 70.3% (1506) were within one standard deviation.

Relative to the mean birth weight, the percentage weight of one standard deviation varied between breeds. For example, for the Collie Rough, one standard deviation was 31.4% of the mean birth weight. For the Pekingese it was 13.5%. The degree of variation did not appear to be related to either the breed group or the number of pups surveyed in each breed.

Table 2:27 Mean birth weight and standard deviation for breeds represented by more than ten litters.

Breed	Number of pups	Mean birth weight (g)	Standard deviation	Range 1 s.d.
Chihuahua	174	117.8	29.9 (25.3%)*	87.8 -147.8
Pekingese	123	189.9	25.6 (13.8%)*	163.8 - 215.8
Australian Silky Terrier	191	156.0	40.0 (25.6%)*	116.0 - 196.0
Shetland Sheepdog	247	203.0	58.0 (28.6%)*	145.0 - 261.0
Golden Retriever	116	468.6	73.5 (15.7%)*	395.6 - 541.6
German Shepherd	282	563.0	126 (22.4%)*	437.0 - 689.0
Collie Rough	104	274.0	86.7 (31.4%)*	187.0 - 361.0
Dachshund Miniature	116	200.7	55.2 (27.5%)*	145.7 - 255.7

Great Dane	195	536.1	148.1 (27.6%)*	388.1 - 684.1
Basset Hound	80	458.2	68.3 (14.9%)*	309.2 - 526.2
Labrador	154	422.2	74.6 (17.7%)*	347.2 - 497.2
Australian Cattle Dog	108	308.6	48.0 (15.5%)*	260.6 - 356.6
Rhodesian Ridgeback	106	471.1	82.4 (17.5%)*	389.1 - 553.1
Border Collie	152	353.4	67.0 (19.0%)*	256.4 - 420.4
Boxer	105	407.4	82.2 (20.2%)*	325.4 - 486.4

* Percentage of mean birth weight that one standard deviation represents for each breed.

The total mortality relative to birth weight is graphed in Figure 2:18. Stillbirths (3.6%) were lowest in pups with birth weights within one standard deviation from the mean. Early neonatal mortality (4.0%) was lowest in pups with birth weights above one standard deviation greater than the mean.

Total mortality in pups of birth weights below one standard deviation of the mean was over three times higher (38.4%) than those within one standard deviation of mean birth weight, for both stillborn (14.1%) and early neonatal deaths (23.0%).

In the statistical analyses the average birth weight was a significant variable with the odds of a pup dying increasing by 0.9981x ($p < 0.007$) for a decrease in average birth weight by ten grams (Appendix 2). This result means that the smaller breeds with the lower average birth weight have greater odds of dying than pups from the larger breeds with a higher average birth weight.

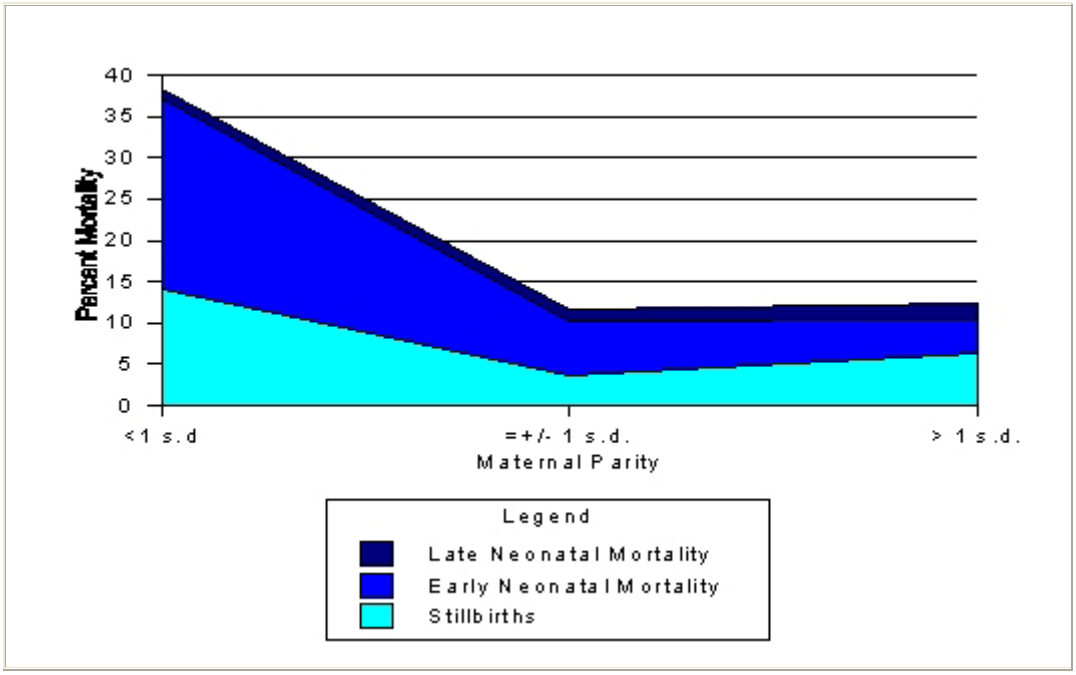

Figure 2:18 Total mortality relative to the mean birth weight

Figure 2:19 graphs the classification of mortality relative to mean birth weight. At birth weights of below one standard deviation of the mean, approximately one third of all deaths are abnormal pups (16.0%). The remaining pups were considered by the breeder to be normal and their deaths attributed to foetal distress and fading puppy syndrome.

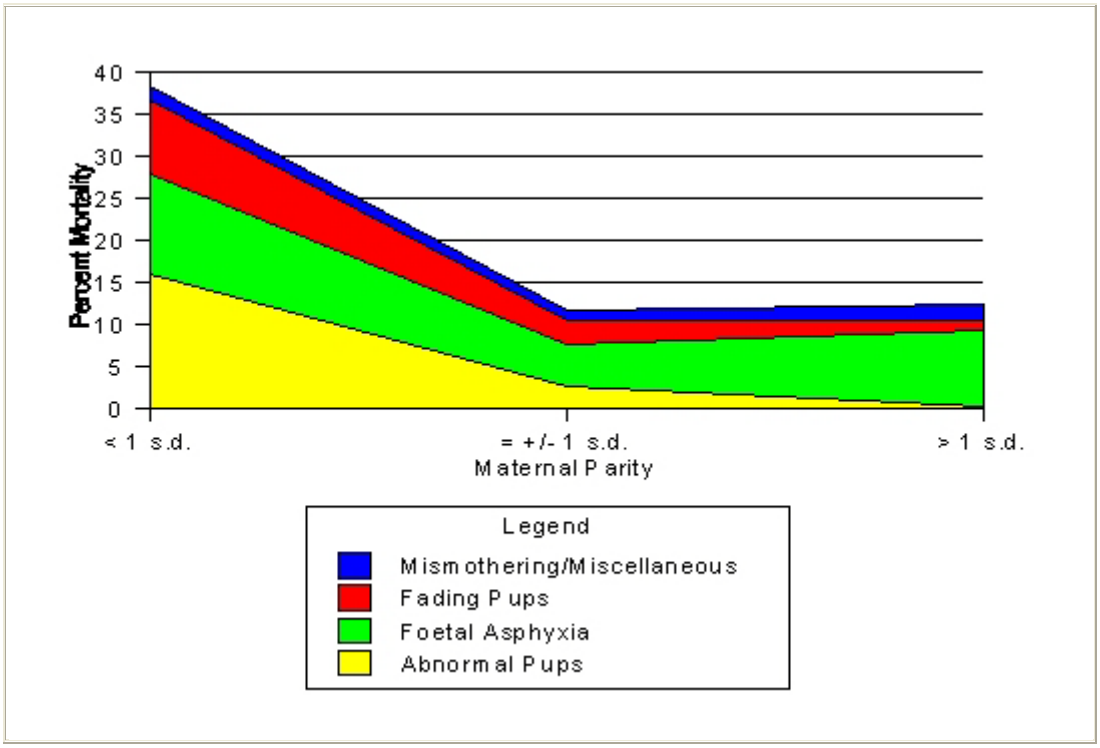

Figure 2:19 Classification of mortality relative to the mean birth weight.

There were 88 pups classified as dying from fading puppy syndrome. Thirty-one of these pups had birth weights below one standard deviation lower than the mean for their breed. This suggested that 35.2% of fading pups may have been growth retarded. Similarly, 86 pups died as a result of foetal asphyxia, during an apparently normal whelping. Twenty-three of these had birth weights below one standard deviation lower than the mean for their breed. This suggested that 26.7% of these pups may have been growth retarded.

In the toy breed group birth weight is a significant predictor of mortality (Appendix 3 and 11). For each ten gram increase in the average birth weight the odds of a pup dying decreased by 0.99x ($p = 0.03$). For each ten gram increase in birth weight the odds of mortality due to the birth of abnormal pups decreased by 0.96x ($p < 0.001$). This is a result of the abnormality rather than a cause. For each ten gram increase in average birth weight the odds of mortality due to fading puppy syndrome decreased by 0.98x ($p = 0.004$). Birth weight was also a significant predictor of losses due to miscellaneous deaths. For each ten gram increase in average birth weight the odds of losses due to miscellaneous causes decreased by 0.96x ($p=0.01$).

In the small breed group birth weight was also a significant predictor of losses due to abnormal pups and fading puppy syndrome (Appendix 12). For every ten gram increase in average birth weight the odds of mortality due to the birth of abnormal pups decreased by 0.97x ($p <0.001$) and for fading puppy syndrome the odds decreased by 0.96x ($p = 0.001$).

In the large breed group the average birth weight was a significant predictor of mortality due to the birth of abnormal pups (Appendix 14). For every ten gram increase in average birth weight the odds of mortality due to the birth of abnormal pups decreased by 0.99x ($p < 0.001$). Similarly in the giant breed group (Appendix 15) birth weight was a significant predictors of mortality due to the birth of abnormal pups. For each ten gram increase in the birth weight the odds of mortality. decreased by 0.96x ($p = 0.3$).

Birth Order

Table 2:28 summarises perinatal mortality relative to birth order (litter position) in litter sizes one through to twelve.

With the exception of litter size six, perinatal mortality was highest for the last pup born at each litter size. There was no pattern of increasing mortality with increasing litter position.

Table 2:28 Percent perinatal mortality relative to birth order

Litter Size	Number of Litters	Litter Position											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	32	43.8											
2	53	28.	32.										

		3	1										
3	67	13.4	10.4	22.4									
4	85	15.3	12.9	14.1	18.8								
5	69	15.9	15.9	20.3	14.5	23.2							
6	67	19.4	16.4	10.4	10.4	13.4	12.4						
7	39	12.8	12.8	20.5	15.4	18.0	18.0	28.2					
8	24	12.5	20.8	8.3	12.5	20.8	12.5	20.8	29.2				
9	30	16.7	13.3	6.7	6.7	6.7	10.0	13.3	23.3	30.0			
10	17	11.8	5.9	0.0	5.9	5.9	23.5	17.7	11.8	23.5	29.4		
11	10	0.0	10.0	0.0	20.0	0.0	0.0	10.0	10.0	30.0	10.0	60.0	
12	5	0.0	20.0	0.0	20.0	0.0	0.0	0.0	0.0	40.0	40.0	20.0	60.0

Table 2:29 summarises the inter-pup whelping interval relative to birth order in the different litter sizes. As previously noted, the time required to whelp the first pup was not recorded because of the difficulty encountered by many breeders to accurately identify the start of second stage labour. In seven of the ll litter sizes, the last born recorded the longest inter-pup interval. There was no pattern of increasing inter-pup intervals with increased litter position.

Table 2:29 Inter-pup whelping intervals (minutes) relative to birth order.

Litter Size	**Number of Litters**	**Litter Position**											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

1	32												
2	53		88.9										
3	67		65.0	62.5									
4	85		58.5	63.3	53.3								
5	69		42.8	51.4	60.0	64.4							
6	67		48.0	49.8	49.8	72.2	70.2						
7	39		55.6	40.1	55.3	42.9	85.6	88.0					
8	24		55.7	38.1	33.5	75.8	83.7	55.9	120.7				
9	30		53.2	48.9	35.7	73.1	43.8	68.7	93.2	95.0			
10	17		40.1	40.0	68.9	27.4	50.3	57.7	68.0	58.2	189.9		
11	10		49.0	49.3	49.8	34.0	52.5	69.3	34.4	66.9	79.5	137.6	
12	5		85.4	13.0	51.0	34.4	69.4	57.0	62.4	61.3	60.0	138.7	220.0

Birth order was a significant predictor of mortality due to foetal asphyxia in the large breed group (Appendix 14). For every increase in birth order the odds of mortality due to foetal asphyxia increased by 9.99x ($p = 0.003$). Similarly, in the giant breed (Appendix 15) for every increase in the birth order the odds of mortality increased by 142.53x ($p = 0.005$).

Pup Sex

The sex of the pup was not a significant predictor variable in any of the statistical analyses performed.

Pup Presentation

The pup presentation was a significant predictor of mortality due to foetal asphyxia and fading puppy syndrome (Appendix 10). The odds of mortality due to foetal asphyxia increase by 1.64x ($p = 0.02$) when the pup is in posterior rather than anterior presentation. The odds of mortality due to fading puppy syndrome decreased by 0.42x ($p = 0.02$) when a pup was born in posterior rather than anterior presentation.

In the toy breed group the odds of mortality due to foetal asphyxia increased by 3.90x ($p = 0.001$) when the pups birth presentation was posterior rather than anterior (Appendix 11).

Placental Attachment

Placental attachment is a significant predictor of mortality due to foetal asphyxia in the toy breed group (Appendix 11). The odds of mortality decreased by 0.34x ($p = 0.01$) when the pup is born with the placenta attached.

2:2:5 DYSTOCIA

The incidence of dystocia encountered in this study was 35.6%, that is, 176 whelpings required some form of assistance (plus six elective caesareans).

Losses attributed to dystocia were 24.4% of total mortality and 4.5% of all pups whelped. In decreasing order, the predominant causes of pup mortality associated with dystocia were maternal secondary uterine inertia (2.0% of all pups born and 10.9% of total mortality), foetal disproportion (1.2% of all pups born and 6.7% of total mortality), maternal primary uterine inertia (0.9% of all pups born and 4.6% of total mortality) and foetal presentation (0.4% of all pups born and 2.1% of total mortality).

The incidence and form of dystocia encountered varied significantly between the breeds surveyed (Table 2.30). In the Pekingese, dystocia occurred in 85.7% of whelpings, with foetal disproportion accounting for 46.7% of the problems.

In the three other toy breeds (Chihuahua, Miniature Dachshund and Australian Silky Terrier), foetal disproportion was again the predominant cause of dystocia (35.2%, 58.8% and 66.7% respectively). There was, however, a marked difference in the incidence of dystocia in these toy breeds, with the Australian Silky Terrier recording the lowest rate of 12.2%.

Table 2:30 The occurrence of dystocia in selected breeds.

Breed	Number of Litters	Chemical Assistance	Manual Assistance	Caesarean Section	Total Dystocia	Primary Cause
Shetland Sheepdog	64	0.0%	1.6%	9.4%	10.9%	MPUI (85.7%)
Chihuahua	53	5.7%	7.5%	18.9%	32.1%	FD (35.2%)
Australian Silky Terrier	49	2.0%	4.1%	6.1%	12.2%	FD (66.7%)
German Shepherd	42	28.6%	9.5%	7.1%	45.2%	MSUI (73.7%)
Pekingese	35	5.7%	20.0%	60.0%	85.7%	FD (46.7%)
Dachshund Miniature	31	3.2%	22.6%	29.0%	54.8%	FD (58.8%)

Border Collie	24	16.7%	12.5%	12.5%	41.7%	MPUI (60.0%)
Great Dane	23	39.1%	0.0%	21.7%	60.9%	MSUI (78.6%)
Golden Retriever	22	0.0%	0.0%	9.1%	9.1%	MPUI (100%)
Labrador	21	0.0%	19.0%	33.3%	52.4%	MPUI (63.6%)
Collie Rough	17	5.9%	5.9%	5.9%	17.6%	MPUI MSUI and FD
Boxer	16	6.3%	0.0%	25.0%	31.3%	MPUI (60.0%)
Australian Cattle Dog	16	0.0%	6.3%	12.5%	18.8%	MSUI FD and FP
Basset Hound	12	8.3%	25.0%	33.3%	66.7%	MSUI and FD
Rhodesian Ridgeback	10	0.0%	10.0%	10.0%	20.0%	MPUI and FD

MPUI: Maternal primary uterine inertia; MSUI: Maternal secondary uterine inertia; FD: Foetal disproportion; FP: Foetal Presentation

The second highest incidence of dystocia was in the Basset Hound (66.7%), primarily due to both maternal secondary uterine inertia and foetal disproportion. This was followed by the Great Dane (60.9%), with maternal secondary uterine inertia being the principal cause of dystocia.

Caesarean Section

The necessity for caesarean section varied between breeds from 60% in the Pekingese to 4.8% in the German Shepherd.

The mortality recorded in litters delivered by caesarean section depended on the reason the caesarean section was performed (Table 2:31).

Forty-eight percent of caesarean sections were performed because of maternal primary uterine inertia.

Foetal disproportion accounted for 17.3% of caesarean sections performed. Both maternal primary uterine inertia and foetal disproportion recorded perinatal mortality rates well below those associated with maternal secondary uterine inertia and foetal disproportion.

Table 2:31 Mortality Relative to Caesarean Section.

Form Of Dystocia	Number of Litters	Stillbirths	Early Neonatal Mortality	Perinatal Mortality
Maternal Primary Inertia	47 (48.0%)	8.7%	9.8%	18.5%
Maternal Secondary Inertia	19 (19.4%)	15.4%	19.5%	35.0%
Foetal Disproportion	17 (17.3%)	10.2%	6.8%	17.0%
Foetal Presentation	9 (9.2%)	18.7%	25.0%	43.8%
Elective	6 (6.1%)	0.0%	40.7%	40.7%

19.4% of the caesarean sections performed were for maternal secondary uterine inertia and was associated with a high level of perinatal mortality (35%). Abnormal foetal presentation was the least frequent form of dystocia encountered (9.2%) but recorded the highest perinatal mortality rate of 43.8%.

Breeders may request elective caesarean sections for a number of reasons such as bitch age, previous whelping history or breed. Perinatal mortality in the elective caesareans was 40.7%. This high loss was associated primarily with mismothering/mismanagement in two litters. The first was a litter of six Saint Bernard pups, where the bitch was euthanased after surgery because of a haemangiosarcoma. Four of the pups died because they were kept in a cardboard box, in front of a fire and fed goat's milk. In a second litter all five pups died. In this case the bitch never settled and nursed the pups, because of continual interference by a number of people including children.

Caesarean section relative to maternal age and parity is summarised in Table 2:32.

There appeared to be no relationship between the necessity for caesarean section and maternal age or parity. This result may be influenced by breeder selection. Breeders tend to cull poor performing bitches and the caesarean sections reported in the higher age and parity groups may reflect this culling.

Table 2:32 Caesarean section relative to maternal age and parity.

Maternal age (years)	Number of litters	Caesarean section	Maternal parity	Number of litters	Caesarean section
1	84	16.7%	1	194	17.0%
2	108	14.8%	2	133	21.8%
3	91	24.2%	3	76	17.1%
4	72	19.4%	4	42	26.2%
5	65	21.5%	5	32	12.5%
6	38	23.7%	6	12	0.0%
7	25	20.0%	7	7	28.6%
8	11	16.2%	8	1	0.0%
9	4	50.0%	9	2	0.0%

The results indicated that at both parity one and two, one and two-year-old bitches had the lowest caesarean section rate. In parity one bitches the number of caesarean sections rose sharply in three-year-old bitches, then dropped. In parity two bitches the number of caesarean sections tended to increase with increasing age. At parity three, the number of caesarean sections decreased with increasing age until five years of age. More litters are necessary to confirm and investigate the significance of this relationship.

Figure 2:20 graphs the caesarean section rate for bitches of different ages in parity one, two and three.

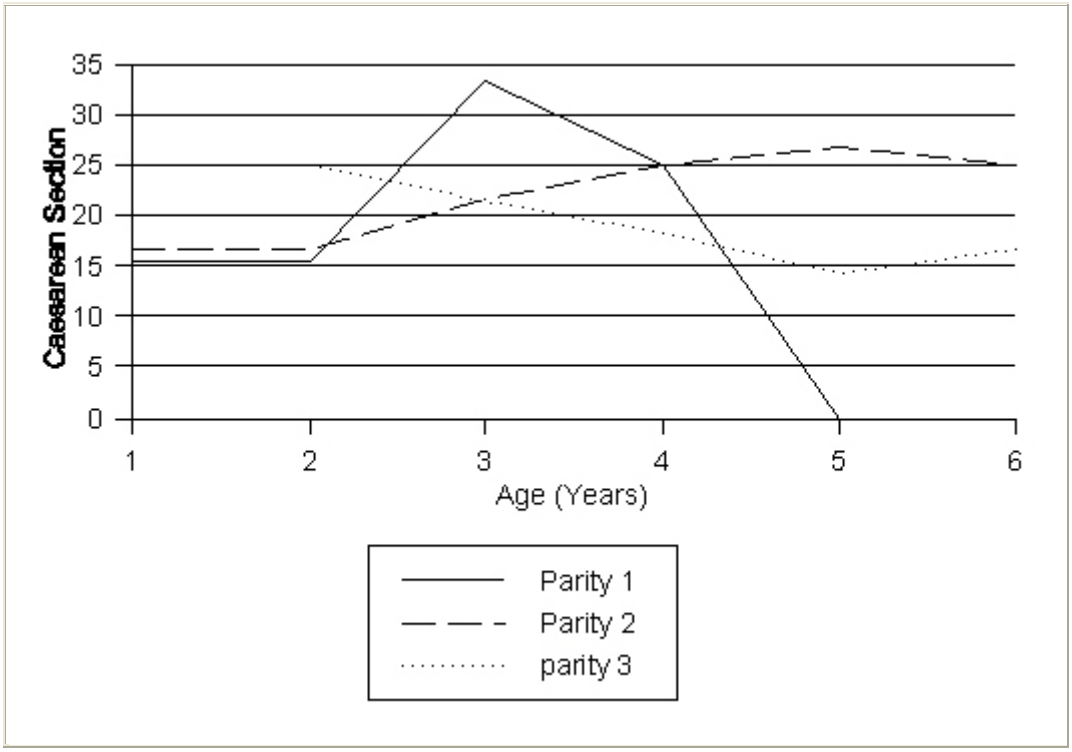

Figure 2:20 Caesarean section relative to Age:Parity relationship

2:3 DISCUSSION

This epidemiology study differed from previous reports on perinatal and late neonatal mortality in the dog in three ways.

Firstly, the whelpings were supervised and therefore detailed histories of both the parturition and the clinical condition of the pup at birth were available.

Secondly, a simple mortality classification based on the clinical assessment of the pup was used. This approach was adopted because the classification mimicked the limited clinical history available to a clinician when requested to investigate a pup mortality problem. Except for gross congenital defects and most of the sudden illnesses, post mortem findings were not used in the mortality classification.

Thirdly, both veterinary and breeder intervention was encouraged. These results therefore identified the current clinical problem that existed and did not provide information on the "natural incidence of pup mortality", as provided in the reports of Andersen (1957) and Potkay and Bacher (1977).

The influence of the breeder on pup mortality could not be measured. In the older age group of bitches, breeders tended to breed from those dogs that have a good whelping and/or mothering history. Therefore the frequency of dystocia and pup mortality in this group may not be a true representation. Similarly it was difficult to assess the influence of mismothering and mismanagement on pup mortality. There was a large variation in breeder experience and competence and most breeders were reluctant to admit to, or were unaware of, factors that constituted mismothering / mismanagement. Confirmed losses attributed to mismothering / mismanagement only accounted for 4.2% of the total mortality.

Late neonatal losses of 1.7% were significantly lower than that previously reported. It is in this age group that infections, accidents and environmental influences (that is, miscellaneous deaths) were the primary causes of pup mortality and the reduction in losses may reflect improved hygiene, vaccination and worming programs and greater attention to pup care by the breeder.

There were significantly higher numbers of late neonatal deaths in Spring. These losses were due to the occurrence of Parvovirus and Herpes virus infections. The prominence of infectious diseases in this period may be the result of the *in utero* transfer of immunoglobulins. It has been shown that even without the ingestion of colostrum, a pup is usually protected for at least one week due to a small amount of *in utero* transfer of immunoglobulins (Appel and Gillespie, 1972).

It was during the perinatal period that the majority of pup deaths occurred (90.9%) and over half of these pups were either stillborn or died in the first 24 hours (66.0%). Losses that could be directly attributable to factors that were present before or during parturition (that is, abnormal pups and deaths attributed to foetal asphyxia) accounted for just over two thirds of the total mortality (68.8%).

The age distribution of pup losses due to maternal illness or mismanagement and fading puppy syndrome (day two and three respectively) may be related to the neonatal hepatic glycogen reserves. The newborn relies almost exclusively on hepatic glycogen for energy for

the first 24 hours. Failure to suck results in rapid depletion of the liver reserve of glycogen and the development of hypoglycaemia by the second day (Center *et al.*, 1990). Severe hypoglycaemia may also lead to a decrease in mean arterial blood pressure which is believed to be related to the inhibition of contractile processes in the myocardium and/or smooth muscle in the peripheral vasculature (Hernandez *et al.*, 1980). This suggests that despite breeder's assessment that these pups were normal at birth and suckling, inadequate nutritional intake may be a important factor in these losses. This result emphasises the necessity to critically evaluate the clinical condition of the pup at birth and in the first 24 hours after birth.

There were significantly higher numbers of early neonatal deaths in Winter. All breeders surveyed whelped the bitches inside the house and provided supplementary heating. Breeders believe that it is easier to keep a pup warm in Winter compared to cooling it in the extreme Summer temperatures. The higher pup mortality in Winter contradicts this theory, but pup losses may not necessarily be directly attributable to the cold. In some instances breeders may inadvertently provide too much heat, making it uncomfortable for the bitch to stay in the whelping box and nurse the pups effectively. It is probable that some of these losses in the Winter period are therefore either management or mothering losses.

In order of importance, the causes of pup mortality in this study were foetal asphyxia due to dystocia, foetal asphyxia with no obstetrical cause, fading puppy syndrome, gross congenital defects, miscellaneous causes, small for date pups, mismothering/ mismanagement, death prior to birth and mummified pups.

Foetal asphyxia, due to recognised dystocia and with no obstetrical cause, accounted for 7.9% of all pups born and 42.5% of the total mortality.

Deaths in this study that could be directly attributed to dystocia accounted for 24.4% of all losses. Without breeder or veterinary intervention these losses would be much higher. The incidence of dystocia in this study 35.6%, that is, approximately one in three whelpings required some form of assistance.

The most frequent form of dystocia encountered was maternal secondary uterine inertia (35.8%) principally in the large and giant breeds of dogs. This was followed by foetal disproportion (31.3%), primarily in toy breeds, maternal primary uterine inertia (26.7%) and foetal presentation (6.3%). These results contrast with those reported by Darvelid and Linde-Forsberg (1994) who reported maternal primary uterine inertia (primary complete uterine inertia: 48.9%) as the most frequently encountered form of dystocia, followed by maternal secondary uterine inertia (primary partial uterine inertia: 23.1%), foetal presentation (malpresentation: 15.4%) and foetal disproportion (birth canal too narrow and oversized foetus: 7.7%). The higher incidence of foetal disproportion reported in this thesis is due to the fact that nearly half of these were relieved by the breeder at home. Lawler (1989) specified dystocia and prolonged labour as significant causes of early death but cited no references.

The incidence of apparent fading puppy syndrome was 3.4% of all pups born and 18.5% of all losses. These losses were well below those reported by Blunden (1986) where deaths attributed to fading puppy syndrome were 15.8% of all pups born, but are comparable to deaths attributed to unknown causes in both Andersen's (1957) and Potkay and Bacher's (1997) reports (4.0% and 2.4% respectively).

Grossly recognised congenital defects accounted for the death of 2.8% of all pups born and 15.5% of total losses. This was higher than previously reported and does not include those defects identified later in life. Not all pups in this study were available for post mortem and it is probable that the incidence of congenital defects was in reality higher.

Intrauterine death prior to whelping was recognised by the birth of mummified pups and pups that had died "prior to birth". Total foetal loss was 1.2% of all pups born. This probably represented only a small proportion of the total intrauterine loss that occurred.

The total mortality in litters whelping mummified pups was 50%. There was a significant association between mummification and losses due to small for date pups and foetal distress. The reason for this was unclear, but these results suggested the possibility of an common intrauterine aetiological agent in some litters.

The total mortality in litters whelping pups which had died prior to birth was 56% and there was a significant association with pups dying from fading puppy syndrome in some of these litters. The cause for this is again unclear, but suggests a common intrauterine agent may be implicated in some litters.

The three most consistent predictors of mortality were birth weight, inter-pup intervals and total whelping time.

Average birth weight were a marginal predicator of mortality with the odds of a pup dying increasing as average birth weight decreased. This analysis did not allow for the variation of normal birth weight in the different breeds and simply indicated that pups from the smaller breeds had marginally increased odds of dying.

More pivotal was the decreasing average birth weights which significantly increased the odds of mortality due to abnormal pups, foetal distress and fading puppy syndrome in almost all the statistical analyses performed.

While these analyses identified the importance of birth weight to the survivability of the pup, they did not identify the critical weight below which the pup is compromised.

Clinically identified small for date pups accounted for the death of 0.9% of all pups born and 4.8% of the total mortality. A more appropriate definition for these small for date pups is intrauterine growth retardation. This identifies the pathological process that compromised these pups. In the infant, the limited nutritional and circulatory reserves of such a foetus make even normal labour and delivery, in itself a hypoxic stimulus, a more stressful event. The higher rate of antepartum and intrapartum stillbirths in this group attests to this concept (Renfield, 1975). Such pups are also at greater risk from hypothermia and can not compete well for milk against their larger litter mates (Mosier, 1978).

For the purpose of this study it was assumed that pups below one standard deviation lower than the mean bodyweight for the breed were growth retarded. Using this definition, 14.5% of pups born were growth retarded and their perinatal mortality was 38.4%. Therefore the death of 5.6% of all pups born and 29.4% of the total mortality may be associated with growth retardation. Also, 35.2% of the apparent fading pups and 26.7% of pups that died because of foetal asphyxia during an apparently normal whelping, were growth retarded.

The possibility that intrauterine factors may be involved with fading puppy syndrome was suggested by Blunden (1986). He found that 75% of fading pups that died by day three had marked growth arrest at the costochondral junction and it was possible that growth retardation may have commenced in late pregnancy. The probability that intrauterine factors are involved in the aetiology of some of the fading pups was also supported by the significant association of fading pups with pups that had died "prior to birth" in the same litter.

This definition of growth retardation presented an discrepancy that appeared to be unrelated to the breed. The percentage of mean birth weight that one standard deviation represented varied between breeds. This variability could not be attributed to either adult weight or insufficient numbers of pups surveyed in each breed. Such a discrepancy challenges the validity of this definition of growth retardation. Similarly, it challenges **Mosier's (1978)** definition of a small for date pups being less than 25% below average birth weight. It does, however, explain the difficulty a breeder may have in identifying clinically growth retarded pups.

The diagnosis of growth retardation relative to standard deviation from the mean birth weight or as a percentage of average birth weight needs to be investigated further. However, this should not detract from the significance of growth retardation and the associated limited nutritional and circulatory reserves, being a primary contributing factor to perinatal mortality in the pup.

Increasing inter-pup intervals were significant predictors of increased mortality in the toy, small and giant breed groups. It was also a significant predictor of increased mortality due to foetal distress in the overall pup data, and in the toy and giant breeds

The total whelping times were a non-significant predictor of the proportion of mortalities within a litter in the overall statistical analysis. However, it was significant in the toy and giant breeds with the odds of a pup dying increasing for every ten minute increase in the total whelping time.

While the statistical analysis identified the significance of whelping times and inter-pup intervals, they did not define the critical times or intervals, above which, foetal health is compromised. There were two findings in the epidemiological study that from a practical point of view, were important. First, in litters where no mortalities were recorded and no assistance was required during the whelping, the average inter-pup interval was less than 60 minutes. Secondly, there was a marked increase in perinatal mortality, primarily due to foetal distress, when the inter-pup interval was greater than one and a half hours. When the inter-pup interval was greater than four hours, all losses reported were stillbirths.

The evaluation of the influence of whelping times on perinatal mortality presented a number of limitations. Breeders had difficulty identifying accurately the start of both first and second stage labour. Therefore, while these times were initially intended to be part of the study, they were abandoned. Breeders were simply requested to record the time each pup was born. This approach made it easier for breeder compliance and reduced the chance of error. It did not however take into account the two parts of the interval between birth, that is, resting and straining. Within these limitations, the results have demonstrated that, without a method of monitoring the pups for foetal distress *in utero*, the currently recommended time constraints are excessive.

Relative to birth order or litter position, perinatal mortality was highest for the last pup born in a litter. This tended to coincide with extended inter-pup intervals. A simplistic explanation for this would be maternal exhaustion and prolonged exposure to intermittent periods of anoxia that occurs during each contraction. If foetal asphyxia was the sole reason then a pattern of increased mortality with increasing litter position would be expected. This did not occur and therefore other factors must also be involved.

It was only in the large and giant breeds that birth order was a significant predictor of mortality due to foetal distress. For every increase in the birth order the odds of mortality increased. Inter-pup intervals greater than 60 minutes in litters with no mortalities occurred after pup seven (Table 2:25, page 128). The inter-pup intervals and mortality relative to litter position (tables 2:28 and 2:29, pages 139 and 140) also suggested pup seven in the whelping order may be the critical number above which foetal asphyxia becomes significant.

Litter size was not an important predictor of the proportion of mortalities within a litter. However, the statistical analysis of litter size did not allow for the different litter size ranges that occurred in the various breed groups.

Litter size was a significant predictor of mortality in the toy breeds with the odds of mortality decreasing as the litter size increased. At litter size one and two mortality was primarily due to foetal asphyxia, principally as a result of foetal disproportion.

In the large breeds, litter size one presented the greatest risk to the pup with a total mortality of 62.5%, which coincided with the highest average birth weight and losses were primarily attributed to foetal distress associated with maternal primary uterine inertia. From this result, the recommendation for a singleton pup in a large breed would be an elective caesarean section. Alternatively, if a singleton occurs in a young bitch, abortion of foetus may be more economically viable.

Maternal age was not an important predictor of the proportion of mortalities within a litter. In this study, the loss in one-year old bitches was 11.1% and at nine years, 33.3%, but this result was not significantly different. Breeder culling for poor reproductive performance may have modified these results and they may not be a true indicator of the natural incidence of pup mortality. Andersen (1965) reported a neonatal mortality of 18.5% when dams were two years old, increasing to 38.9% at seven years and 76.3% at nine years of age. These two contradictory results suggest that, while pup mortality increases with maternal age, older bitches with good reproductive histories may still be bred from.

Parity was significant with the odds of a pup dying increasing for each subsequent parity. Why increasing parity and not age was a significant variable is difficult to explain.

Of possibly greater significance was the influence of the age:parity relationship on perinatal mortality. Parity one bitches recorded increasing perinatal mortality with increasing age. It would therefore be recommended that bitches should have their first litter between the age of one and two years. Deferring breeding for show reasons etc., resulted in increased pup losses.

The results also showed that the average age for the first litter in the toy breeds was 1.5 years and in the large breeds it was 2.1 years. This pattern of breeding reflected the breeder's belief that large breeds matured slower than the small breeds. There were insufficient litters available to investigate the validity of this observation.

Bitches whelping their second litter had a perinatal mortality lower than parity one bitches. This reduction in mortality may have been due to both improved maternal care of pups by an experienced dam and possibly an easier whelping associated with a second litter. In addition to this, the breeder may have been more aware of the bitch's capabilities and corrected any obvious causes of previous pup losses.

Toy breeds were bred from more frequently than the large breeds. The age:parity three relationship indicated that breeding too frequently increased the caesarean section rate or perinatal mortality and bitches should have their third litter at four or five years of age.

The age:parity relationship was not statistically analysed because of it's complexity and insufficient numbers of litters in some of the groupings. The gross results suggest that an analysis is warranted.

Posterior presentation was a significant predictor of mortality due to foetal asphyxia with the odds increasing when the pup is in posterior rather than anterior presentation. However, in the breed group analysis it is only in the toy group that presentation was a significant predictor with the odds of mortality nearly four times more, if it is born in posterior presentation. Similarly, placental attachment was only a significant predictor of mortality in the toy breeds. The odds of mortality decreased by when the pup was born with the placenta attached. In the toy breeds it is the combination of small litter size, posterior presentation and detachment of the placenta that present the greatest risk in this group.

The diagnosis of fading puppy syndrome was depended the clinical assessment of the pup as normal at birth. The birth weights of 35.2% of these apparently normal pups were below one standard deviation lower than the mean for the breed, that is, growth retarded. This appeared to be a significant problem in the toy breeds with large litters. For the remaining fading pups there were very few significant predictors of mortality identified. Parity was a significant predictor with the odds of mortality increasing for each subsequent parity. Unexplainably, the odds of mortality decreased by 0.416 when the pup was in posterior presentation. In the medium breeds, maternal age was a significant predictor with the odds increasing for each increase in age by one year. There was no obvious reason to explain this singular result.

These results validate the statistical analysis of whelping data but the analysis also points out the limitations in the conclusions that can be drawn. These limitations are a consequence of the variability of the mortality pattern that occurred between breeds, litter sizes and birth weights and a complex interaction of predictor variables that increase the odds of mortality. In addition, breeders influenced the age and frequency at which bitches were bred from and breeder or veterinary intervention altered outcomes in some litters.

The interpretation of the statistical results is best done on an individual breed basis in those breeds where the mortality pattern has been identified. If we examine five breeds the value of this approach can be demonstrated.

The **Miniature Dachshund** recorded the highest total mortality of 28.5%. The principal cause of mortality was fading puppy syndrome followed foetal asphyxia. The incidence of dystocia in this breed was 54.8%, of which foetal disproportion was the primary problem. The risks identified in this breed occur in the larger litter sizes, with growth retardation and apparent fading puppy syndrome. In the smaller litters of one and two pups it is foetal disproportion in combination with posterior presentation, detached placenta and a long inter-

pup whelping interval. However, this finding did not correlate with average birth weights greater than one standard deviation above the mean for the breed at these litter sizes. The diagnosis of foetal disproportion was made when the history indicated that the pup was too big, but there was no distinction between foetal oversize and small maternal birth canal. The birth weights of these pups suggested that the fault was bitch size and more careful selection of brood bitches by the breeder would be required.

The **Shetland Sheepdog** has a total mortality of 21.8% and the principal cause of loss was the birth of abnormal pups followed by foetal asphyxia. The incidence of dystocia in this breed was 10.9%, of which maternal primary uterine inertia was the principal problem. The Shetland Sheepdog recorded high losses due to mummification (1.6%), small for date (3.2%) and gross congenital defects (3.6%). There were no significant predictors of mortality due to the birth of abnormal pups except birth weight. The odds increased with decreasing birth weight, however this is the result of the abnormality rather than a contributing cause. This breed has limited genetic diversity in Australia and inbreeding may be a contributory factor. The principal diagnostic problem associated with maternal primary uterine inertia was that anticipated whelping dates were based on mating dates and not ovulation. It is not know what number of these caesarean sections were unnecessary.

The **German Shepherd** and the **Rhodesian Ridgeback** also had the birth of abnormal pups as the primary cause of mortality (8.5% and 6.6% respectively). In the Rhodesian Ridgeback this could be attributed to the presence of the dorsal sinus which is a specific genetic problem in the breed. In the German Shepherd, congenital defects were the principal component (4.5%) of mortality. This could not be attributed to inbreeding or line breeding because there was a continuous infusion of imported German stud dogs into Australia. It would be interesting to determine the occurrence of congenital defects in this breed in Germany as many, but not all, congenital defects are genetically caused (Leipold, 1978).

The **Great Dane** has a total mortality of 23.1%, and the principal cause of mortality was foetal distress which occurred during apparently normal whelpings and fading puppy syndrome. The incidence of dystocia 60.9% with maternal secondary uterine inertia the primary problem. Both birth order and inter-pup intervals were significant predictors of mortality due to foetal distress. Therefore the risk in this breed is in litter sizes greater than seven pups and prolonged inter-pup intervals. This suggested that earlier veterinary intervention in the form of either chemical assistance (oxytocin with or without calcium) or caesarean section would be necessary to reduce losses. There were no significant predictors of mortality due to fading puppy syndrome in the Great Dane.

When the definition of breed groups was based on the adult body weight, there was no significant difference in the total mortality between the breed groups.

Each breed studied exhibited a specific mortality pattern and the results of one breed could not be used to anticipate the outcome in another breed, with the exception of the predictor variables identified in the breed groups. The veterinary profession requires a vastly expanded data base of the whelping performance for the different breeds of dogs before the full extent of perinatal mortality can be appreciated and the causes defined. Further, any recommendations to reduce perinatal mortality must be breed specific.

CHAPTER 3 : PATHOLOGY OF PERINATAL MORTALITY IN THE DOG

3:1 INTRODUCTION

Pup losses attributed to foetal asphyxia and fading puppy syndrome accounted for 61.0% of the total mortality reported in the epidemiology study (Chapter 2). The diagnosis of these probable causes of pup death was based on both the whelping history and clinical assessment of the pup by the breeder and/or the veterinarian in the immediate postpartum period.

Foetal asphyxia was assumed to be the aetiological factor responsible for death of pups in the intrapartum period (stillborn) and pups born in a clinically distressed condition which subsequently died. These pups were considered to be of normal development and foetal health compromised during the whelping process. Just under a half of these were deaths with no obstetrical explanation.

Losses attributed to fading puppy syndrome were based on the clinical assessment of the pups by the breeder as, "normal at birth", failure to thrive, and subsequent death in the post partum period. It was hoped that the accurate clinical assessment and monitoring of pups would eliminate intrapartum and maternal factors such as birth injuries, poor mothering instinct, agalactia etc., and give a reliable representation of pups that "fail to thrive".

Since these diagnoses are subjective, a pathological study was conducted to investigate the validity of the clinical diagnosis of foetal asphyxia and fading puppy syndrome. This involved a detailed post mortem and histopathological investigation of pups presumed to have died from foetal asphyxia and fading puppy syndrome.

3:2 MATERIALS AND METHODS

The pups examined in this study were from from whelpings reported in the epidemiology study and therefore the same problems of incomplete histories occurred (Chapter 2, section 2:2:1). Not all pups included in the epidemiology study were available for post mortem as either breeder compliance or distance made them unavailable.

Breeders were requested to store dead pups in the refrigerator and to deliver them to the clinic as soon as possible. The time between death and post mortem varied from minutes to a number of days and consequently a number of pups, and more commonly a large number of brains, were too autolysed for use in the study.

Some pups were examined before they died and medical treatment and supplementary feeding instigated.

The Post Mortem

The skin was cut along the mid line and removed from the body. The subcutaneous tissue was examined for evidence of bruising, haemorrhage or oedema.

The abdomen and chest were opened along the midline and abdominal and thoracic viscera examined for normality. The rectum was examined for presence or absence of meconium.

When peritoneal haemorrhage was identified, the umbilicus and viscera were examined for evidence of traumatic injury. Similarly in the chest cavity, the ribs were checked for evidence of fractures.

The oral cavity was examined and the opening extended by cutting through the temperomandibular area. The length and shape of the soft palate, as well as the larynx, cervical trachea and oesophagus were examined. The trachea was opened to the level of the bifurcation to check for evidence of meconium aspiration, haemorrhage or other obstructive plugs.

Abnormal organs were collected and preserved in formalin. Lung sections which floated in formalin were considered indicative of a live birth.

The brain was exposed by removing the frontal and parietal bones and removed by first cutting through the olfactory bulbs, and tipping out the brain while systematically cutting the cranial nerves. The entire cerebrum, cerebellum and brain stem were collected in this manner. The brain was preserved intact in formalin.

Post mortem findings including pup weight, evidence of external trauma and gross changes were recorded.

Staining

The collected samples were embedded in paraffin wax, sectioned, mounted on glass slides and stained routinely with haematoxylin and eosin. These sections were then examined for histopathological changes. Because of financial constraints, bacteriology and virology were not performed.

3:3 PATHOLOGY OF FOETAL ASPHYXIA : STILLBORN PUPS

3:3:1 Introduction

Apparently normal but stillborn pups were presumed to have died as a result of foetal asphyxia in the intrapartum period. The gross post mortem and histopathological features of foetal asphyxia of stillborn pups is poorly documented in the veterinary literature.

Because the dog has been used extensively as an experimental model, we know that the intra-uterine response of the canine foetus to asphyxia is the same as the human foetus (Fazekas *et al*., 1941; Himwich *et al*., 1941; Glass *et al*., 1944; Mott, 1961; Shelly, 1961; Arango and Rowe, 1971; Duffy *et al*., 1981; Monheit *et al*., 1988). Consequently, it is assumed that asphyxial changes in the stillborn human and canine neonate are similar.

The purpose of this section of the thesis was to identify the post mortem and histological changes in stillborn pups and determine if the changes found are comparable to those reported in the asphyxiated stillborn infant or if there is evidence to incriminate other aetiological agents in these intrapartum deaths.

3:3:2 Materials and Methods

Fourteen apparently normal, stillborn pups were available for post mortem.

Dystocia was reported during the birth of eight of these pups. The other six pups died during whelpings in which no obstetrical cause was identified by the breeder. However, risk factors identified in the epidemiological study, which predispose pups to intrapartum asphyxial injury, were present in the whelping history of these six pups. They either were outside one standard deviation of the mean birth weight for their breed, were the last born in the litter or had inter-pup whelping intervals greater than one and a half hours.

3:3:3 Results

Gross Post Mortem Findings

The consistent post mortem finding was that the lungs showed no evidence of inflation. They were collapsed, a uniform dark red colour and when the sections of lung were put into formalin, they sank (Figure 3:1).

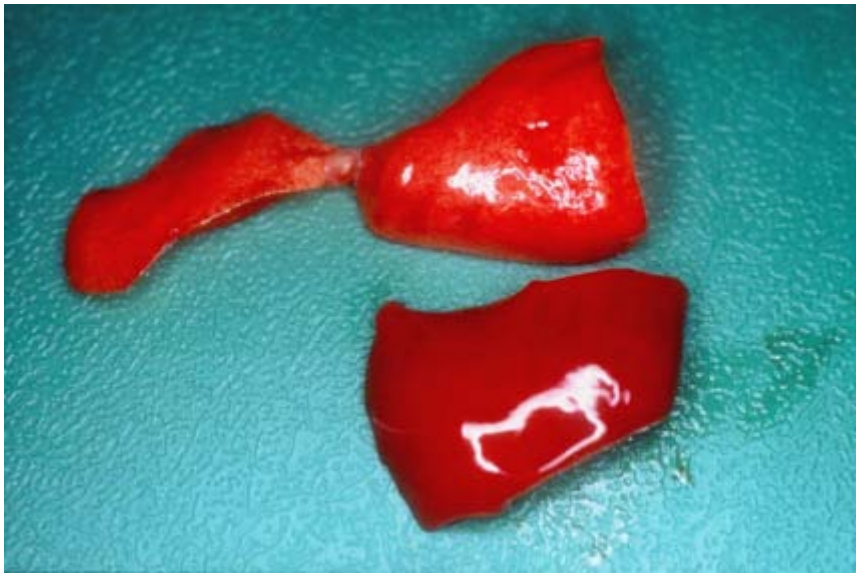

Figure 3:1 Gross appearance of the perinatal lung. Top: fully inflated lung from a live, day old pup. Bottom : uninflated lung from stillborn pup.

Leptomeningeal congestion and/or haemorrhage was present in thirteen of the fourteen pups (Figure 3:2).

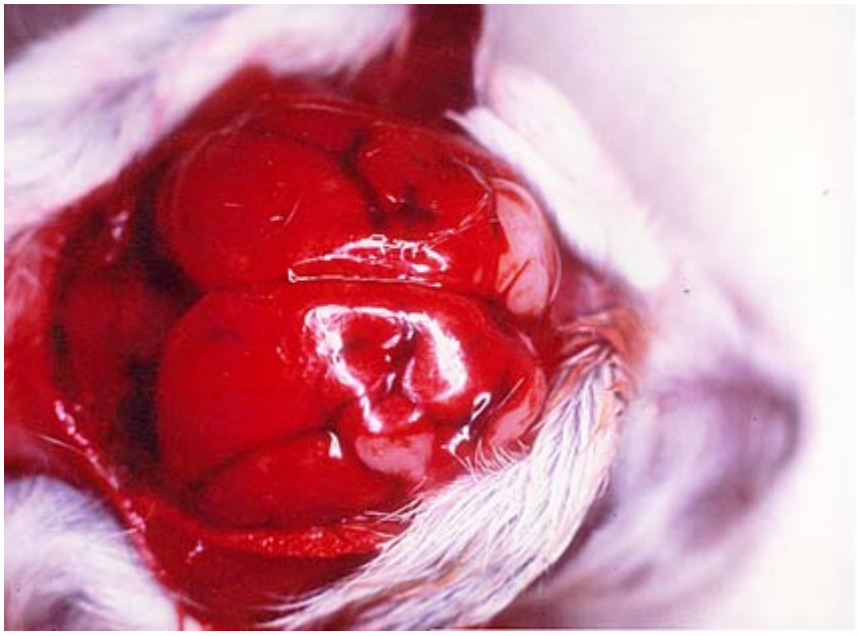

Figure 3:2 Gross leptomeningeal congestion and haemorrhage in a stillborn pup.

Subcutaneous oedema and/or bruising was identified in five pups. This was a consistent finding in those pups which required manual assistance for delivery (foetal disproportion). It occurred in one of the four pups whose death was attributed to maternal secondary uterine inertia and in one of the six pups where dystocia was not identified.

Table 3:1 Gross post mortem findings of stillborn pups (n=14)

Gross Post Mortem Finding	Incidence
Noninflated Lung	100%
Leptomeningeal congestion and/or haemorrhage	92.9%
Rectal evacuation of meconium	35.7%
Meconium staining	24.1%
Peritoneal haemorrhage	35.7%
Subcutaneous oedema and/or bruising	21.4%
Evidence of trauma	21.4%
Tracheal Meconium	14.3%

Evidence of trauma (liver rupture, peritoneal haemorrhage and clotting over the rupture site) was present in three pups. Peritoneal haemorrhage was also present in two other pups, but the site of haemorrhage could not be identified (Figure 3:3). Peritoneal haemorrhage was found in two of the three pups manually assisted (foetal disproportion), in one pup whelped after an inter-pup interval of 213 minutes, and in two pups whelped with no clinical evidence of dystocia.

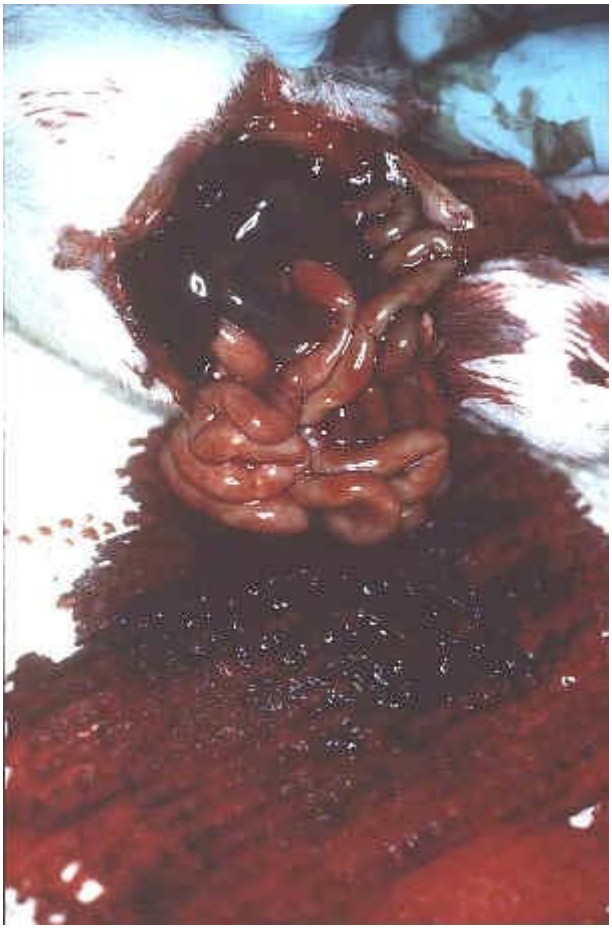

Figure 3:3 Gross abdominal haemorrhage. (Pup 271: Stillborn pup with no history of dystocia or evidence of trauma).

Rectal evacuation of meconium was detected in five pups, but breeders had recorded meconium staining in only three. Inhalation of meconium into the trachea was evident in two of these pups.

Histopathology

The histopathological changes identified stillborm pups is summarised in Appendix 20:1, 20:2 and 20:3. Acute systemic congestion was a consistent finding. This was evident in the liver, heart, kidney and brain. In 21.4% of pups petechial haemorrhages were seen in the renal medulla and/or the liver.

Table 3:2 The incidence of histopathological findings in stillborn pups (n=14)

Histopathological Finding	**Incidence**
Acute systemic congestion	100%
Leptomeningeal congestion/oedema	100%

Pulmonary parenchymal congestion	100%
Congestion/oedema of the choroid plexus of the lateral ventricles	100%
Alveolar macrophages	100%
Neonatal atelectasis	92.3%
Alveolar squames	92.3%
Cerebral vascular congestion	90%
Cerebral perivascular ring haemorrhages	80%
Alveolar meconium	46.2%
Petechial haemorrhages (renal medulla / liver / brain)	21.4%
Alveolar neutrophils	14.2%

Congenital (neonatal) atelectasis with variable degrees of lung inflation indicative of *in utero* premature respiration occurred in 12 of the 13 lungs examined (Figures 3:4 and 3:5). In the remaining pup there was almost complete inflation of the lung. There were alveolar squames in 12 pups (92.3%) and alveolar meconium in six (46.2%) pups (Figure 3:6). Pulmonary parenchymal congestion and the presence of alveolar macrophages were consistent findings to varying degrees in all pups. The severity of pulmonary congestion was greater in those pups with a history of dystocia during the whelping. There appeared to be no association with dystocia and the presence of alveolar macrophages. In two pups small numbers of alveolar neutrophils were identified.

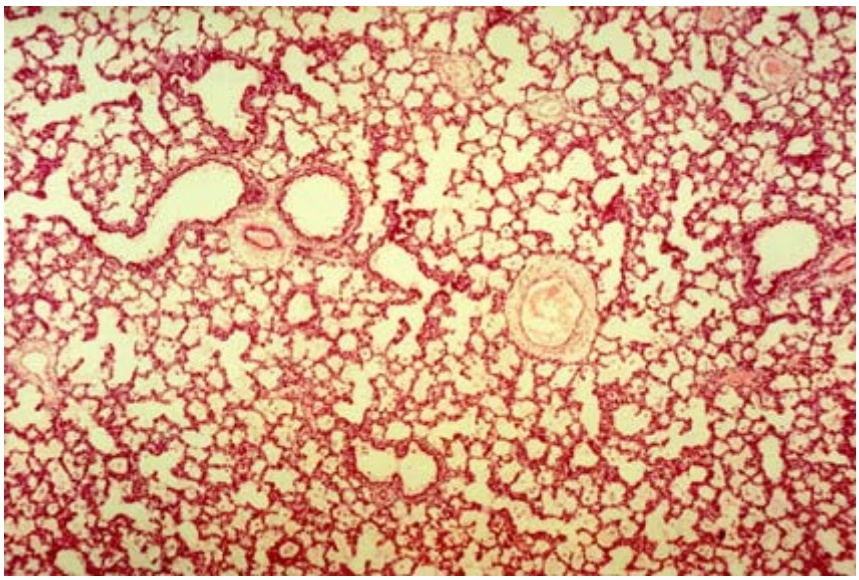

Figure 3:4 Normal inflated lung of a day old pup.

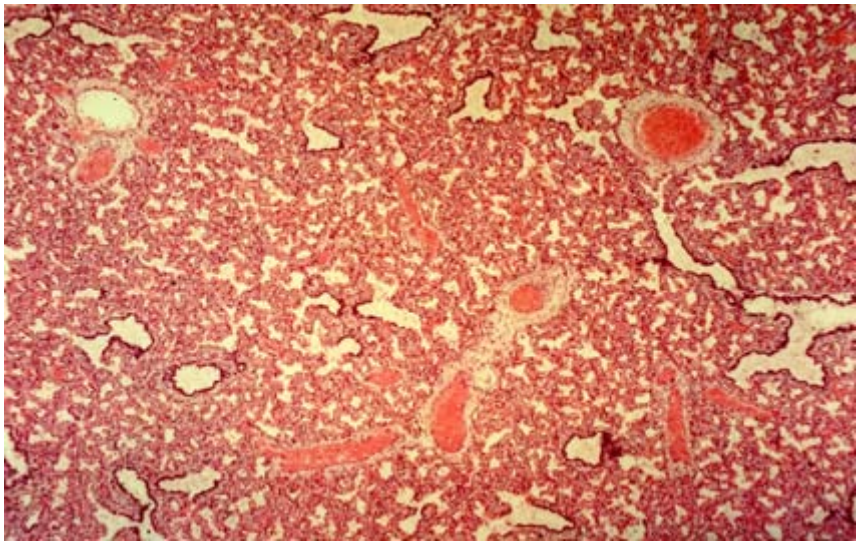

Figure 3:5 Neonatal atelectasis (Pup 1616)

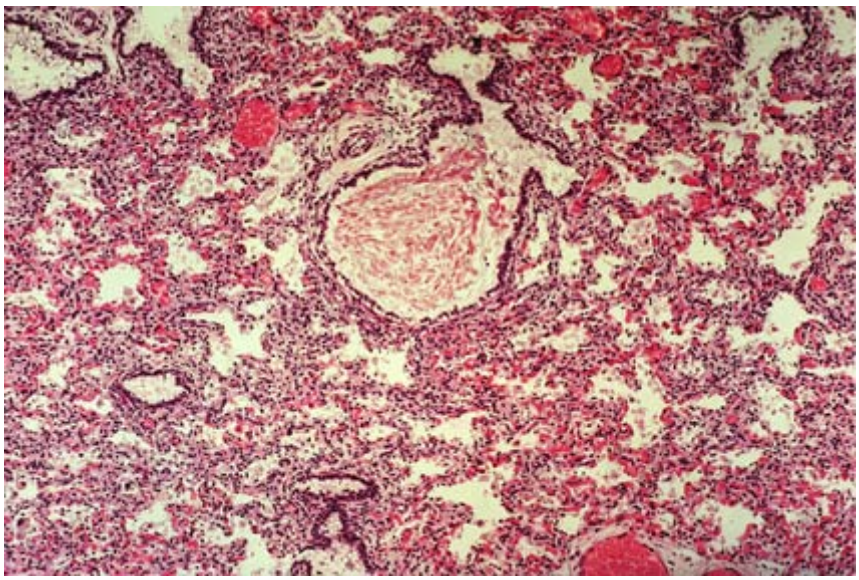

Figure 3:6 Neonatal atelectasis with aspiration of squames into both bronchi and alveolar lumina (Pup 1616)

The brains of ten of the pups were available for histopathology. The remaining four were too autolysed for examination. Mild to moderate leptomeningeal congestion and congestion/oedema of the choroid plexus of the lateral ventricles were consistent findings (Figure 3:7). Congestion of the cerebral blood vessels, perivascular ring haemorrhages and petechial cerebral haemorrhages were seen in 90%, 80% and 10% of brains examined.

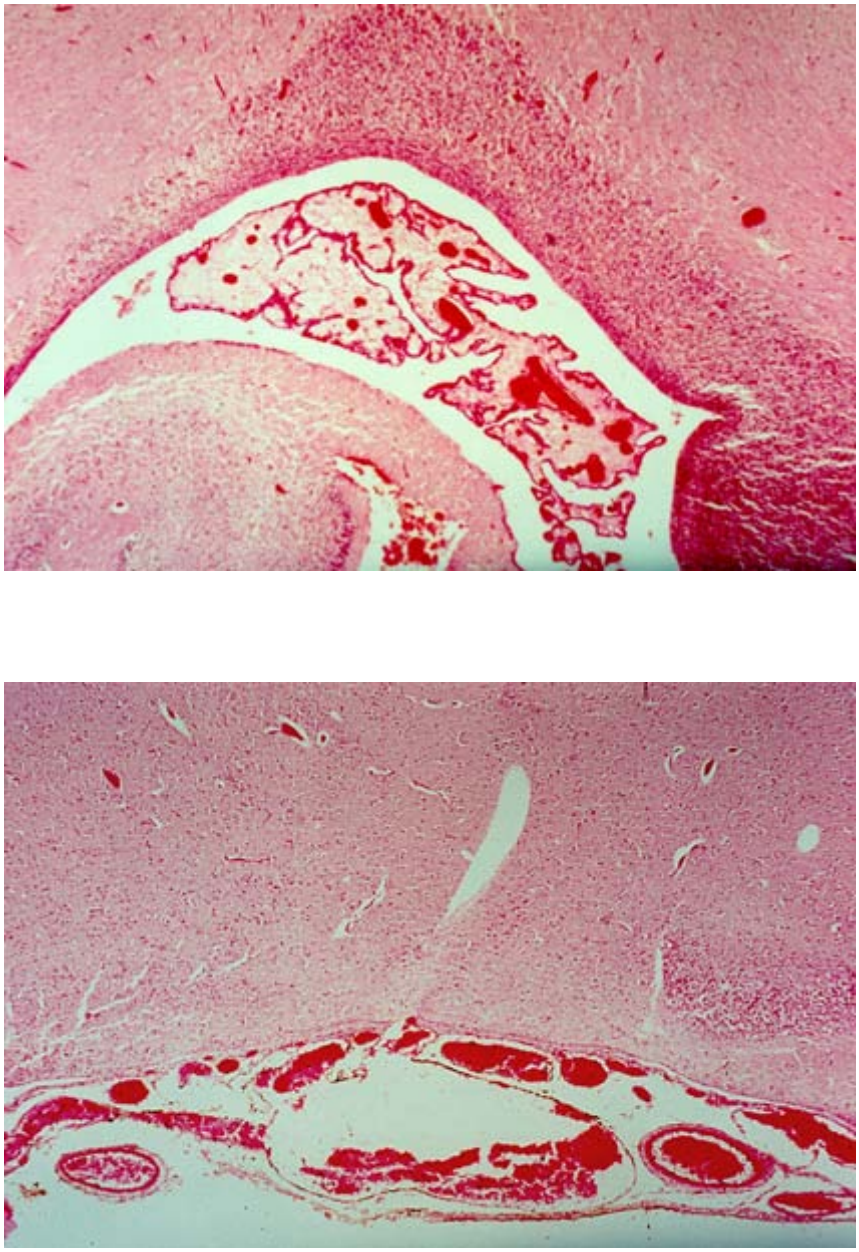

Figure 3:7 Severe diffuse, leptomeningeal and choroid plexus congestion and haemorrhage in a stillborn pup (Pup 646).

3:3:4 Discussion

The main internal features of acute asphyxia in the infant are congestion, with petechial haemorrhages into internal organs (Wigglesworth, 1991). In these stillborns pups, multi-system congestion, including pulmonary parenchymal congestion and congestion/oedema of the leptomeninges and choroid plexus of the lateral ventricles were consistent findings. Petechial haemorrhages were only occasionally.

The lung changes reported in the asphyxiated stillborn infant are acute congestion and haemorrhages seen at the gross level with capillary engorgement and bleeding into the interstitial tissue and alveoli histologically (Wigglesworth, 1991). Air spaces show a variable degree of expansion and there are often masses of inhaled squames and granular debris within the bronchi, bronchioles and down to the saccular level in some lobules. The lung changes identified in these stillborn pups were parenchymal congestion, variable degrees alveolar expansion, alveolar macrophages and alveolar squames.

All the pup lungs examined showed either variable degrees of alveolar inflation or alveolar squames which indicated that, at some stage, intrauterine premature respiration had occurred. Alveolar macrophages were present in all of the pup lungs examined. Mobilisation of alveolar macrophages into bronchial and particularly alveolar lumina can be an acute change and there was no evidence of subacute or more chronic pulmonary pathology predating parturition. The number of mobilised macrophages may be a reflection of both the volume of aspirated fluid and the interval between aspiration and death (J. Charles, personal communication). Small numbers of neutrophils were present in two pups that suggested early amniotic fluid infection.

Variable degrees of pulmonary congestion with capillary engorgement were consistent findings in pup lungs, however the interstitial haemorrhage, reported in the infant, was not identified in the pup and alveolar haemorrhage an infrequent finding.

The brain changes reported in the asphyxiated stillborn infant are usually non-specific, comprising little more than a generalized congestion without apparent brain swelling (Wigglesworth, 1991). In the stillborn pup, leptomeningeal congestion and haemorrhage that varied from mild congestion with the brain bathed in a bloody serous fluid to more significant haemorrhage along the transverse sinus were consistent findings. Histology confirmed the gross findings as well as oedema of the choroid plexus of the lateral ventricles. Congestion of the cerebral blood vessels and perivascular ring haemorrhages were also frequent findings.

Morison (1970) reports that most of the intrapartum stillborn infants will show relatively non-specific lesions of anoxia and the recognition of the conditions producing anoxia must depend on the clinical history rather than positive autopsy findings. The histopathological changes identified in these stillborn pups exhibited, for the most part, comparable non-specific lesions. There was no evidence to incriminate other aetiological agents in these intrapartum deaths.

The variable gross changes seen in these asphyxiated stillborn pups were meconium staining, peritoneal haemorrhage and subcutaneous oedema and/or bruising. They were not reliable indicators of foetal asphyxia or dystocia.

Anoxia causes increased intestinal peristalsis, relaxation of the anal sphincter and the passage of meconium (Potter and Craig, 1976). Meconium staining of the coat was reported for 35.7% of the stillborn pups examined. Obvious rectal evacuation and tracheal meconium were seen in 21.4% and 14.3% of stillborn pups respectively. Alveolar meconium was identified in 46.2% of the lungs examined but only two of these pups were reported to be meconium stained at birth. This suggested that breeders did not see meconium in the amniotic fluid, possibly because the bitch cleans the pups herself. These results indicated that 64.3% of the stillborn pups had passed meconium in the intrapartum period.

Peritoneal haemorrhage was present in five pups (35.7%). In three pups there was gross evidence of a liver rupture. These three pups had varying whelping histories, with two born during apparently normal births. The site of haemorrhage in the remaining two pups could not be determined. Peritoneal haemorrhage has been reported in the infant (Wigglesworth, 1991). Rupture of a subcapsular haematoma of the liver with resultant peritoneal haemorrhage is one of the most frequently recognised forms of soft tissue trauma in the infant. The risk of this lesion is enhanced by hepatic congestion or by operative delivery.

Subcutaneous oedema and/or bruising occurred in the pups requiring manual assistance for delivery because of foetal disproportion and one of four pups subjected to maternal secondary uterine inertia. It was also found in one of the six pups born during an apparently normal whelping.

In summary, the death of these apparently normal pups in the intrapartum period was confirmed to be due to asphyxia. On post mortem and histopathology pups had variable degrees of neonatal atelectasis, *in utero* hypoxia and multi-system congestion which are consistent with that reported in the hypoxic stillborn infant. There appeared to be no association between the severity of post mortem findings and the clinical histories of the whelpings. Pups that died during whelpings where no obstetrical cause was identified had gross and histological changes comparable to those stillborn pups with a history of dystocia.

3:4 PATHOLOGY OF FOETAL ASPHYXIA : LIVE DISTRESSED PUPS

3:4:1 Introduction

At birth, the presence of asphyxia (foetal distress) in the infant is recognised by the observation of respiratory activity and heart rate, muscle tone, skin colour and reflex activity (Meadow and Smithells, 1983). These clinical observations may be evaluated by the Apgar Scoring System and this method is still the most widely accepted indicator of intrauterine asphyxia.

For the epidemiology study (Chapter 2) it was assumed that these same criteria are indicative of intrauterine asphyxia in the canine neonate. Therefore the mortality classification of a pup born with clinical signs of hypotonia, cyanosis, slow to gasping respiration and/or a slow heart rate, which subsequently died, was foetal asphyxia.

The purpose of this section of the thesis was to identify the clinical progression, post mortem and histological changes in these distressed pups and to determine if they are comparable to those reported in the distressed infant or if there is evidence to incriminate other aetiological agents.

3:4:2 Materials and Methods

Thirty pups which were born distressed and subsequently died were available for post mortem. A pup showing any evidence of viability at birth, regardless of how long it lived, was judged liveborn.

Eleven pups were clinically distressed following a whelping where no obstetrical problem was identified by the breeder. Three pups were outside one standard deviation from the mean birth weight for their breed. Four pups were the last born in their litter and one pup had an inter-pup interval greater than 90 minutes. Therefore factors recognised in the epidemiology study as presenting a risk to the pup during whelping were present for eight of these 11 pups.

Seventeen pups were born distressed as a result of whelpings where clinical evidence of dystocia was noted by the breeder. Eleven of these were born by caesarean section.

3:4:3 Results

Clinical History

The clinical picture of the live distressed pup varied from those born gasping, hypotonic and cyanotic and died soon after birth through to pups which were revived successfully, appeared normal and subsequently died.

Nine pups were born gasping and hypotonic and could not be revived. Two other pups were born alive with good muscle tone but deteriorated rapidly and died within five minutes of delivery.

Thirteen pups, which were resuscitated, were never considered good by the breeder. One of these cried persistently and was euthanased on day one. Another pup was reported by the breeder as having persistent moist lungs and died on day three.

After resuscitation, the principal problem identified by the breeders was the inability of the neonate to suck properly. These pups had difficulty finding and staying on the teat or they were easily knocked off by a stronger pup. Often there was a persistence of hypotonia and poor locomotion in the whelping box. Sometimes the pups were observed to be disoriented in the box, crawling behind or away from the bitch and becoming chilled. The terminal stage was weight loss, dehydration, hypotonia and hypothermia.

One pup lived to ten days of age. This was a singleton pup from a large breed of dog, born by caesarean section because of primary maternal uterine inertia. The pup was severely distressed at birth, required very aggressive resuscitation and only survived to ten days of age, because of breeder assistance. Initially this pup could not suck of the bitch, but could suck from a bottle until day eight. When euthanased at day ten, the pup was non-responsive to external stimuli, was hypotonic and had no righting reflexes.

Six pups were considered normal following resuscitation and were found dead in the whelping box. Two other pups developed sudden respiratory distress after supplementary feeding. Inhalation of milk was thought to be the cause of the respiratory distress.

Gross and Histopathology of Pups Dying in the First 48 Hours

Twenty-one pups died in the first 48 hours after birth. Three of these were revived and then considered to be normal by the breeder. They were later found dead in the whelping box. All the remaining pups had persistent clinical problems.

Lung consolidation / poor inflation / haemorrhage, leptomeningeal congestion, peritoneal haemorrhage and subcutaneous oedema and/or bruising were the principal gross findings in these pups.

Gross lung changes varied from poor inflation through to petechial and lung lobe haemorrhage (Figure 3:8).

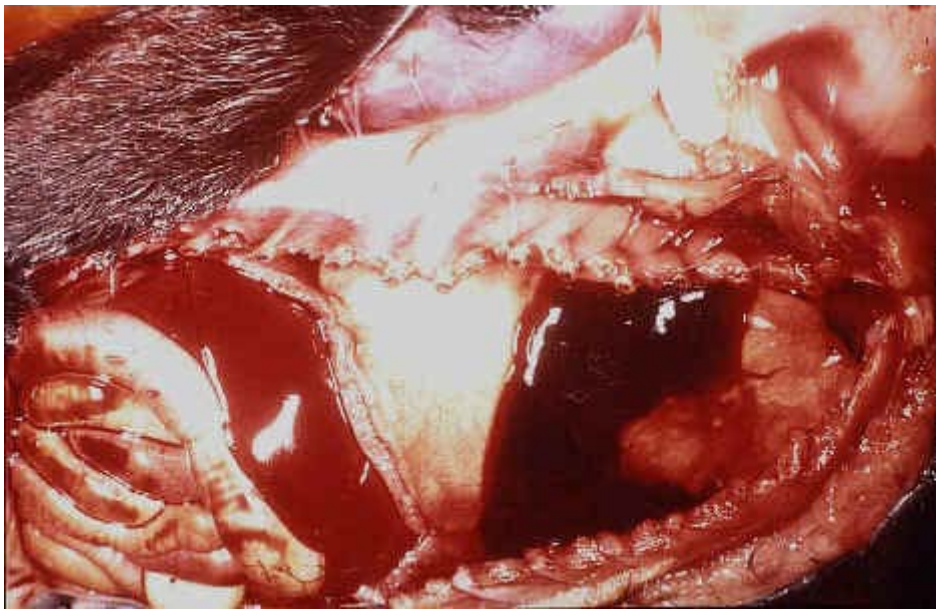

Figure 3:8 Pulmonary haemorrhage of the cardiac lobe of the lung.

The severity of leptomeningeal congestion ranged from mild through to severe congestion and haemorrhage (Figure 3:9). Pup 1861 appeared to have an under-developed brain with flattened sulci (lissencephaly).

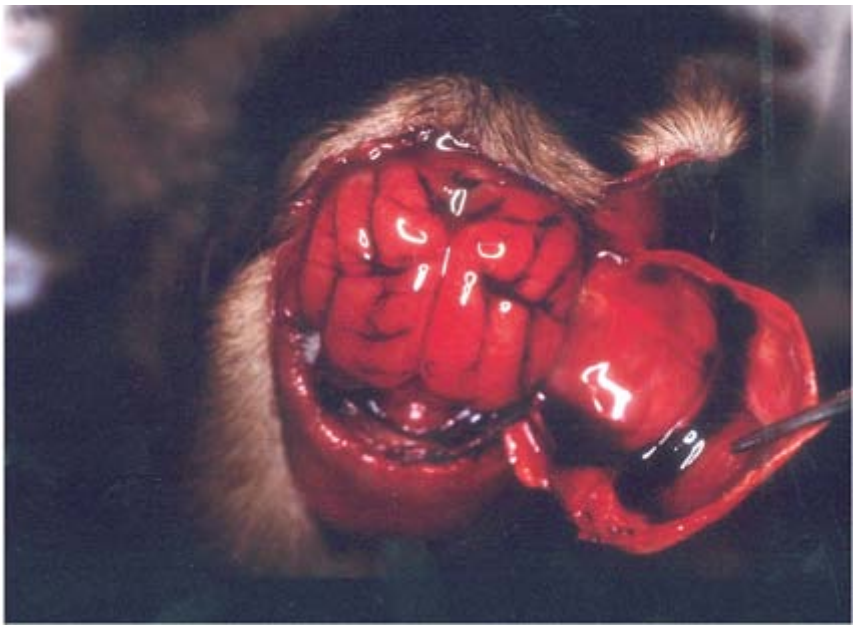

Figure 3:9 Severe leptomeningeal congestion and haemorrhage in the live distressed pup.

In the pups with peritoneal haemorrhage there was no evidence of traumatic damage to any of the abdominal organs and the umbilical blood vessels were intact. All these pups were delivered by caesarean section and either could not be revived or, in the case of two pups, were born alive with good muscle tone but deteriorated quickly and died within five minutes**.**

Table 3:3 Gross post mortem findings of live distressed pups dying in the first 24 hours (n=21)

Gross Post Mortem Finding	**Incidence**
Lung consolidation/haemorrhage/poor inflation	52.3%
Leptomeningeal congestion and/or haemorrhage	47.9%
Peritoneal haemorrhage	28.6%
Subcutaneous oedema and/or bruising	23.8%

Cardiac anomalies were not detected. The heart of one pup appeared large and pale and the liver was also enlarged with rounded edges. Another pup appeared to have a pale heart.

One pup appeared normal on post mortem.

Appendices 21:1, 21:2 and 21:3 summarise the histopathological changes identified in the live distressed pups which died in the first 24 hours.

The predominant histopathological findings in the lungs were neonatal atelectasis (80.9%), alveolar macrophages (100%), alveolar squames and meconium (71.4%), alveolar haemorrhage (71.4%), alveolar oedema and/or connective tissue oedema or haemorrhage (50%), alveolar neutrophils (19.1%) and distended terminal bronchioles (14.3%). Alveolar neutrophils were present in large enough numbers in three pups to diagnose bronchopneumonia. One pup (1821) died 1½ hours after birth and two others on day two.

Table 3:4 Incidence of histopathological findings in five distressed pups dying within the first 48 hours after birth.

Histopathological Finding	**Incidence**
Leptomeningeal congestion/ haemorrhage	100%
Alveolar macrophages	100%
Neonatal atelectasis	80.9%
Alveolar squames and/or meconium	71.4%
Alveolar haemorrhage	71.4%
Oedema of the choroid plexus of the lateral ventricles	62.5%
Alveolar oedema and/or connective tissue oedema and/or haemorrhage	50%
Perivascular ring haemorrhages	43.8%
Acute systemic congestion	42.9%
Alveolar neutrophils	19.1%
Distended terminal bronchioles	14.3%
Petechial cerebral haemorrhage	12.5%
Ventricular haemorrhage	6.3%
Cerebral brain haemorrhage	

When the post mortem reports were compared to the histological results it was evident that there was no consistency in the gross evaluation of lung expansion. This appeared primarily due to the severity of parenchymal congestion, alveolar haemorrhage and oedema that was also present in the lungs.

Moderate to severe acute systemic congestion was a feature of 42.9% of the pups examined.

The predominant histopathological findings in the brain were leptomeningeal congestion/haemorrhage (100%), oedema of the choroid plexus of the lateral ventricles (62.5%), perivascular ring haemorrhages (43.8%), petechial brain haemorrhages (12.5%) and ventricular haemorrhage (6.3%).

Cerebral brain haemorrhage was identified in one pup (1821). Haemorrhage occurred in the hippocampus. Clinically, the pup was born with gasping respiration, could not be revived and died 1.5 hours after birth. No brain necrosis was identified.

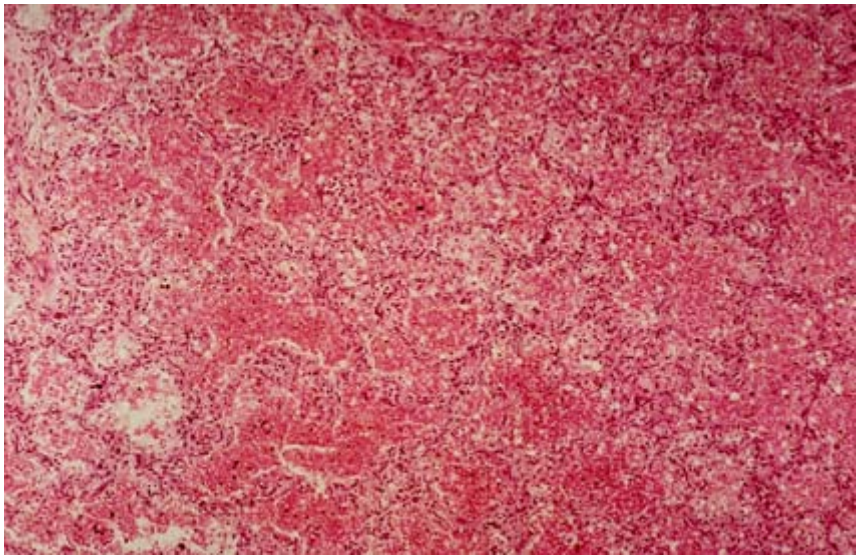

Figure 3:10 Pulmonary haemorrhage (pup 249)

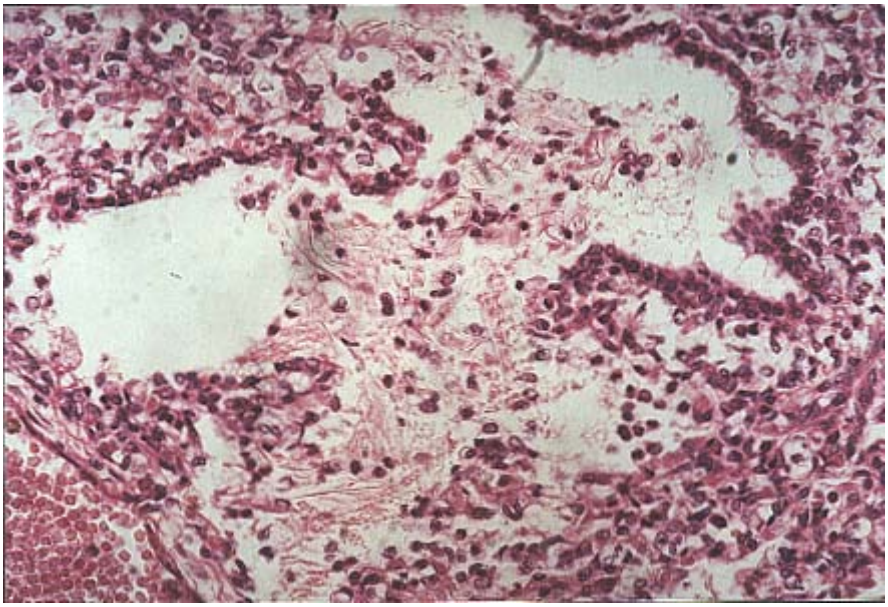

Figure 3:11 Aspiration of infected amniotic fluid (pup 1821)

Gross and Histopathology of Pups Dying After the First 48 Hours

A summary of the histopathological changes identified in distressed pups dying after 24 hours are in Appendices 22:1, 22:2 and 22:3.

Nine pups died after two days of age. Four died because of either bronchopneumonia or plural infection. Two of these were associated with evidence of *in utero* hypoxia and the inhalation of meconium. The other two may have been associated with milk inhalation during supplementary feeding.

In three other pups neonatal atelectasis was the predominant finding and it was surprising that considering the degree of lung expansion that these pups lived to three and four days of age. The lungs of remaining pups were inflated but alveolar haemorrhage and oedema and connective tissue oedema and haemorrhage were prominent features. One pup, whose lungs appeared grossly over-expanded, had on histopathology, congenital pulmonary over-inflation with *in utero* hypoxia.

Very mild leptomeningeal congestion occurred in three pups and mild to moderate oedema of the choroid plexus in two pups.

One pup (Pup 450) exhibited severe, non-inflammatory, multifocal, subacute to chronic, periventricular leukoencephalomalacia. This pup was delivered by caesarean section, required very aggressive resuscitation, and only lived to ten days of age because of intensive breeder assistance. The full histological report is in Appendix 22:4.

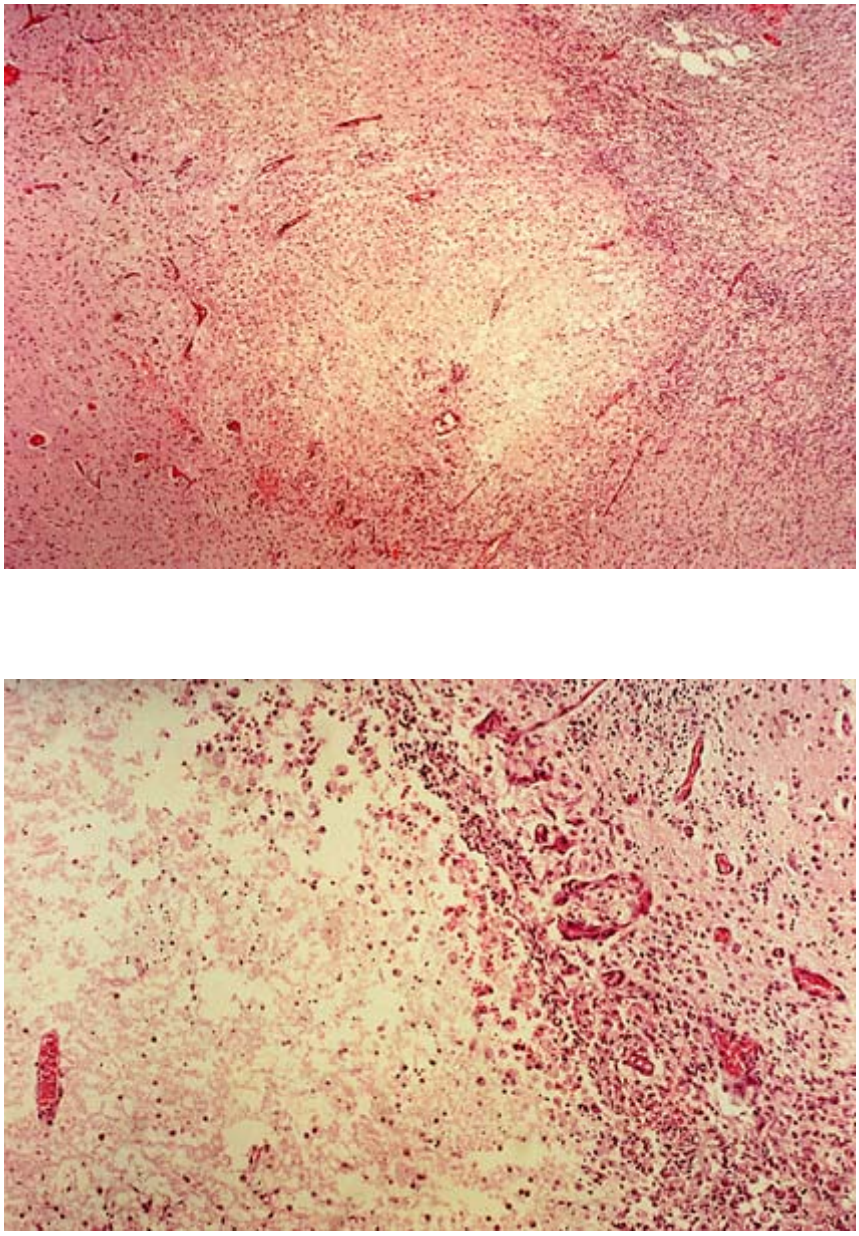

Figure 3:12 Subacute to chronic, periventricular leukoencephalomalacia.

No hypoxic lesions of the kidney and heart, as described in the distressed infant, were identified.

Figure 3:13 is a picture of a perforated small intestine, possibly associated with ischaemia. Unfortunately, the specimen was lost after photography. It came from an Australian Silky Terrier pup, delivered by caesarean section because of foetal disproportion. Its birth weight was 354 grams (116.9-192.9 grams) and resuscitation of the pup was required. The pup appeared to progress until day four when acute abdominal pain developed. The pup died quickly over approximately six hours from the onset of clinical illness.

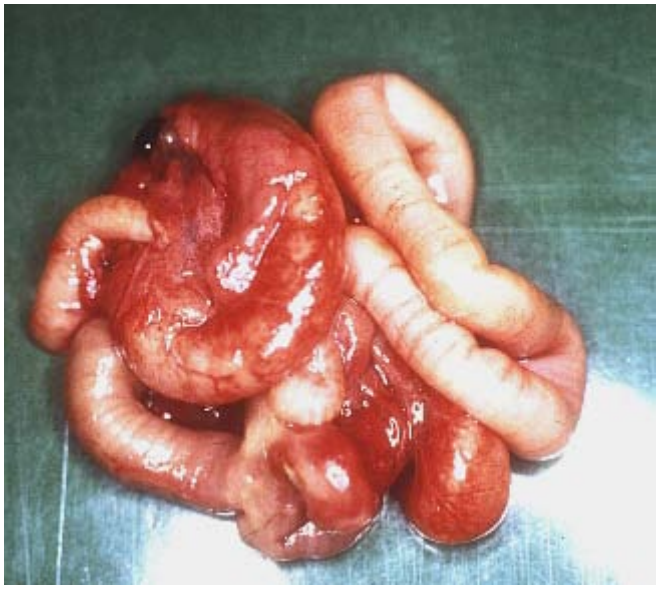

Figure 3:13 Suspect ischaemic bowel disease in the pup.

Figure 3:14 is a picture of an American Pit Bull pup which was born by caesarean section and required both aggressive resuscitation and postnatal assistance. The pup progressed well, but was presented at day five with the fore paws sloughing off. This may represent an ischaemic lesion in the pup. Occasionally, some pups will slough individual toes or tip of the tail.

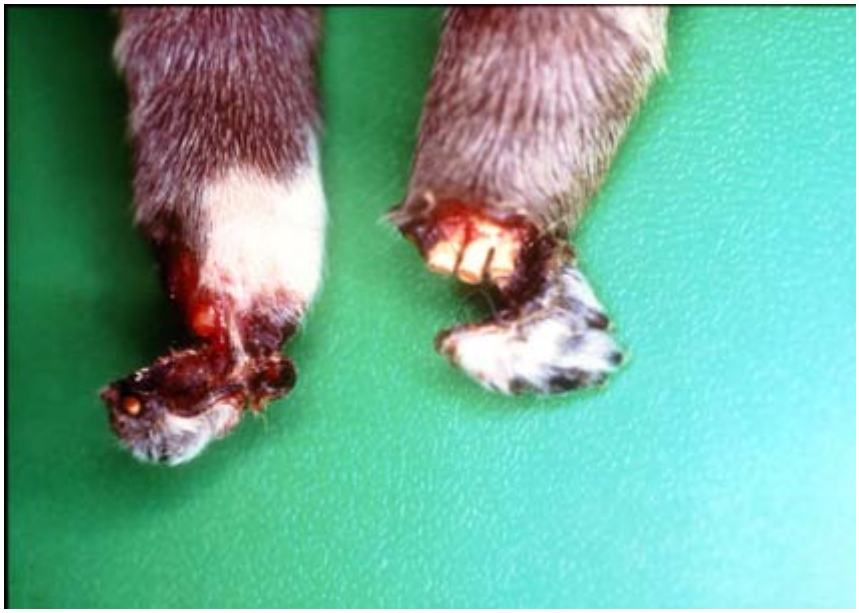

Figure 3:14 Suspect ischaemic necrosis of the fore paws of a pup.

3:4:4 Discussion

The clinical observations of the pup immediately after birth showed that breeders recognised muscle tone, respiratory pattern and sometimes mucous membrane colouration in distressed pups. Breeders checked for the presence of a heart beat, but heart rate was not measured. No breeders reported meconium staining.

Not all pups born in a clinically distressed condition died. In the epidemiology study (Chapter 2), 10.2% of those pups surveyed were reported to be born in a distressed condition and 22.1% of these survived. However, there was no system in place that allowed for a critical clinical evaluation of these pups. Therefore, the clinical condition of those pups that died was not accurately assessed, nor could their condition be contrasted to that of surviving pups.

The time of death of these pups varied from soon after birth to ten days of age. Most of those with long survival times required breeder assistance in the form of supplementary feeding.

The primary clinical problem identified in pups that were revived was their inability to suck properly. Persistence of hypotonia, abnormal respiratory patterns and apparent inability to seek heat and crawl away from the bitch were also noted in some pups. Whether this was a manifestation of neurological disfunction or related to hypoglycaemia was unclear. Some breeders also described a possible convulsive state where the pups would go rigid or stiff for some minutes at a time. Fox (1963) has previously reported a muscle flaccidity followed by tetanic rigours tonic-clonic "walking" with the hind and fore limbs. The clinical progression in these pups is similar to that reported in the infant when the central nervous system is the principal organ affected (Brann and Dykes, 1977). "After a difficult but successful resuscitation the infant, if not closely observed, may appear to be moderately well for the first few hours. However, if examined closely, the infant may have some mild hypotonia and poor sucking response during the transitional period. At approximately eight to ten hours of age episodes of lip smacking and/or eye blinking may be noted with the appearance of a tonic-clonic seizure at 12 to 16 hours".

The post mortem and histological changes found in the majority of the pups that died in the first 48 hours after birth, were similar to those found in the stillborn pups and infants. These changes were acute systemic congestion, neonatal atelectasis, alveolar macrophages, squames and meconium, leptomeningeal congestion and/or haemorrhage and choroid plexus congestion and haemorrhage.

The differences in the lungs of the live distressed pup compared to the stillborn were primarily alveolar haemorrhage, alveolar oedema and / or connective tissue oedema or haemorrhage, bronchopneumonia and distended terminal bronchioles. Alveolar and interstitial haemorrhage has been reported in the infant and is not an uncommon finding in the lungs of infants who have suffered birth asphyxia or some other perinatal stress (Askin, 1991).

One of the pups diagnosed with bronchopneumonia died at 1½ hours after birth, and the other pups on day two. This indicated that the bronchopneumonia developed either intrauterine or was acquired during the birth by the inhalation of infected amnion or cervical fluids. Congenital intrauterine pneumonia in the infant is essentially a form of aspiration pneumonia and usually accompanies ascending infection of the amniotic fluid from a vaginal source. The

membranes may have ruptured prematurely, but it has been suggested that in many cases infection is the cause rather than the result of membrane rupture (Askin, 1991).

The differences in the brain histopathology between the live distressed pup and the stillborn were that the changes tended to be more advanced in the live distressed pups. In addition, brain haemorrhage occurred in two pups and ventricular haemorrhage in one. There was no evidence of brain necrosis. This was not surprising because the death of the pups, within 48 hours after birth, did not allow sufficient time for nerve cell changes to be recognised. Rapid autolysis of the brain also limited availability and reliability of histopathological changes.

The results support the assumption that these pups were subjected to anoxia in the intrapartum period and the clinical signs of hypotonia, cyanosis, slow to gasping respiration and slow heart rate can be attributed to foetal distress. The clinical progression and pathological findings were similar to those reported in the infant.

Pups which were born distressed and died after the first 48 hours exhibited a more varied pathological picture. Four pups died because of either bronchopneumonia or pleural infection possibly acquired *in utero* from the inhalation of infected amniotic fluid, or from the inhalation of infected cervical fluids or milk. Three other pups had marked neonatal atelectasis and it is surprising that they lived this long. A further pup had congenital pulmonary overinflation suggesting an obstructive plug in the bronchi (Thibeault *et al*., 1984).

The final pup in this group was of specific interest because it probably represents the only documented case of hypoxic-ischaemic encephalopathy in the pup. There was severe, multifocal, subacute to chronic periventricular leukoencephalomalacia. The presence of gemistocytic astrocytes indicated a lesion age approaching 14 day. This suggested hypoxia at, or prior to, the caesarean section (J. Charles, personal communication).

In the full-term infant there is a varied clinical course following a prolonged period of intrauterine asphyxia (Brann and Dykes, 1977). This variation results from the multiplicity of organ systems affected when blood flow is redistributed to preserve flow and oxygen delivery to the brain and myocardium during asphyxia. The organ systems involved in decreasing order of frequency in the infant are the pulmonary system, cardiovascular system, central nervous system, gastrointestinal system and renal system. The pathology study of distressed pups identified the respiratory system as the primary target organ of dysfunction following asphyxia. The clinical observations by the breeders of these pups suggested that there may also be neurological dysfunction, but because the majority of these pups died within 48 hours of birth, this observation could not be supported with histopathological evidence.

Asphyxial damage to the central nervous system has been reported in sheep (starvation - mismothering - exposure complex) and horses (convulsive foal syndrome). The different clinical picture between these two species is thought to be due to the difference in the degree of maturation of the central nervous system at birth (Johnson and Rossdale, 1975). In only one pup in this study was neuronal necrosis identified and the changes seen were compatible with that described in the infant , that is hypoxic-ischaemic encephalopathy.

Ischaemic myocardial necrosis, ischaemic bowel disease (necrotising enterocolitis) and renal necrosis, described in the infant, were not identified in this study. I have however included

the two photos (Figures 3:13 and 3:14) as they may represent ischaemic lesions. These were a possible ischemic bowel disease and ischaemic distal limb necrosis.

A significant influence on pup mortality that could not be assessed in a pathological study was the immaturity of the neonatal pup. If a pup can not or does not suck within the first 24 hours, hepatic glycogen stores will be depleted and the ensuing hypoglycaemia and cardiovascular failure will be the final clinical outcome. It is therefore possible that the transient respiratory insufficiency reported in the infant (Thibeault et al., 1984) may be lethal to the pup and only minor histopathological changes identified. Further, death within 24 hours may not allow sufficient time for multi-system organ damage reported in the infant, to be clinically or histologically identifiable in the pup.

3:5 PATHOLOGY OF FADING PUPPY SYNDROME

3:5:1 Introduction

Regardless of which definition of Fading Puppy Syndrome is adopted, the primary observation is that these pups are "apparently born healthy" and usually die in the first seven days. For many of these pups no obvious cause of death is found (Blunden, 1998).

In contrast, most human infant deaths in the first week are either, a direct or indirect consequence of factors, that were present before or during parturition (Baird and Thomson, 1969).

The purpose of this section of the thesis was to investigate the cause of death in those pups for which mortality was attributed to Fading Puppy Syndrome. To determine, where possible, the cause of death and if there was any evidence of prepartum or intrapartum factors that may have contributed to the death of the pup.

3:5:2 Materials and methods

Fading Puppy Syndrome was identified as the probable cause of mortality in 3.4% of all pups born and 18.5% of all losses (Chapter 2). Fifty-five pups, considered on clinical histories to be fading pups, were available for post mortem (Table 3:5). These pups came from 17 litters consisting of 100 pups, from which 71 died, 66 of which were considered to have died from Fading Puppy Syndrome. Litter sizes varied from 1 to 12 pups and losses varied from one pup to the entire litter.

In seven litters, all pups died. In two of these litters, a whelp that had died prior to birth, was also present. The association of death prior to birth and Fading Puppy Syndrome was identified in the epidemiology study (Chapter 2).

Mortalities attributed to Fading Puppy Syndrome are grouped relative to their probable aetiological cause as identified by both their clinical histories and pathological findings.

Table 3:5 Fading Puppy Losses.

Litter	Number of pups in litter	Survived	Died	Fading Puppy Syndrome	Available for Post Mortem
AG	12	3	9	7	6
BE	5	0	5	5	2
BJ	3	0	3	2	2
BT	5	0	5	5	5
CW	5	2	3	2	3

CY	1	0	1	1	1
Dan	8	0	8	8	1
FG	2	0	2	2	2
FY	5	2	3	2	2
GH	6	4	2	2	2
GJ	10	3	7	3	3
IT	5	3	2	1	1
KL	4	0	4	4	2
QA	8	0	8	5	5
QV	7	4	3	2	3
RR	7	0	7	7	7
SP	7	0	7	7	7
	100	**21 (21%)**	**79 (79%)**	**66 (66%)**	**55**

3:5:3 Results

1. Growth Retardation

In five litters, consisting of 27 pups (Litters FG, GH, IT, RR and SP), there were only seven survivors (25.9%). Eighteen of these pups were considered by the breeders to be fading pups. There was no evidence of distress at birth, nor was dystocia or other problems encountered during the whelping.

The breeders considered the birth weight of these pups to be normal however, all pups were below one standard deviation of the mean birth weight for their breeds, that is they were growth retarded (Chapter 2).

All 18 pups had abnormal lungs as the principal gross post mortem finding. Histopathology was done on 14 of these and the changes identified are summarised in Appendix 23. All the lungs examined exhibited varying degrees of neonatal atelectasis and moderate to severe pulmonary congestion. The majority also had alveolar oedema (92.8%), a moderate to marked mononuclear infiltration (92.8%) and alveolar haemorrhage (41.4%). Alveolar

squames (21.4%), small numbers of alveolar neutrophils (21.4%) and alveolar meconium (7.1%) were also identified.

2. Lung Pathology

In seven litters and a total of 35 pups, (Litters BJ, CY, KL, GJ, FY, CW and QV), 18 were considered to be fading pups, nine died from other causes and eleven survived. Fourteen of these fading pups were available for post mortem.

The fading pups in five litters were within one standard deviation of the mean birth weight for their breed. The birth weights were not recorded in the other two litters, but they were considered to be normal pups by the breeders.

The principal abnormal organs identified in the post mortem of these pups were the lungs. The histopathological changes identified are summarised in Appendix 24.

The lung pathology identified was varied. Neonatal atelectasis with congestion and alveolar macrophage infiltration occurred in ten of the lungs examined (71.4%). Concurrently, evidence of *in utero* hypoxia occurred in four, alveolar haemorrhage in four and bronchopneumonia in three.

The lungs of three pups were fully inflated, but showed evidence of the pup having being subjected to *in utero* anoxia by the presence of alveolar meconium. In one lung there was concurrent milk inhalation and low grade bronchopneumonia.

The lungs of the final pup were again fully inflated, with severe, acute pulmonary haemorrhage.

In two litters where fading pups occurred, the death of a pup prior to birth occurred. These were litters BJ and KL.

3. Overwhelming Sepsis

From a litter of eight Boxer pups (Litter QA), five were considered to be fading pups, one had died prior to birth and the remaining two were white pups, euthanased immediately after birth. The birth weight of three of the pups were within one standard deviation and two were below one standard deviation of the mean birth weight for the breed.

The pups appeared to be doing well in the first 24 hours and then lost weight in the next 24 hours. The owners reported that pup QA 2153 developed, sudden onset of distress indicated by persistent crying and died before it could be examined. At this time the remaining four pups were examined. They were dehydrated and had yellow diarrhoea with a trace of blood. Treatment consisted of trimethroprim/sulfadiazine orally (Septrin Elixir®) and subcutaneous fluids. Despite this, all four remaining pups died, the last one on day 16.

The gross pathological changes identified in the four pups dying in the neonatal period were similar with peritonitis and rupture of either the stomach or small intestine. The intestinal systems were gas filled, dilated and discoloured. The intestinal contents were bloody and the mucosa appeared to be sloughing. The only significant finding in the pup that died on day 16 was lung haemorrhage and fresh blood in the trachea.

The histopathological findings in these pups are summarised in Appendix 25 and include severe, acute, gastric venous infarction with gastric perforation and acute, fibrinosuppurative, septic peritonitis. Acute, segmental, necrotohaemorrhagic enteritis. Intestinal dysbacteriosis with severe intestinal mesenteric congestion and haemorrhage and focal, acute, fibrinosuppurative, septic peritonitis. Acute to early subacute, diffuse interstitial pneumonia with mild atelectasis, severe post mortem bacterial invasion, possible *in utero* hypoxia and possible terminal aspiration of gastrointestinal contents.

The pup which survived to day 16 following antibiotic administration had mild to moderate, chronic, diffuse, interstitial pneumonia, very mild, chronic, active, tricuspid valvular endocarditis and moderately severe leptomeningitis, cerebral and lateral ventricular choroid plexus congestion with very mild, neutrophilic/lymphocytic/histocytic leptomeningitis and periventriculitis. Similarly the pup dying on day four had moderate to severe leptomeningeal, cerebral and lateral ventricular choroid plexus congestion with oedema, multifocal, acute, petechial haemorrhage and mild neutrophilic-lymphocytic-histocytic leptomeningitis.

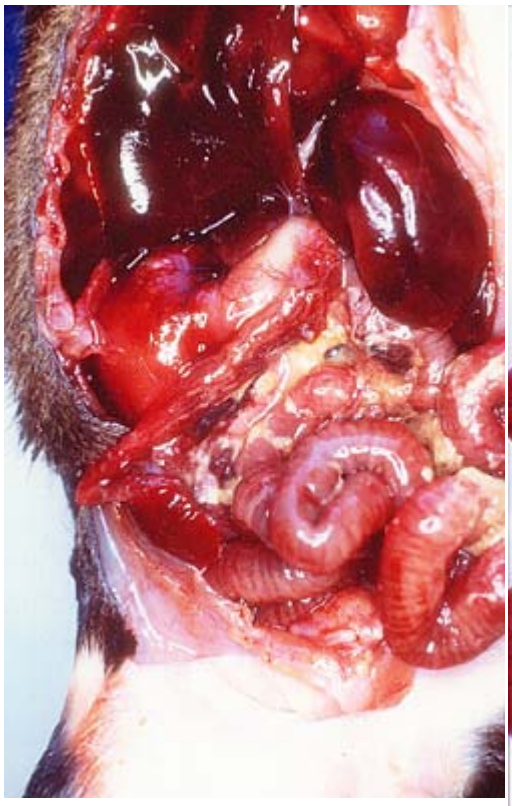

Figure 3:15 Gross pathology from QA 2150

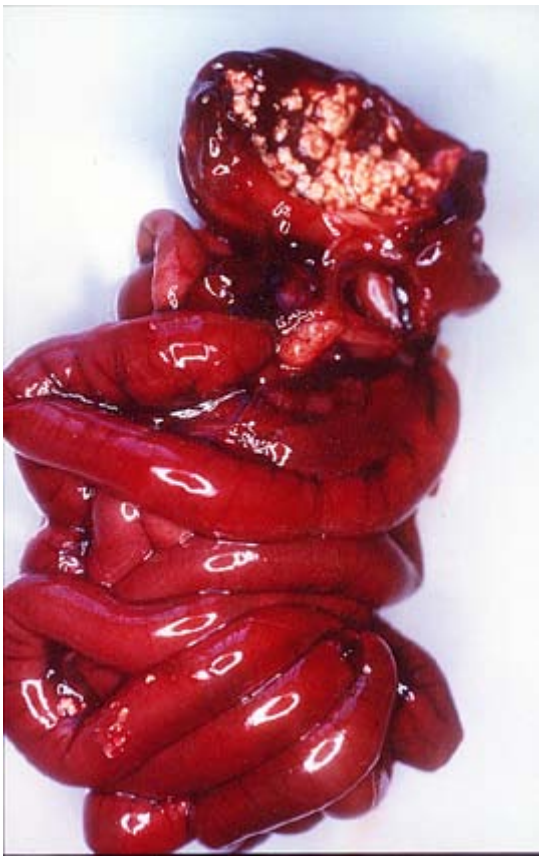

Figure 3:16 Gross pathology from QA 2154

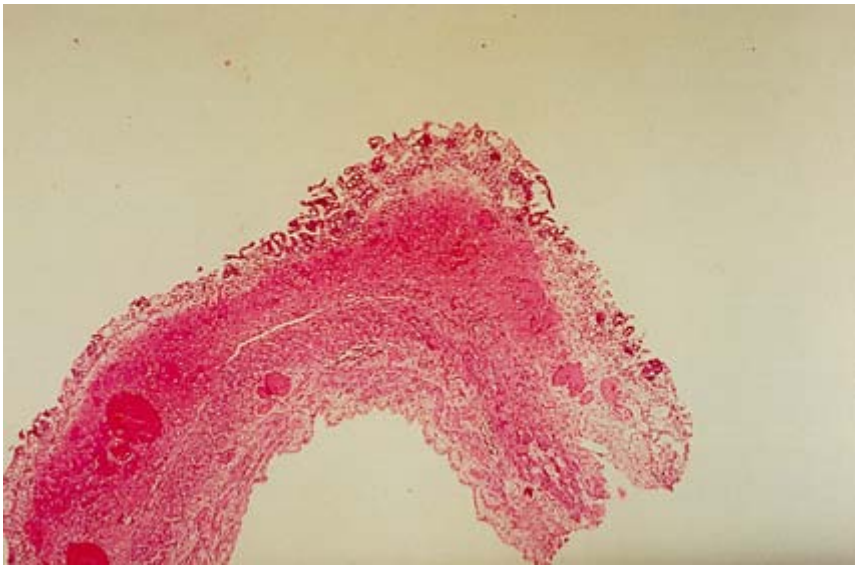

Figure 3:17 Gastric histopathology QA 2150

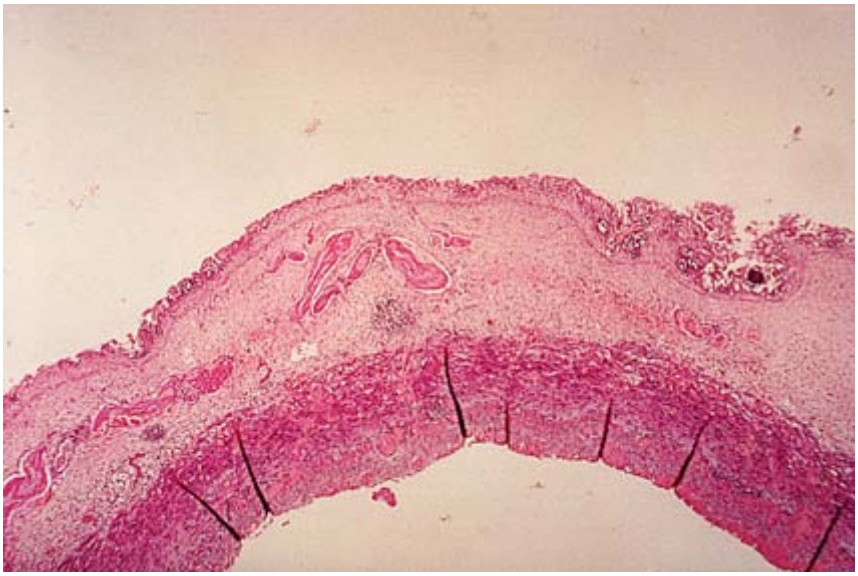

Figure 3:18 Gastric histopathology QA 2154.

4. Consecutive Litters

This Australian Silky bitch whelped four litters. The first litter was raised without any problem. All the pups in the second litter were killed by the bitch. The breeder believed that this occurred because of a management problem. All pups from the third and fourth litters (litters BE and FG) died and the histological changes identified in these pups are summarised in Appendix 26.

Litter BE consisted of five pups, all of which died. Their birth weights were not recorded but the breeder considered them to be normal. The pups died on days one, two, three, five and six. Only the last two pups were available for post mortem.

The histopathological changes identified in the first of these pups (BE 95) were subendocardial myocardial necrosis, subepicardial oedema and reactive fibroplasia/fibrosis, leptomeningitis and interstitial pneumonia.

In the second pup (BE 96) the changes identified were suppurative leptomeningitis, diffuse interstitial pneumonia and myocardial myofiber necrosis.

Litter FG consisted of two pups which died days one and two. The birth weight of both pups were below one standard deviation from the mean for their breed, that is, they were growth retarded. The pathological changes identified in the first pup (FG:105) were neonatal atelectasis with acute intra-alveolar haemorrhage. In the second pup (FG:106) the changes identified were neonatal atelectasis with *in utero* hypoxia.

5. Iatrogenic Mortality

The predominant gross pathological finding in two litters (AG and BT) was iatrogenic damage and the histological changes identified in these pups are summarised in Appendix 27.

In a litter of 12 Great Dane pups (AG) two were stillborn and seven either died or were euthanased between the ages of two and ten days. The principal gross and histopathological findings in six of the seven pups were inhalation pneumonia, thought to be a consequence of bottle feeding which was started when the pups failed to gain weight. The primary cause of failure to thrive could not be determined in these pups. The histopathological findings in the seventh pup was congenital (neonatal) atelectasis with *in utero* hypoxia and moderate congestion of the leptomeninges, choroid plexus, cerebrum and heart.

In a litter of five Miniature Dachshund pups (BT) all pups died. The bitch's first litter of five pups all died between 3 and 4 days of age. These pups from her second litter, were examined by a veterinarian 48 hours after birth and were cold, dehydrated and poorly responsive to handling. They received subcutaneous fluids (5% dextrose), trimethroprim/sulfadiazine orally (Septrin Elixir), and were warmed gradually in a humidicrib. The pups appeared to respond and, although still mildly hypotonic, were returned to the breeder who supplementary fed with a stomach tube. The breeder presented three pups dead the next day and two the following day.

The pathology findings these pups indicated three contributing causes for the deaths. The first was improper tube feeding technique in three pups. One pup had pleurisy from a ruptured oesophagus and two others had gastric haemorrhage. The brains were abnormal in all five pups. Three pups had large dural clots while the other two pups had a marked, diffuse leptomeningeal congestion and haemorrhage (Figure 3:15). Histopathology showed no organisation of the clots and indicated that the haemorrhage was recent (occurred after birth). Other brain changes in these pups included oedema and haemorrhage of the choroid and ventricles as well as grey matter haemorrhage in one pup. There was no history or evidence to indicate that trauma was involved, but this can not be ruled out especially in those pups with large dural haemorrhages.

On gross post mortem the lungs of all five pups exhibited various degrees of consolidation. The histopathology of two indicated neonatal atelectasis, with evidence of *in utero* hypoxia in one. In the third lung examined, there was adequate inflation again with evidence of *in utero* anoxia and the remaining two pups had low grade bronchopneumonia.

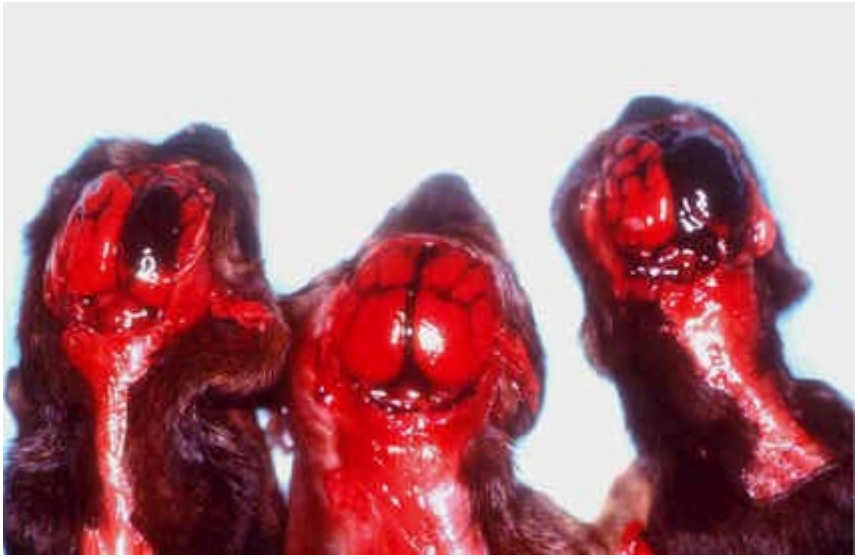

Figure 3:19 Gross leptomeningeal haemorrhage and clots found in litter BT.

6. The Diagnostic Dilemma (Litter Dan)

This pup exemplifies the diagnostic problems that exist when a veterinarian is asked to investigate a suspected Fading Puppy Syndrome. The caesarean section in a maiden British Bulldog was elective based on breed problems. All eight pups were delivered alive and in apparent good health. The post whelping history of the bitch and pups was, at best, sketchy with all pups dying over the first three days. Of the eight pups that died, only the last was available for post mortem. Birth weights and growth rates had not been measured.

The stomach and intestinal system were empty and there was no white fat around the coronary vessels, which contradicted the breeder's observation that the pup had been feeding well.

The histological diagnosis was congenital (neonatal) atelectasis with *in utero* hypoxia and moderate multi-systemic congestion of the leptomeninges, choroid plexus, heart and liver which may reflect cardiovascular collapse or hypoxia (J. Charles, personal communication).

If this pup was indicative of the rest of the litter then a tentative diagnosis of *in utero* anoxia, neonatal atelectasis, amniotic fluid inhalation, respiratory distress and starvation were the combination of causes which resulted in the death of these whelps.

3:5:4 DISCUSSION

Previous theories on fading puppy syndrome have produced a confusing picture to both the veterinarian and breeder. This confusion has arisen for two reasons. First, all conditions leading to poor weight gain and ill-thrift in the first months of life have been incorporated under this one syndrome. Secondly, the pups were considered normal at birth and often no obvious cause of death is identified.

Either gross post mortem or histopathological changes, not caused by iatrogenic damage, were found in 83.6% of fading pups available for investigation. This result differed significantly to that reported by Blunden (1986). This large discrepancy may be due to the fact both the whelpings and the immediate post partum period in this study were supervised. Because of this, death caused by mismothering/mismanagement may have been reduced. It would be anticipated that if a pup died from mismothering/mismanagement, no gross or histological changes would be found.

If the diagnosis of intrauterine growth retardation is based on birth weights below one standard deviation of the mean for a breed then 35.2% of fading pups in the epidemiological study (Chapter 2) were growth retarded. The pathological changes in these growth retarded, apparent fading pups, were comparable with those pups dying as a consequence of foetal distress. As in the growth retarded human foetus (Renfield, 1975), these pups have limited nutritional and circulatory reserves making labour and delivery, in itself a hypoxic stimulus, a more stressful event.

Clinically these pups were classified as normal at birth, growth retardation was not recognised, there was apparently no evidence of respiratory problems nor other indicators of *in utero* hypoxia.

The majority of apparent fading pups of normal birthweight in this study died because of lung pathology. The changes found were for the most part comparable to those identified in pups dying as a consequence of foetal asphyxia. However as with the previous growth retarded litters, there was no clinical evidence of distress at birth, no dystocia or other problems encountered during the whelping and no respiratory or feeding problems recognised post whelping by the breeder.

These pups were neither normal nor healthy at birth. Unless more specific criteria for the clinical evaluation of the pup at birth are established, then a large number of pups will be incorrectly assessed as fading pups by the breeder.

Not all fading pups died as a result of hypoxia. Bacterial infections, probably of maternal origin, occurred in two litters. Overwhelming sepsis commencing with a form of necrotising gastroenteritis was found in one litter and meningitis, pneumonia and myocardial necrosis in the second.

In the Boxer litter (QA) the described pathology supported a common bacterial pathogenesis (J. Charles, personal communication). From what was presumed to be a primary alimentary tract route of infection, there appeared to have been variable haematogenous dissemination to the lungs, leptomeninges and, in QA 2156, the tricuspid valve leaflets. The source of the bacterial infection was unknown, but could have been intrauterine, with the ingestion of amniotic fluid, or post partum with the ingestion of infected milk or environmental.

There are, however, two other possible primary causes that may lead to necrotising gastroenterocolitis in the pup.

The first is the syndrome ischaemic bowel disease ("necrotising enterocolitis") described in the infant (deSa, 1991). A full description of this syndrome, clinical signs and histopathology changes can be found in "Textbook of Fetal and Perinatal Pathology". The changes described in these pups are consistent with many of those described in the infant. The primary cause of this in the infant was hypoxia. In the hypoxic newborn infant a splanchnic shunt mechanism can be demonstrated in which the less vital organs, such as the gut undergo a reduction of blood flow to protect the brain and heart (deSa, 1991).

In a recent case investigated, acute necrotising enteritis and peritonitis and acute interstitial pneumonia was identified (personal observation). This occurred in one pup, the last born from a litter of ten Golden Retrievers. This pup was born distressed (hypotonic and gasping respiration), never sucked properly and died on day two.

A second possible aetiological explanation for ischaemic necrosis could be hypothermia. A similar shunt system operates when the pup is subjected to prolonged hypothermia (Sheffy, 1978). Bowel necrosis was identified in three pups whelped outside in Winter (personal observation). The bitch whelped four pups inside the house and was allowed outside for approximately one hour before being brought back inside. In the morning three almost dead, pups were found under the house. These pups were moribund and very cold to touch when presented at the clinic (rectal temperature was too low to register on the thermometer). They improved over four hours with gradual warming (humidicrib) and intravenous 5% dextrose solution. They became active, mobile and hungry and then rapidly deteriorated, crying in pain and passing bloody stools. On post mortem there was necrosis of the intestinal system and kidneys. Whether less extreme hypothermia can initiate necrotising enterocolitis in the dog is unknown.

Meningitis, pneumonia and myocardial necrosis was identified in litter BE. Haematogenous insult by bacteria or bacterial endotoxins is considered the most likely cause (J. Charles, personal communication). Although *in utero* infection can not be excluded, subepicardial fibroplasia/fibrosis and subtle pulmonary interstitial fibrosis in pup BE 95 and the fibrous connective tissue thickening of the interstitium of pup BE 96 are not inconsistent with insults sustained in the very early neonatal period. Multifocal myocardial necrosis was identified in both pups. Whether this is the same as ischaemic myocardial necrosis described in the infant (Rosenberg and Donnelly, 1991) is unknown.

Blunden (1998) identified a tendency for certain dams to have successive fading litters. Only one case of consecutive litter losses was available. The pathological findings differed between these two litters. The first litter died from meningitis, pneumonia and myocardial necrosis and losses in the second litter attributed to growth retardation and consequent anoxia during parturition. It would be difficult to argue that these two disease processes could be attributed to the same cause. A possible explanation would be endometritis and/or placentitis.

The epidemiological study (Chapter 2) identified a significant risk of fading puppy syndrome in litters whelping a pup which had died prior to birth. Apparent fading pups whelped from litters with pups which had died prior to birth were all growth retarded with consequent neonatal atelectasis and *in utero* hypoxia. A possible explanation is that those intrauterine

factors that which caused death prior to birth may also have interfered with the nutrition and growth of other pups within the litter.

Iatrogenic damage was identified in pups from two litters. In litter AG this was due to the inhalation of milk from bottle feeding in six of the seven pups. The primary cause of failure to thrive could not be determined in these pups nor was there was evidence of congenital (neonatal) atelectasis with *in utero* hypoxia, which was seen in the seventh pup.

In litter BT there were three contributing causes. These were neonatal atelectasis, leptomeningeal haemorrhage and traumatic tube feeding. The probable course of events leading to the death of these pups was lung pathology, due to *in utero* hypoxia, which interfered with feeding and led to the weight loss, dehydration and hypothermia. Warming the pups and the subcutaneous 5% dextrose corrected the hypoglycaemia, dehydration and cardiovascular collapse. Leptomeningeal haemorrhage occurred because of the advanced vascular collapse at the time of rehydration. Inappropriate feeding technique simply complicated the post mortem picture.

The results in litter BT emphasised a number of important points. Firstly, the gross pathological picture can be deceptive, as in this case death would have been deemed to be iatrogenic, if the underlying lung pathology had not been identified. Secondly, the brain, though often unrewarding, must be included in the pathological study. Thirdly, Fox (1963) describes a therapeutical irreversible syndrome of hypothermia and cardiopulmonary failure that develops in pups and, regardless of the cause, is characteristically fatal. The leptomeningeal haemorrhage in these pups and the intestinal necrosis seen following extreme hypothermia are examples of this irreversible syndrome.

In summary, this study has indicated that the majority of apparent fading pups are not normal at birth and the primary insult has occurred prior to or during labour.

To investigate apparent fading puppy losses satisfactorily the veterinarian would require the same historical and clinical material needed to investigate infant mortality, that is, the combined assessments of maternal health, foetal health *in utero*, the clinical events surrounding the birth of the infant, the evaluation of anatomical maturity, growth and development of the infant, placental examination and adequate dissection and microscopic examination of tissue sections (Wigglesworth, 1991).

In addition to this the clinical assessment of the pup at birth must be improved. Particular attention should be paid to both the birthweight and the respiratory system and failure to gain weight in the immediate post partum period should be investigated immediately. The diagnostic dilemma that exists with fading pups is that by the time veterinary assistance is sought, almost all investigative opportunities have been lost.

CHAPTER 4 : GENERAL DISCUSSION AND CONCLUSIONS

In this study total pup mortality, including elective euthanasia for show reasons, was 20.2%. If we assume that the average price of a pup (2001) is $500, then this translates into a $259,500 loss over 500 litters, or $519 per litter.

Total mortality, excluding elective euthanasia, was 18.5%. This study has determined that 90.9% of pup mortality occurred in the perinatal period and the death of the majority of these pups was a consequence of factors that were present during the pregnancy or the intrapartum period. This concurs with the aetiology of perinatal mortality in human medicine where most infants that die do so as a direct or indirect consequence of factors that were present before or during parturition. Early neonatal deaths can therefore be added to stillbirths to give a measure of total foetal loss (Baird and Thomson, 1969).

Foetal asphyxia (defined as the condition of hypoxemia, hypercarbia and acidosis) was the principal cause of mortality. Pups were either, stillborn or born in a distressed condition and subsequently died. Foetal asphyxia caused the death of 7.9% of all pups born and 42.5% of total mortality. Over half the losses associated with foetal asphyxia (57.4%) could be directly attributed to dystocia. The remaining pups died either during or after what appeared to be a normal whelping. In addition to these losses was a large proportion of the apparent fading pups which had on post mortem lung pathology consistent with foetal asphyxia.

The primary clinical problem identified in asphyxiated pups that were revived was their inability to suck properly. Persistence of hypotonia, abnormal respiratory patterns and apparent inability to seek heat and crawl away from the bitch were also noted in some pups. The pathological picture of foetal asphyxia was predominantly one of neonatal atelectasis, with or without evidence of *in utero* hypoxia, and systemic congestion, particularly evident in the leptomeninges and choroid plexus. Clinical evidence suggested that neurological dysfunction occurred but neurological damage could only be confirmed in one case studied. This was hypoxic - ischaemic encepalopathy, similar to that reported in the infant. Lesions indicative of the ischaemic bowel and distal limbs necrosis were also seen.

A factor which may have limited the pathological picture of foetal asphyxia was the immaturity of the neonatal pup. Any pup that fails to suck develops hypoglycaemia and subsequent cardiovascular collapse. This cycle may not allow adequate time for the consequences of hypoxic damage to be identified histologically.

Infectious diseases were not a common feature of perinatal mortality. Pneumonic complications of foetal distress associated with the inhalation of meconium and possibly infected amniotic fluid and the inhalation of milk supplements were seen in the first week. Infectious diseases occurred principally in the late neonatal period and the reduction of these losses in this thesis compared to previous reports (Lawler, 1989) possibly reflects improved management and vaccination programs.

The birth of abnormal pups (defined as mummified, death prior to birth, small for date and congenital defects) was the second principal cause of mortality. Abnormal pups accounted for 4.9% of all pups born and 26.3% of the total mortality. They are a consequence of either *in utero* or genetic factors (Leipold, 1978).

The definition of small for date in the epidemiology study was dependent on the breeder recognising the pup as small. When birth weights were accurately defined relative to one standard deviation from the mean for the breed, the contribution of intrauterine growth retardation to mortality was significantly higher. The pathological study identified lung pathology in the growth retarded pup similar to that found in the live distressed pup. In addition to this, the growth retarded pup has to compete with other normal litter mates and can be easily bullied off the teat. In the growth retarded infant, death from intrapartum asphyxia alone is ten times higher for the appropriately grown infant. Normal labour may severely compromise the infant. Meconium staining with possible aspiration and severe neonatal depression with acidosis may result (Renfield 1965).

What was unclear, and requires further investigation was how to define growth retardation in general terms. The overall mortality for pups of birth weights less than one standard deviation lower than the mean was 38.4%. For pups with birth weights of less than 25% of the mean the total mortality was 39.1%. There was, however, a marked discrepancy between the weight at minus one standard deviation and at 25% less than the mean in some of the individual breeds investigated. Greater numbers of observations would be required for individual breeds before one could accurately define growth retardation.

Fading puppy syndrome has in the past been considered to be a significant cause of pup mortality. Sturgess (1998) defined fading puppy syndrome as a clinical description and covered a multitude of infectious and non-infectious conditions of the neonate which cause animals born apparently healthy to gradually become inactive, loose their suck reflex and die in the first two weeks. In Chapter Two of this study, losses attributed to fading puppy syndrome, based on the clinical history of the pup, occurred primarily in the first week, peaked at day three and accounted for the death of 3.4% of all pups born and 18.5% of the total mortality. In contrast to Blunden's findings (1986), an obvious cause of death was identified in 83.6% of the apparent fading pups examined and the majority of illness could be attributed to pre or intrapartum factors. These pups were not normal at birth. Growth retardation and the consequent increased susceptibility to foetal hypoxia, lung pathology indicative of foetal asphyxia and possibly primary intrauterine and/or very early neonatal infections were the principal causes of mortality attributed to fading puppy syndrome identified in this study.

To investigate apparent fading puppy syndrome, diagnostic aids should be directed towards identifying maternal illness, placental pathology, foetal distress during the whelping, birth weights of the pups and maternal care (including milk quality and quantity) of the pups. It is then necessary to correlate these findings with the clinical and post mortem findings in the pups.

To achieve a reduction in canine perinatal mortality it would be necessary to achieve four goals. These are:

1. The identification and management of pregnancies that are at increased "risk" to the foetus.

2. The unhealthy foetus must be identified prior to labour.

3. Abnormal labour must be prevented because even a healthy foetus may succumb to repetitive non-physiologic stresses.

4. The clinical identification and treatment of the sick neonate.

These are dealt with separately below:

1. Identification and management of pregnancies that are at increased "risk" to the foetus.

The first stage of human risk assessment is to identify a population subset that requires special attention with the ultimate aim of maintaining maternal health and delivery of a healthy neonate (Depp, 1986). This form of risk scoring has shown that the predicative ability is limited until the onset of labour and risk status must be reassessed at the onset of labour.

Subset populations in the dog can be identified by their breed. Examination of the four worst performing breeds in the survey demonstrated that different management techniques would be necessary to reduce mortality.

In the Miniature Dachshund the total mortality was 28.5% and the principal cause of loss was fading puppy syndrome. Identifying the reasons for these apparent fading pups would be the key to reducing mortality in this breed. Risks of mortalities due to apparent fading puppy syndrome, identified in the toy breeds, were litter size and birth weights, that is, large litters with growth retarded pups and consequent hypoxic injury.

In the Pekingese total mortality was 24.4% with dystocia occurring in 85.7% of whelpings and the principal cause of loss was foetal asphyxia. Measures to reduce losses in this breed would require elective caesarean section or modification of the breed standards and selection from self-whelping bitches.

Total mortality in the Great Dane was 23.1% primarily due to foetal asphyxia. Dystocia occurred in 60.9% of whelpings primarily due to maternal secondary uterine inertia. A strategy to reduce losses would possibly involve more aggressive and earlier use of oxytocin to shorten inter-pup whelping intervals in the large litters or elective caesarean section.

Total mortality in the Shetland Sheepdog was 21.8% with the birth of abnormal pups the primary cause of mortality. In my experience this represents only a small percentage of the intrauterine loss that occurs in this breed as resorption of all or part of the litter appears common (personal observation). This breed has limited genetic diversity in Australia and inbreeding may be a contributory factor.

These four examples emphasise the need for individual breed whelping performance evaluation and the identification of the principal causes of mortality and significant predictor variables within each breed.

An alternative approach to subset identification could be based on the whelping performance of individual breeds. Where a breeder or breed line have losses either lower or higher than the average for the breed, then factors such as bitch selection, age and frequency of breeding and management practices, for example, diet, should be investigated to try and identify the reason(s) for the discrepancy.

Individual whelpings can be targeted as at risk to the foetus. Examples of this are singleton pups in large or giant breeds, litter size one and two in the toy breeds particularly if the pups

are in posterior presentation, four and five-year-old bitches whelping their first litter, high parity bitches and litters of seven or more pups. The clinical identification of most of these risk litters is dependent on access to ultrasonography.

2. Identification of the Unhealthy Foetus

The veterinary profession does not currently have access to the techniques used in human medicine to assess the health of the foetus *in utero* and during the birth process. Multiple foetuses and a great variation in foetal sizes between breed groups, makes cephalic measurement, as an indicator of intrauterine growth rate, impractical at this stage.

The epidemiology results implicated growth retardation as a contributory cause in the death of 29.4% of the total pup mortality. Therefore the primary problem was intrauterine and investigative procedures must be directed to bitch health and diet and uterine and placental integrity. Where growth retardation was identified as a breed problem, for example the Shetland Sheepdog, genetic counselling may be indicated. It would be valuable to collect comparable data on Shetland Sheepdogs overseas to determine if the same problem exists.

Attention to the diet of the bitch in the last three weeks of pregnancy may improve both pup birth weight and survivability. Preliminary studies have suggested that increased carbohydrate loading may achieve this in some cases (personal observation). The reasoning for carbohydrate loading was the close correlation between foetal cardiac carbohydrate concentration and the survival time during anoxia (Mott, 1961).

3. Prevention of Abnormal Labour

Foetal asphyxia was the principal cause of perinatal mortality in the dog. The canine foetus has been used extensively as an experimental animal in medical research and therefore its tolerance and response to anoxia has been documented (Fazekas *et al*., 1941; Himwich *et al*., 1941; Glass *et al*., 1944; Mott, 1961; Shelly, 1961; Arango and Rowe, 1971; Duffy *et al*., 1981; Monheit *et al*., 1988). The only diagnostic technique currently available to the veterinary profession is foetal heart monitoring, using ultrasonography. However, the diagnostic value of foetal heart rate measurements in the infant is dependent on continuous monitoring and demonstration of a persistent unresponsive bradycardia. This form of monitoring using ultrasonography in the dog is impracticable.

The level of acceptable bradycardia, both pre- and post- whelping, that is considered to be diagnostic of foetal hypoxia in the canine perinate has yet to be defined and validated. On current data available, the lowest acceptable foetal heart rate was 104 ±9 beats per minute (Monheit *et al*., 1988). Where slow foetal heart rates are detected, bitch behaviour, anticipated whelping dates and the clinical examination of the bitch must be included in the assessment.

The incidence of dystocia encountered in the epidemiology study was 35.2%, that is, 176 whelpings required some form of assistance. Clinically recognised dystocia contributed to the deaths of 4.5% of all pups born and 24.4% of the total mortality. Specific breeds may be targeted based on the occurrence of dystocia. The breeds with the highest frequency of dystocia in the survey were the Pekingese, Basset Hound, Great Dane and Miniature Dachshund.

To reduce mortality due to dystocia, more frequent and earlier caesarean sections would be necessary. If this policy were adopted, the results would have to be carefully scrutinised as other factors such as mismothering, agalactia and breeder incompetence may significantly affect the survival rate of these pups. Alternatively breeders may be able to reduce the caesarean section rate and mortality by greater care in their selection of future breeding stock. By buying in, or keeping, bitch pups from brood bitches which were good whelpers with low pup mortality, the reproductive performance within the kennel may gradually improve. Maternal rearing efficiency in ewes has a strong heritable basis (Haughey, 1981). The heritability in the dog is unknown.

There were, however, a large number of pups which died as a result of foetal asphyxia, during what the breeder or veterinarian considered a normal whelping. Because of a current inability in veterinary science to assess the health of the foetus *in utero* prior to and during whelping, it may be necessary to redefine acceptable normal whelping times. The inter-pup intervals of pups from litters with no mortalities indicated that the frequency of pup delivery can be expected to be approximately one per hour and that there was a marked increase in mortality after an inter-pup interval of two hours. This suggested that a possible means of reducing perinatal loss would be the chemical control of parturition, thus reducing the inter-pup interval, particularly in the large and giant breeds with litters of seven pups or more. This approach has been suggested in pigs (Sprecher *et al*., 1974). Adopting this recommendation would necessitate earlier veterinary intervention and, if combined with proper neonatal assessment, mortality rates may be reduced.

4. The Sick Neonate

Neonatal disease can be divided into five gross groups. These are pups with congenital defects, the asphyxiated neonate, the growth retarded pup, apparent fading pups and miscellaneous causes. Undoubtedly a pup may be simultaneously classified in a number of groups, eg. a growth retarded, asphyxiated pup.

A large number of congenital defects were obvious at birth and breeders usually elected to euthanase these pups. Other defects may be detected by the veterinarian when the pup was vaccinated at six weeks of age. There were, however, a large number of defects not obvious at birth, but which caused the death of the neonate. If no post mortem had been done, these pups would be classified as fading pups. Examples of such defects are small intestinal atresia, peritoneal-pericardial hernias and soft palate hypoplasia. A breeder or veterinarian should be alerted to the possibility of an internal congenital defect when a single pup, in an otherwise normal litter, fails to thrive.

When there are repeated losses from successive litters by the same bitch, or unusual patterns of pup mortality in related bitches, the possibility of inherited metabolic diseases should be considered (Lawler, 1989). The diagnosis of metabolic diseases involves biochemical screening of urine and blood, placental histology and other specific diagnostic tests which are either not available or, beyond the financial means of breeders. Histological investigation of the placenta would be required as an enlarging number of inborn errors of metabolism are associated with placental pathology (Dimmick and Applegarth, 1991). Until the incidence of metabolic diseases in the canine perinate is defined, it is probable that an unknown number of these pups will be classified as fading puppy syndrome with no obvious cause of death.

The pathological study confirmed that the clinically asphyxiated neonate, the growth retarded pup and the majority of apparent fading pups were subjected to *in utero* hypoxia. There was no system in place for the clinical evaluation of these pups at birth. Consequently, the clinical condition of those distressed pups which died could not be compared to the surviving pups and the majority of growth retarded and fading pups had been assessed by the breeder as normal at birth.

From the published reports on the response of the canine foetus to anoxia (Monheit *et al*., 1988) and the results of this study, it can be assumed that the consequences of asphyxia in the canine perinate is similar to that reported in the infant.

The quantitative assessment of the newborn infant described by Virginia Apgar remains the most simple and best way to evaluate the condition of the infant at birth (Tooley and Phibbs, 1975). While Apgar Scores are not specific for hypoxia and do not always appear to correlate with intrapartum indicators of asphyxia, they do direct medical attention (Jacobs and Phibbs, 1989). Such a system may be adopted for the canine neonate with modifications. The significant level of bradycardia has yet to be defined and birth weights relative to the mean for the breed must be included in the assessment. There are, however, a number of obvious restrictions that may limit its application. These are breeder compliance and ability, temperament of the bitch, rate of pup delivery and identification of individual pups.

Resuscitative techniques used for the infant including oxygenation and correction of metabolic acidosis and hypoglycaemia need to be adopted. I currently use intravenous jugular bolus injections of 5% glucose (personal observation). The clinical response of most of the distressed neonates has been dramatic.

In both the distressed pups and those considered normal at birth, the lungs were the principal organ affected. In the clinical evaluation this was the most frequent condition missed. Therefore the immediate post-natal assessment of the pup must include auscultation of the lungs. Neonatal atelectasis, lung congestion, meconium and/or amniotic debris and alveolar macrophages were frequent findings. Pneumonia was an occasional finding and possibly justifies the use of antibiotics. These pups require dietary support, in the form of tube feeding, as well as specific medication directed at the lung pathology.

In the infant a transient respiratory insufficiency is recognised. Perinatal asphyxia, severe acidosis and hypoxia, in the absence of meconium aspiration, are known to cause increased pulmonary vascular resistance with right to left shunting through foetal channels and depression of surfactant metabolism. These infants had right to left shunting and may well have had a temporary pulmonary hypertension. Diffuse pulmonary haemorrhage may develop and may in part be related to ischaemic myocardial dysfunction or a bleeding diathesis as well as pulmonary vascular damage from profound acidosis (Thibeault *et al*., 1984). It may be that some of the pups assessed at birth as normal developed this transient respiratory distress syndrome.

The incidence and severity of neurological problems in the canine neonate was difficult to evaluate. A comprehensive neurological examination of the pup is difficult and may be compounded by hypoglycaemia and hypothermia which occurs in the sick neonate. Clinically, hypotonia and poor sucking reflexes and possibly disorientation and crawling away from the bitch are recognised.

While the pathology study of the canine did not associate neonatal distress with ischemic bowel necrosis, it did suggest that a similar pathological entity may exist in the pup. However, it must also be remembered that a lot of sick neonates found by the breeder, or presented to the veterinarian, are hypothermic and bowel stasis is a common clinical complication in the pup. The hypothermic puppy's ability to suck effectively is impaired and the gastrointestinal tract motility decreases when the rectal temperature falls below 34°C (Sheffy, 1978). Breeders will often bottle or tube feed milk supplements to hypothermic neonates. Consequently milk aspiration is a frequent complication. Also, on post mortem, milk will be present in the stomach, often hours after the pup was last fed.

One of the simplest and most significant, but often overlooked, assessments of neonatal health is weight gain. As previously reported (Mosier, 1978) and supported by the observations in this study, a healthy neonate does not lose weight. Failure to gain weight in the first 24 hours is indicative of inadequate nutrition. This is significant in that the newborn relies almost exclusively on hepatic glycogen for energy for the first 24 hours and failure to suck results in rapid depletion of the liver reserve of glycogen and the development of hypoglycaemia by the second day (Center *et al.*, 1990). As a consequence of severe hypoglycaemia, mean arterial blood pressure decreases by approximately 50% (Hernandez *et al.*, 1980). This is believed to be related to the inhibition of contractile processes in the myocardium and/or smooth muscle in the peripheral vasculature. This is the probable pathway of the therapeutically irreversible syndrome of hypothermia and cardiopulmonary failure described by Fox (1963). The significance of the hepatic reserves was demonstrated in those pups identified as dying as a result of maternal ill health and/or mismanagement. Fifty-five percent of these pups died on day two.

Leptomeningeal haemorrhage, gastrointestinal and renal necrosis may be the clinical consequences of trying to revive pups that have gone past this point of no return.

The results of this study demonstrated that the cause of illness can be identified in the majority of pups on post mortem and if no gross or histological lesions are present then consideration should be given to mismothering/mismanagement as a potential cause of death.

Where pups have been confirmed as gaining weight and suddenly become ill or are found dead, trauma and infectious causes must be the primary differential diagnosis.

The canine perinate is totally dependent on the bitch both in the uterus and in the immediate post partum period. The investigation of pup mortality can not be divorced from the assessment of maternal health, the influence of the whelping process and the post whelping care of the immature pups by the bitch. These factors must be correlated with gross and histological changes identified in dead pups to determine the sequence of events that contributed to the death of the whelp.

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Appendix 1
Regression Analysis: Breedgroup.breed
Try Drop Breedgroup.breed (Giant Breed reference)

**** Regression Analysis****

Response variate: Totalmor
Binomial totals: Littersi
Distribution: Binomial
Link function: Logit
Fitted Terms: Constant + Breed group

*** Summary of analysis ***

	d.f.	deviance	mean deviance	deviance ratio	approx F.pr
Regression	4	16.9	4.235	2.12	**0.077**
Residule	430	858.5	1.997		
Total	434	875.5	2.017		
Change	**10**	**26.8**	**2.680**	**1.35**	**0.200**

Dispersion parameter is estimated to be 2.00 from the residule deviance
* MESSAGE: The following units have large standardized residules:

Unit	Response	Residual
33	7.00	3.32
36	7.00	3.32
317	9.00	3.30
387	6.00	3.07
417	8.00	3.50

* MESSAGE:The following units have high leverage:

Unit	Response	Leverage
155	4.00	0.046
159	1.00	0.046
160	1.00	0.077
161	0.00	0.041
162	0.00	0.036
163	1.00	0.051
164	2.00	0.056
165	5.00	0.067
166	3.00	0.036
167	2.00	0.077
169	3.00	0.056
170	9.00	0.062
171	4.00	0.046
172	2.00	0.062
173	3.00	0.041
176	2.00	0.036
317	9.00	0.046
318	0.00	0.038
330	1.00	0.038

***** Estimates of parameters*****

	estimate	s.e.	t(430)	t pr	antilog of estimate
constant	-1.204	0.240	-5.01	<.001	0.3000
breedgrp L	-0.539	0.273	-1.98	**0.049**	0.5831
breedgrp M	-0.472	0.340	-1.39	**0.166**	0.6240
breedgrp S	-0.070	0.324	-0.22	**0.830**	0.9326
breedgrp T	-0.109	0.278	-0.39	**0.694**	0.8964

MESSAGE s.e.s are based on the residule deviance

Appendix 2
Litter Data - Regression (fitted model)

**** Regression Analysis****

Response variate: Totalmortotalmort
Binomial totals: Littersilittersize
Distribution: Binomial
Link function: Logit
Fitted Terms: Constant + avgipi + parity + avgbirth + littersi + assistcl

*** Summary of analysis ***

	d.f.	deviance	mean deviance	deviance ratio	approx **F.pr**
Regression	7	58.6	8.368	5.20	<.001
Residule	295	474.9	1.610		
Total	302	533.5	1.767		

Dispersion parameter is estimated to be 1.61 from the residule deviance
* MESSAGE: The following units have large standardized residules:

Unit	Response	Residual
33	7.00	3.91
36	7.00	3.56
422	6.00	3.46

* MESSAGE:The following units have high leverage:

Unit	Response	Leverage
14	2.00	0.078
35	0.00	0.321
87	2.00	0.084
88	3.00	0.105
89	1.00	0.151
166	3.00	0.683
167	2.00	0.198
170	9.00	0.082
172	2.00	0.083
311	1.00	0.080
319	1.00	0.107
342	1.00	0.081
380	2.00	0.143
384	1.00	0.104

***** Estimates of parameters*****

	estimate	s.e.	t(430)	**t pr**	antilog of estimate
Constant	-2.591	0.352	-7.36	<.001	0.07498
avgipi	0.01038	0.00240	4.338	**<.007**	1.010
parity	0.1283	0.0587	2.19	**0.030**	1.137
avgbirth	-0.001852	0.000679	-2.73	**0.007**	0 .9981
littersi	0.0631	0.0357	1.77	0.078	1.065
assistcl 7	0.407	0.253	1.61	0.109	1.502
assistcl C	0.466	0.900	0.52	0.605	1.594
assistcl M	-0.260	0.348	-0.75	0.455	0.7709

MESSAGE s.e.s are based on the residule deviance

Appendix 3
Toy Breed Group : Litter Data

Model:

$$\text{logit}\left(\frac{\textit{total mortality}}{\textit{litter size}}\right) = \beta_0 + \beta_1 avgbirth + \beta_2 assistcode_T + \beta_3 assistcode_C + \beta_4 assistcode_M + \beta_5 whelping\ time + \beta_6 parity$$

Output:

```
***** Regression Analysis *****

 Response variate: totalmortotalmort
  Binomial totals: littersilittersize
     Distribution: Binomial
    Link function: Logit
     Fitted terms: Constant + avgbirth + assistcl + whelptim +
                   parity

*** Summary of analysis ***
                                          mean  deviance approx
              d.f.     deviance      deviance     ratio  F pr.
Regression       6         46.2         7.705      4.40  <.001
Residual        98        171.4         1.749
Total          104        217.7         2.093

Dispersion parameter is estimated to be 1.75 from the residual
deviance
* MESSAGE: The following units have large standardized residuals:
         Unit     Response    Residual
           33         7.00        3.57
           36         7.00        3.13
* MESSAGE: The following units have high leverage:
         Unit     Response    Leverage
           28         3.00       0.225
           35         0.00       0.368
           38         0.00       0.281
          260         5.00       0.296
          348         0.00       0.181
          381         0.00       0.203
          382         2.00       0.201
          383         0.00       0.222


*** Estimates of parameters ***

                                                              antilog
                                                                 of
                  estimate         s.e.     t(98)  t pr.    estimate
Constant             -1.38         1.24     -1.11  0.269      0.2516
avgbirth          -0.01483      0.00672     -2.21  0.030      0.9853
assistcl 7           1.316        0.670      1.96  0.053       3.729
assistcl C            -3.4         16.6     -0.20  0.838     0.03331
assistcl M           1.515        0.668      2.27  0.025       4.551
whelptim           0.00392      0.00180      2.17  0.032       1.004
parity               0.243        0.117      2.09  0.039       1.276
 * MESSAGE: s.e.s are based on the residual deviance
```

Appendix 4
Small Breed Group : Litter Data

Model:

$$\text{logit}\left(\frac{\textit{total mortality}}{\textit{litter size}}\right) = \beta_0 + \beta_1 \textit{avgipi} + \beta_2 \textit{avgbirth} + \beta_3 \textit{litter size}$$

Output:

```
***** Regression Analysis *****

 Response variate: totalmortotalmort
  Binomial totals: littersilittersize
     Distribution: Binomial
    Link function: Logit
     Fitted terms: Constant + avgipi + avgbirth + littersi


*** Summary of analysis ***

                                    mean  deviance approx
             d.f.     deviance     deviance     ratio  F pr.
Regression      3        25.19        8.395      6.24  0.001
Residual       41        55.18        1.346
Total          44        80.36        1.826

Dispersion parameter is estimated to be 1.35 from the residual
deviance
* MESSAGE: The following units have high leverage:
         Unit     Response    Leverage
           92         0.00        0.37
          133         4.00        0.21
          135         2.00        0.32
          136         1.00        0.26


*** Estimates of parameters ***


                                                          antilog
                                                          of
                  estimate         s.e.     t(41)  t pr.  estimate
Constant             -1.02         1.37     -0.75  0.460     0.3604
avgipi            0.02195      0.00699      3.14  0.003      1.022
avgbirth         -0.01474      0.00580     -2.54  0.015     0.9854
littersi            0.234        0.212      1.10  0.276      1.264
* MESSAGE: s.e.s are based on the residual deviance
```

Appendix 5
Medium Breed Group : Litter Data

Model:

$$\text{logit}\left(\frac{\textit{total mortality}}{\textit{litter size}}\right) = \beta_0 + \beta_1 assistcode_7 + \beta_2 assistcode_M + \beta_3 age + \beta_4 avgbirth + \beta_5 parity$$

Output:

```
* MESSAGE: Term assistcl cannot be fully included in the model
  because 1 parameter is aliased with terms already in the model

  (assistcl C) = 0

***** Regression Analysis *****

 Response variate: totalmortotalmort
  Binomial totals: littersilittersize
     Distribution: Binomial
    Link function: Logit
     Fitted terms: Constant + assistcl + Age + avgbirth + parity


*** Summary of analysis ***

                                      mean  deviance approx
             d.f.     deviance     deviance     ratio  F pr.
Regression      5        12.40        2.479      2.19  0.092
Residual       22        24.88        1.131
Total          27        37.27        1.381

Dispersion parameter is estimated to be 1.13 from the residual
deviance
* MESSAGE: The following units have large standardized residuals:
         Unit     Response    Residual
          324         3.00        2.72
          413         3.00        2.17
* MESSAGE: The following units have high leverage:
         Unit     Response    Leverage
          319         1.00        0.65
          325         2.00        0.58
          399         1.00        0.61
          413         3.00        0.45
          416         1.00        0.68


*** Estimates of parameters ***

                                                          antilog
                                                             of
                estimate         s.e.     t(22)  t pr.  estimate
Constant            1.80         2.73      0.66  0.516     6.055
assistcl 7         0.386        0.714      0.54  0.594     1.471
assistcl C             0            *         *      *     1.000
assistcl M          -7.8         27.2     -0.29  0.778 0.0004160
Age                0.760        0.450      1.69  0.106     2.137
avgbirth         -0.01616      0.00894     -1.81  0.084    0.9840
parity             -0.577        0.560     -1.03  0.313    0.5614
* MESSAGE: s.e.s are based on the residual deviance
```

Appendix 6
Large Breed Group : Litter Data

Model:

$$\text{logit}\left(\frac{\textit{total mortality}}{\textit{litter size}}\right) = \beta_0 + \beta_1 \textit{avgipi}$$

Output:

```
***** Regression Analysis *****

 Response variate: totalmortotalmort
  Binomial totals: littersilittersize
     Distribution: Binomial
    Link function: Logit
     Fitted terms: Constant, avgipi


*** Summary of analysis ***

                                        mean  deviance approx
              d.f.     deviance     deviance     ratio  F pr.
Regression       1          1.3        1.313      0.99  0.323
Residual       109        145.3        1.333
Total          110        146.6        1.333

Dispersion parameter is estimated to be 1.33 from the residual
deviance
* MESSAGE: The following units have large standardized residuals:
         Unit     Response    Residual
          422         6.00        3.53
* MESSAGE: The error variance does not appear to be constant:
            large responses are less variable than small responses
* MESSAGE: The following units have high leverage:
         Unit     Response    Leverage
           89         1.00       0.167
          313         1.00       0.084
          341         1.00       0.063
          342         1.00       0.073
          430         1.00       0.059
          431         1.00       0.064


*** Estimates of parameters ***

                                                          antilog of
                 estimate         s.e.    t(109)  t pr.    estimate
Constant           -2.069        0.255     -8.10  <.001      0.1263
avgipi            0.00358      0.00355      1.01  0.316       1.004
* MESSAGE: s.e.s are based on the residual deviance
```

Appendix 7
Giant Breed Group (Great Dane) : Litter Data

Model:

$$\text{logit}\left(\frac{\textit{total mortality}}{\textit{litter size}}\right) = \beta_0 + \beta_1 \textit{whelping time} + \beta_2 \textit{avgbirth} + \beta_3 \textit{age} + \beta_4 \textit{assistcode}_7 + \beta_5 \textit{assistcode}_C$$

Output:

```
***** Regression Analysis *****

 Response variate: totalmortotalmort
  Binomial totals: littersilittersize
     Distribution: Binomial
    Link function: Logit
     Fitted terms: Constant + whelptim + avgbirth + Age + assistcl

 *** Summary of analysis ***

                                      mean  deviance approx
             d.f.     deviance     deviance     ratio  F pr.
Regression      5        22.70        4.539      3.04  0.053
Residual       12        17.92        1.494
Total          17        40.62        2.389

Dispersion parameter is estimated to be 1.49 from the residual
deviance
* MESSAGE: The following units have large standardized residuals:
         Unit     Response    Residual
          167         2.00       -2.09
          170         9.00        2.05
* MESSAGE: The following units have high leverage:
         Unit     Response    Leverage
          155         4.00        0.69


*** Estimates of parameters ***

*** Estimates of parameters ***

                                                          antilog of
                  estimate         s.e.     t(12)  t pr.   estimate
Constant             -2.11         1.43    -1.48  0.165      0.1213
whelptim           0.00254      0.00108     2.35  0.036       1.003
avgbirth           -0.0046      0.00261    -1.76  0.104      0.9954
Age                  0.365        0.176     2.07  0.060       1.440
assistcl 7          -0.537        0.652    -0.82  0.427      0.5847
assistcl C           1.425        0.841     1.70  0.116       4.159
assistcl M               0            *        *      *       1.000
* MESSAGE: s.e.s are based on the residual deviance
```

Appendix 8
Chihuahua : Litter Data

Model:

$$\text{logit}\left(\frac{\textit{total mortality}}{\textit{litter size}}\right) = \beta_0 + \beta_1 \textit{litter size} + \beta_2 \textit{avgbirth} + \beta_3 \textit{avgipi} + \beta_4 \textit{parity}$$

Output:

```
***** Regression Analysis *****

 Response variate: totalmortotalmort
  Binomial totals: littersilittersize
     Distribution: Binomial
    Link function: Logit
     Fitted terms: Constant + littersi + avgbirth + avgipi +
                   parity


*** Summary of analysis ***

                                        mean  deviance approx
             d.f.     deviance      deviance     ratio  F pr.
Regression      4        25.09        6.2735      6.63  <.001
Residual       35        33.12        0.9463
Total          39        58.22        1.4927

Dispersion parameter is estimated to be 0.946 from the residual
deviance
* MESSAGE: The following units have high leverage:
         Unit     Response    Leverage
          347        0.000        0.32
          380        2.000        0.47
          384        1.000        0.38


*** Estimates of parameters ***

                                                              antilog
                                                                 of
                 estimate         s.e.     t(35)   t pr.    estimate
Constant             0.48         2.18      0.22   0.826       1.620
littersi           -0.634        0.262     -2.41   0.021      0.5306
avgbirth          -0.0142       0.0113     -1.26   0.217      0.9859
avgipi            0.00931      0.00396      2.35   0.025       1.009
parity              0.265        0.143      1.86   0.071       1.304
* MESSAGE: s.e.s are based on the residual deviance
```

Appendix 9
Shetland Sheepdog : Litter Data

Model:

$$\text{logit}\left(\frac{\textit{total mortality}}{\textit{litter size}}\right) = \beta_0 + \beta_1 \textit{avgipi} + \beta_2 \textit{avgbirth} + \beta_3 \textit{litter size}$$

Output:

```
***** Regression Analysis *****

 Response variate: totalmortotalmort
  Binomial totals: littersilittersize
     Distribution: Binomial
    Link function: Logit
     Fitted terms: Constant + avgipi + avgbirth + littersi


*** Summary of analysis ***

                                        mean  deviance approx
              d.f.     deviance     deviance     ratio  F pr.
Regression       3        25.19        8.395      6.24  0.001
Residual        41        55.18        1.346
Total           44        80.36        1.826

Dispersion parameter is estimated to be 1.35 from the residual
deviance
* MESSAGE: The following units have high leverage:
         Unit     Response    Leverage
           92         0.00        0.37
          133         4.00        0.21
          135         2.00        0.32
          136         1.00        0.26


*** Estimates of parameters ***


                                                          antilog
                                                          of
                  estimate         s.e.     t(41)  t pr.  estimate
Constant             -1.02         1.37     -0.75  0.460     0.3604
avgipi            0.02195      0.00699      3.14  0.003      1.022
avgbirth         -0.01474      0.00580     -2.54  0.015     0.9854
littersi            0.234        0.212      1.10  0.276      1.264
* MESSAGE: s.e.s are based on the residual deviance
```

Appendix 10
Pup Data : Nominal Logistic Regression (final model)

Model:

$$\log\left\{\frac{\pi_{1x}}{\pi_{1o}}\right\} = \beta_{o4} + \beta_{14}interpup\ interval_{i1} + \beta_{24}birthweight_{i2} + \beta_{34}parity_{i3} + \beta_{44}presentation + \beta_{54}littersize_{i5}$$

Output

Reference Group = MORT 0

```
MTB > NLogistic 'MORT' = 'interpup_int' birthweight parity &
CONT>     Presentat          Littersize;
SUBC>  Factors 'presentat'
SUBC    Reference    MORT 0;
SUBC    Iteration    1000
SUBC>  Brief 2
```

Nominal Logistic Regression: MORT versus interpup_int, birthweight,...

Response Information

Variable	Value	Count	
Mort	0	1330	(Reference Event)
	4	14	
	3	58	
	2	100	
	1	64	
	Total	1566	

Logistic Regression Table

					Odds	95% CI	
Predictor	Coef	SE Coef	Z	**P**	Ratio	Lower	Upper
Logit 1: (4/0)							
Constant	-3.8266	0.9293	-4.12	0.000			
Interpup	-0.006041	0.007944	-0.76	0.447	0.99	0.98	1.01
Birthwei	-0.004051	0.002323	-1.74	0.081	1.00	0.99	1.00
Parity	-0.1756	0.2298	-0.76	0.445	0.84	0.53	1.32
Presenta							
P	0.2990	0.5624	0.53	0.595	1.32	0.45	4.06
Littersi	0.1483	0.1013	1.46	0.143	1.16	0.95	1.41
Logit 2 (3/0)							
Constant	-4.0782	0.4872	-8.37	0.000			
Interpup	- 0.001091	0.003056	-0.36	0.721	1.00	0.99	1.00
Birthwei	**- 0.003380**	0.001060	-3.19	**0.001**	1.00	0.99	1.00
Parity	**0.38747**	0.07739	5.01	**0.000**	1.47	1.27	1.71
Presenta							
P	**-0.8777**	0.3734	-2.35	**0.019**	0.42	0.20	0.86
Littersi	**0.18225**	0.05454	3.34	**0.001**	1.20	1.08	1.34
Logit 3 (2/0)							
Constant	-2.5225	0.3550	-7.11	0.000			
Interpup	**0.007460**	0.001440	5.18	0.000	1.00	1.00	1.01
Birthwei	**-0.0018785**	0.0007509	2.50	**0.012**	1.00	1.00	1.00
Parity	0.02272	0.07109	0.32	0.749	1.02	0.89	1.18
Presenta							
P	**0.4939**	0.2175	2.27	**0.023**	1.64	1.07	2.51
Littersi	-0.01214	0.04366	-0.28	0.781	0.99	0.91	1.08
Logit 4 (1/0)							
Constant	-2.5297	0.4238	-5.97	0.000			
Interpup	**0.004119**	0.002065	1.99	**0.046**	1.00	1.00	1.01
Birthwei	**- 0.002991**	0.001009	-2.96	**0.003**	1.00	1.00	1.00
Parity	0.06113	0.08384	0.73	0.466	1.06	0.90	1.25
Presenta							
P	-0.2320	0.2970	-0.78	0.435	0.79	0.44	1.42
Littersi	0.02639	0.05411	0.49	0.626	1.03	0.92	1.14

Log-likelihood = -903.376
Test that all slopes are zero: G = 101.635, DF = 20, P-Value = 0.000

Appendix 11
Toy Bréed Group : Pup Data

Model :

$$\log\left(\frac{\pi_{ik}}{\pi_{i0}}\right) = \beta_{0k} + \beta_{1k} litter\ size_{i1} + \beta_{2k} birth\ weight_{i2} + \beta_{3k} placental\ attachment_{i3} + \beta_{4k} inter\text{-}pup\ interval_{i4} + \beta_{5k} presentation_{i5}$$

Output:

```
MTB > NLogistic 'MORT' = littersize birthweight 'plac_attach'  &
CONT>     'interpup_int' presentat;
SUBC>   Factors 'plac_attach' 'presentat';
SUBC>   Reference 'MORT' 0;
SUBC>   Iteration 1000;
SUBC>   Brief 2.
```

Nominal Logistic Regression: MORT versus littersize, birthweight, ...

```
Response Information

Variable  Value       Count
MORT      0             336  (Reference Event)
          4               4
          3              23
          2              31
          1              11
          Total         405


Logistic Regression Table
                                                           Odds         95% CI
Predictor        Coef    SE Coef         Z      P       Ratio    Lower    Upper
Logit 1: (4/0)
Constant       -0.777      3.423    -0.23  0.820
littersi        0.3995     0.4753     0.84  0.401        1.49     0.59     3.79
birthwei     -0.04281    0.01742    -2.46  0.014        0.96     0.93     0.99
plac_att
 Y             -1.367      1.142    -1.20  0.232        0.25     0.03     2.39
interpup     -0.00307    0.01653    -0.19  0.853        1.00     0.97     1.03
presenta
 P              2.066      1.250     1.65  0.098        7.90     0.68    91.52


Logit 2: (3/0)
Constant       -6.194      1.872    -3.31  0.001
littersi        1.2232     0.2470     4.95  0.000        3.40     2.09     5.51
birthwei    -0.020753   0.007236    -2.87  0.004        0.98     0.97     0.99
plac_att
 Y             1.0169     0.8153     1.25  0.212        2.76     0.56    13.67
interpup    -0.010951   0.008829    -1.24  0.215        0.99     0.97     1.01
presenta
 P           -0.6829     0.6796    -1.00  0.315        0.51     0.13     1.91


                                                           Odds         95% CI
Predictor        Coef    SE Coef         Z      P       Ratio    Lower    Upper
Logit 3: (2/0)
Constant        0.067      1.070     0.06  0.950
littersi       -0.7065     0.1729    -4.09  0.000        0.49     0.35     0.69
birthwei    -0.002075   0.004007    -0.52  0.605        1.00     0.99     1.01
plac_att
 Y             -1.0765     0.4372    -2.46  0.014        0.34     0.14     0.80
interpup     0.011912   0.003342     3.56  0.000        1.01     1.01     1.02
presenta
 P             1.3600     0.4275     3.18  0.001        3.90     1.69     9.01

Logit 4: (1/0)
Constant        3.298      1.713     1.93  0.054
littersi       -0.1347     0.2639    -0.51  0.610        0.87     0.52     1.47
birthwei    -0.04368    0.01025    -4.26  0.000        0.96     0.94     0.98
plac_att
 Y             -1.7031     0.7342    -2.32  0.020        0.18     0.04     0.77
interpup     0.007736   0.004646     1.67  0.096        1.01     1.00     1.02
presenta
 P             0.5608     0.7299     0.77  0.442        1.75     0.42     7.33

Log-likelihood = -191.449
Test that all slopes are zero: G = 150.168, DF = 20, P-Value = 0.000


Goodness-of-Fit Tests

Method      Chi-Square    DF      P
Pearson      18963.534  1488  0.000
Deviance       377.353  1488  1.000
```

Appendix 12
Small Breed Group : Pup Data

Model:

$$\log\left(\frac{\pi_{ik}}{\pi_{i0}}\right) = \beta_{0k} + \beta_{1k} birth\ weight_{i1}$$

Output:

```
MTB > NLogistic 'MORT' = birthweight;
SUBC>   Reference 'MORT' 0;
SUBC>   Iteration 1000;
SUBC>   Brief 2.
```

Nominal Logistic Regression: MORT versus birthweight

```
Response Information

Variable  Value       Count
MORT      0             174  (Reference Event)
          4               2
          3               6
          2              17
          1              13
          Total         212

Logistic Regression Table
                                                          Odds         95% CI
Predictor       Coef    SE Coef         Z      P         Ratio    Lower    Upper
Logit 1: (4/0)
Constant       0.840      2.558      0.33  0.743
birthwei    -0.03029    0.01717     -1.76  0.078         0.97     0.94     1.00

Logit 2: (3/0)
Constant       3.027      1.663      1.82  0.069
birthwei    -0.03836    0.01198     -3.20  0.001         0.96     0.94     0.99

Logit 3: (2/0)
Constant      -1.313      1.012     -1.30  0.195
birthwei   -0.004885   0.004860     -1.01  0.315         1.00     0.99     1.00

Logit 4: (1/0)
Constant       2.435      1.122      2.17  0.030
birthwei   -0.028358   0.007084     -4.00  0.000         0.97     0.96     0.99

Log-likelihood = -125.310
Test that all slopes are zero: G = 37.930, DF = 4, P-Value = 0.000

Goodness-of-Fit Tests

Method       Chi-Square    DF      P
Pearson         130.253   244  1.000
Deviance         95.664   244  1.000
```

Appendix 13
Medium Breed Group : Pup Data

Model:

Model:

$$\log\left(\frac{\pi_{ik}}{\pi_{i0}}\right) = \beta_{0k} + \beta_{1k} age_{i1} + \beta_{2k} inter\text{-}pup\ interval_{i2}$$

Output:

```
MTB > NLogistic 'MORT' = Age 'interpup_int';
SUBC>   Reference 'MORT' 0;
SUBC>   Iteration 1000;
SUBC>   Brief 2.
```

Nominal Logistic Regression: MORT versus Age, interpup_int

Response Information

Variable	Value	Count	
MORT	0	153	(Reference Event)
	4	2	
	3	4	
	2	9	
	1	4	
	Total	172	

Logistic Regression Table

Predictor	Coef	SE Coef	Z	P	Odds Ratio	95% CI Lower	95% CI Upper
Logit 1: (4/0)							
Constant	-4.441	1.652	-2.69	0.007			
Age	-0.2211	0.5412	-0.41	0.683	0.80	0.28	2.32
interpup	0.01509	0.01569	0.96	0.336	1.02	0.98	1.05
Logit 2: **(3/0)**							
Constant	-12.856	5.280	-2.43	0.015			
Age	1.7636	0.7635	2.31	**0.021**	5.83	1.31	26.05
interpup	-0.01197	0.01652	-0.72	0.469	0.99	0.96	1.02
Logit 3: **(2/0)**							
Constant	-2.1097	0.8092	-2.61	0.009			
Age	0.1044	0.2183	0.48	0.632	1.11	0.72	1.70
interpup	-0.05453	0.02458	-2.22	**0.026**	0.95	0.90	0.99
Logit 4: (1/0)							
Constant	-4.089	1.191	-3.43	0.001			
Age	-0.1174	0.3676	-0.32	0.750	0.89	0.43	1.83
interpup	0.01533	0.01080	1.42	0.156	1.02	0.99	1.04

Log-likelihood = -66.987
Test that all slopes are zero: G = 32.947, DF = 8, P-Value = 0.000

Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	207.233	420	1.000
Deviance	78.803	420	1.000

Appendix 14
Large Breed Group : Pup Data

Model:

$$\log\left(\frac{\pi_{ik}}{\pi_{i0}}\right) = \beta_{0k} + \beta_{1k} birth\ weight_{i1} + \beta_{2k} birth\ order_{i2}$$

Output:

```
MTB > NLogistic 'MORT' = birthweight 'birth order';
SUBC>   Reference 'MORT' 0;
SUBC>   Iteration 1000;
SUBC>   Brief 2.
```

Nominal Logistic Regression: MORT versus birthweight, birth order

```
Response Information

Variable  Value       Count
MORT      0             540  (Reference Event)
          4               6
          3              16
          2              27
          1              32
          Total         621

Logistic Regression Table
                                                          Odds         95% CI
Predictor       Coef    SE Coef         Z      P        Ratio    Lower    Upper
Logit 1: (4/0)
Constant      -3.324      1.671     -1.99  0.047
birthwei   -0.001962   0.003233     -0.61  0.544         1.00     0.99     1.00
birth or      -0.520      1.459     -0.36  0.722         0.59     0.03    10.39

Logit 2: (3/0)
Constant      -3.328      1.090     -3.05  0.002
birthwei   -0.000255   0.001995     -0.13  0.898         1.00     1.00     1.00
birth or     -0.1280      0.8932     -0.14  0.886         0.88     0.15     5.07

Logit 3: (2/0)
Constant     -3.5169      0.9127     -3.85  0.000
birthwei   -0.002100   0.001581     -1.33  0.184         1.00     0.99     1.00
birth or      2.3015      0.7741      2.97  0.003         9.99     2.19    45.55

Logit 4: (1/0)
Constant     -1.2564      0.7389     -1.70  0.089
birthwei   -0.005463   0.001463     -3.73  0.000         0.99     0.99     1.00
birth or      1.2871      0.6715      1.92  0.055         3.62     0.97    13.51

Log-likelihood = -326.692
Test that all slopes are zero: G = 29.429, DF = 8, P-Value = 0.000

Goodness-of-Fit Tests

Method      Chi-Square    DF      P
Pearson       1983.518  1960  0.350
Deviance       568.579  1960  1.000
```

Appendix 15
Giant Breed Group (Great Dane) : Pup Data

Model:

$$\log\left(\frac{\pi_{ik}}{\pi_{i0}}\right) = \beta_{0k} + \beta_{1k} birth\ order_{i1} + \beta_{2k} birth\ weight_{i2} + \beta_{3k} litter\ size_{i3} + \beta_{4k} inter\text{-}pup\ interval_{i4}$$

Output:

```
MTB > NLogistic 'MORT' = 'birth order' birthweight littersize  &
CONT>     'interpup_int';
SUBC>   Reference 'MORT' 0;
SUBC>   Iteration 1000;
SUBC>   Brief 2.
```

Nominal Logistic Regression: MORT versus birth order, birthweight, ...

Response Information

Variable	Value	Count	
MORT	0	127	(Reference Event)
	3	9	
	2	16	
	1	4	
	Total	156	

Logistic Regression Table

Predictor	Coef	SE Coef	Z	P	Odds Ratio	95% CI Lower	95% CI Upper
Logit 1: (3/0)							
Constant	-0.793	3.324	-0.24	0.811			
birth or	0.336	1.441	0.23	0.816	1.40	0.08	23.56
birthwei	-0.004333	0.003633	-1.19	0.233	1.00	0.99	1.00
littersi	0.0094	0.1470	0.06	0.949	1.01	0.76	1.35
interpup	0.001405	0.007569	0.19	0.853	1.00	0.99	1.02
Logit 2: (2/0)							
Constant	-7.388	3.260	-2.27	0.023			
birth or	4.960	1.777	2.79	**0.005**	142.53	4.38	4643.33
birthwei	-0.003300	0.003253	-1.01	0.310	1.00	0.99	1.00
littersi	0.2174	0.1382	1.57	0.116	1.24	0.95	1.63
interpup	0.014326	0.005320	2.69	**0.007**	1.01	1.00	1.03
Logit 3: (1/0)							
Constant	25.06	10.70	2.34	0.019			
birth or	-8.732	5.180	-1.69	0.092	0.00	0.00	4.14
birthwei	-0.04242	0.01889	-2.25	**0.025**	0.96	0.92	0.99
littersi	-0.9246	0.4028	-2.30	**0.022**	0.40	0.18	0.87
interpup	0.02692	0.01897	1.42	0.156	1.03	0.99	1.07

Log-likelihood = -70.389
Test that all slopes are zero: G = 64.990, DF = 12, P-Value = 0.000

Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	860.005	453	0.000
Deviance	140.778	453	1.000

Appendix 16
German Shepherd : Pup Data

Model:

$$\log\left(\frac{\pi_{ik}}{\pi_{i0}}\right) = \beta_{0k} + \beta_{1k} birth\ weight_{i1} + \beta_{2k} birth\ order_{i2}$$

Output:

```
MTB > NLogistic 'MORT' = birthweight 'birth order';
SUBC>   Reference 'MORT' 0;
SUBC>   Iteration 1000;
SUBC>   Brief 2.
```

Nominal Logistic Regression: MORT versus birthweight, birth order

```
Response Information

Variable  Value       Count
MORT      0             182  (Reference Event)
          4               3
          3               5
          2              11
          1              16
          Total         217

Logistic Regression Table
                                                          Odds          95% CI
Predictor       Coef    SE Coef         Z      P        Ratio     Lower     Upper
Logit 1: (4/0)
Constant       0.358      2.532      0.14  0.888
birthwei   -0.006375   0.004447     -1.43  0.152       0.99      0.99      1.00
birth or      -2.270      2.410     -0.94  0.346       0.10      0.00     11.62

Logit 2: (3/0)
Constant      -0.731      2.110     -0.35  0.729
birthwei   -0.004924   0.003585     -1.37  0.170       1.00      0.99      1.00
birth or      -0.367      1.661     -0.22  0.825       0.69      0.03     17.97

Logit 3: (2/0)
Constant      -3.867      1.888     -2.05  0.041
birthwei   -0.003009   0.002705     -1.11  0.266       1.00      0.99      1.00
birth or       3.932      1.470      2.68  0.007      51.01      2.86    909.67

Logit 4: (1/0)
Constant       0.537      1.290      0.42  0.677
birthwei   -0.008642   0.002264     -3.82  0.000       0.99      0.99      1.00
birth or       2.261      1.107      2.04  0.041       9.59      1.10     84.01

Log-likelihood = -120.899
Test that all slopes are zero: G = 34.656, DF = 8, P-Value = 0.000

Goodness-of-Fit Tests

Method       Chi-Square    DF      P
Pearson         564.050   680  1.000
Deviance        205.755   680  1.000
```

Appendix 17
Shetland Sheepdog : Pup Data

Model:

$$\log\left(\frac{\pi_{ik}}{\pi_{i0}}\right) = \beta_{0k} + \beta_{1k} birth\ weight_{i1}$$

Output:

```
MTB > NLogistic 'MORT' = birthweight;
SUBC>   Reference 'MORT' 0;
SUBC>   Iteration 1000;
SUBC>   Brief 2.
```

Nominal Logistic Regression: MORT versus birthweight

```
Response Information

Variable  Value       Count
MORT      0             124  (Reference Event)
          3               2
          2              13
          1              12
          Total         151

Logistic Regression Table
                                                          Odds          95% CI
Predictor       Coef    SE Coef         Z      P        Ratio     Lower     Upper
Logit 1: (3/0)
Constant      -0.636      2.767     -0.23  0.818
birthwei    -0.01762    0.01501     -1.17  0.241      0.98      0.95      1.01

Logit 2: (2/0)
Constant      -0.047      1.231     -0.04  0.970
birthwei   -0.010689   0.006092     -1.75  0.079      0.99      0.98      1.00

Logit 3: (1/0)
Constant       3.593      1.308      2.75  0.006
birthwei   -0.032806   0.008054     -4.07  0.000      0.97      0.95      0.98

Log-likelihood = -82.245
Test that all slopes are zero: G = 26.200, DF = 3, P-Value = 0.000

Goodness-of-Fit Tests

Method       Chi-Square    DF       P
Pearson         102.235   138   0.990
Deviance         67.552   138   1.000
```

Appendix 18

Congenital defects

Defect	Number of pups affected	Breeds recorded in	Sex
Anencephaly	3	1 British Bulldog 2 Pekingese 1 Great Dane	4 M
Anascara	2	1 Pekingese 1 Australian Silky	1 M 1 F
Hydrocephalus	3	1 Shetland Sheepdog 1 German Shepherd 1 Great Dane	2 F 1 M
Cleft Palate	20	1 British Bulldog 6 German Shepherds 2 Keeshonds 2 Boxers 4 Pekingese 1 Pug 3 Hungarian Vizslas 2 Australian Silkies 2 Border Collie 1 Golden Retriever 2 Labradors 1 Australian Cattle Dog	13 M 13 F 1 NR
Cleft Palate + other defects	2	1 German Shepherd 1 Boxer	2 F
Atresia ani	1	1 Shetland Sheepdog	1 M
Anury	2	1 Shetland Sheepdog 1 Schnauzer	2 F
Atresia ani + Anury	2	1 Rhodesian Ridgeback 1 Great Dane	1 M
Dorsal Sinus	6	6 Rhodesian Ridgebacks	1 M 5 F
Foot Abnormalities	5	1 Chihuahua 1 Border Collie 1 Dachshund (min) 1 Shetland Sheepdog 1 German Shepherd	5 F
Omphalocoel	6	2 German Shepherds 2 Labradors 1 Dachshund (min)	3 M 3 F

		1 Shetland Sheepdog	
Megaoesophageous	1	1 German Shepherd	
Spinal Dysraphism	2	1 Shetland sheepdog 1 Chihuahua	1 M 1 F
White/ Deaf/ Blind	3	3 Shetland Sheepdogs	2 M 1 F
Small Intestinal Atresia	3	1 German Shepherd 1 Basset Hound 1 Boxer	2 M 1 F
Renal Agenesis	1	1 Chihuahua	1 F
Soft Palate Hypoplasia	1	1 Cocker Spaniel	1 F
Peritoneal - pericardial Hernia	1	1 Cattle Dog	1 M

Appendix 19
Reproductive performance in all breeds surveyed

Breed	Number of Litters	Number of Pups	Average Litter Size	Mean Birth Weight	Range +/- 1 s.d.	Sex M:F	Presentation A : P	Placenta With:Without	Total Mortality	Stillborn Loss	Early Neonatal Mortality	Perinatal Mortality	Percent Caesarean
Toys	**183**	**664**	**3.6**			**59:41**	**70:30**	**70:30**	**20.5%**	**6.3%**	**12.5%**	**18.8%**	**24.5%**
Australian Silky Terrier	49	191	3.9	156.0	116.0-196.0	59:41	77:23	68 : 32	15.2%	2.1%	9.9%	12.0%	6.1%
Chihuahua	53	174	3.3	117.8	87.8 - 147.8	54:46	65 : 35	82 : 18	20.7%	10.3%	9.8%	20.1%	18.9%
Chinese Crested	9	39	4.3	185.1	129.1-241.1	59:41	71:29	14:86	7.7%	0.0%	5.1%	5.1%	0.0%
Dachshund Miniature	31	116	3.7	200.7	145.7-255.7	48:53	84:16	77:23	28.5%	6.9%	21.6%	28.4%	22.6%
Japanese Chin	1	3	3.0	170.0	170.0 - 170.0	67:33	50 : 50	100 : 0	0.0%	0.0%	0.0%	0.0%	0.0%
Maltese Terrier	1	4	4.0	210.0	129.0 - 291.0	75:25	100 : 0	100 : 0	0.0%	0.0%	0.0%	0.0%	0.0%
Miniature Pinscher	1	5	5.0	98.0	90.0 - 106.0	60:40	60 : 40	100 : 0	60.0%	0.0%	60.0%	60.0%	0.0%
Pekingese	35	123	3.5	189.9	163.8-215.8	53:47	49:51	53:47	24.4%	8.9%	13.8%	22.8%	60.0%
Pomeranian	2	4	2.0	100.0	100.0 - 100.0	75:25	0 : 100	0 : 100	50.0%	25.0%	25.0%	50.0%	100.0%
Poodle Toy	1	5	5.0	234.0	215.0 - 253.0	40:60	100 : 0	100 : 0	0.0%	0.0%	0.0%	0.0%	0.0%
Small	**85**	**340**	**4.0**			**50:50**	**68:32**	**85 : 15**	**21.5%**	**8.5%**	**12.6%**	**21.2%**	**11.8%**
Dachshund Standard	2	11	5.5	265.5	214.5 - 316.5	27:73	75 : 25	75 : 25	0.0%	0.0%	0.0%	50.0%	50.0%
Shetland Sheepdog	64	247	3.9	203.0	145.0-261.0	48:52	69:31	86:14	21.8%	10.5%	11.3%	21.9%	9.4%
Cavalier King Charles Spaniel	5	22	4.4	198.5	168.5 - 228.5	68:32	22 : 78	71 : 29	40.9%	4.5%	36.4%	40.9%	20.0%
Cairn Terrier	2	5	2.5	178.6	113.6 - 243.6	20:80	100 : 0	100 : 0	20.0%	0.0%	20.0%	20.0%	0.0%
Bichon Frise	2	4	2.0	214.0	100.8 - 240.0	67:33	67 : 33	0 : 100	25.0%	0.0%	25.0%	25.0%	50.0%
Schnauzer Miniature	7	36	5.1	161.6	111.6 - 211.6	64:36	86 : 14	100 : 0	13.9%	0.0%	11.1%	11.1%	14.3%
Pug	2	10	5.0	197.2	152.2 - 242.2	40:60	70 : 30	80 : 20	30.0%	20.0%	10.0%	30.0%	0.0%
Lhasa Apso	1	5	5.0	242.0	228.0 - 256.0	40:60	80 : 20	60 : 20	0.0%	0.0%	0.0%	0.0%	0.0%

Appendix 19
Reproductive performance in all breeds surveyed

Breed	Number of Litters	Number of Pups	Average Litter Size	Mean Birth Weight	Range +/- 1 s.d.	Sex M:F	Presentation A : P	Placenta With:Without	Total Mortality	Stillborn Loss	Early Neonatal Mortality	Perinatal Mortality	Percent Caesarean
Medium	**56**	**339**	**6.1**			**51:49**	**74:25**	**82:18**	**18.3%**	**5.9%**	**8.3%**	**14.2%**	**14.2%**
Australian Cattle Dog	16	108	6.8	306.6	260.6-356.6	49:51	75:25	86 : 14	21.3%	4.6%	7.4%	12.0%	12.5%
Basenji	1	5	5.0			60:40	80 : 20	100 : 0	0.0%	0.0%	0.0%	0.0%	0.0%
Beagle	1	6	6.0	316.7	255.7 - 377.7	83:17	50 : 50	50 : 50	33.3%	33.3%	0.0%	33.3%	100.0%
Border Collie	24	152	6.3	353.4	286.4-420.4	48:52	80:20	84:16	11.8%	5.9%	5.3%	11.2%	12.5%
British Bulldog	3	13	4.3	386.3	355.3 - 417.3	38:62			69.2%	0.0%	69.2%	69.2%	33.3%
Bull Terrier	2	10	5.0	432.2	379.2 - 485.2	70:30	70 : 30	60 : 40	10.0%	10.0%	0.0%	10.0%	0.0%
Cocker Spaniel	1	2	2.0	212.5	191.5 - 233.5	50:50	100 : 0	100 : 0	50.0%	0.0%	0.0%	0.0%	0.0%
Keeshond	5	22	4.4	273.2	201.2 - 345.2	55:45	77 : 23	55 : 45	27.2%	13.6%	9.1%	22.7%	0.0%
Staffordshire Bull Terrier	2	13	6.5	229.6	161.6-297.6	69:31	25:75	100 : 0	15.4%	0.0%	7.7%	7.7%	50.0%
Whippet	1	8	8.0	236.3	203.3 - 269.3	50:50	13 : 17	100 : 0	0.0%	0.0%	0.0%	0.0%	0.0%
Large	**152**	**1030**	**6.8**			**52:48**	**71 : 29**	**79 : 21**	**15.1%**	**6.1%**	**7.7%**	**13.8%**	**15.8%**
Basset Hound	12	80	6.7	458.2	390.2 - 526.2	44:56	44 : 56	92 : 8	13.8%	3.8%	7.5%	11.3%	33.3%
Bearded Collie	2	12	6.0	257.1	226.1 - 288.1	50:50	100 : 0		41.7%	16.7%	8.3%	25.0%	0.0%
Belgian Shepherd	2	14	7.0	400.3	296.3 - 504.3	64:36	36 : 64	86 : 14	14.2%	0.0%	7.1%	7.1%	0.0%
Borzoi	1	6	6.0	523.3	480.3 - 566.3	67:33	33 : 67	67 : 33	0.0%	0.0%	0.0%	0.0%	0.0%
Boxer	16	105	6.6	407.4	325.4-489.4	57:46	76 : 24	79 : 21	20.0%	4.8%	14.3%	19.1%	25.0%
Boxer*									33.3%	4.8%	28.6%	32.4%	
Collie Rough	17	104	6.1	274	187.0-361	50:50	78 : 22	81 : 19	10.6%	4.8%	4.8%	9.6%	5.9%
German Shepherd	42	282	6.7	563	437.0-689.0	61:39	74:26	70:30	19.9%	9.2%	8.9%	18.1%	4.8%
German Shorthaired Pointer	3	16	5.3	427.4	358.4 - 496.4	50:50	79 : 21	60 : 40	6.3%	0.0%	6.3%	6.3%	33.3%

Appendix 19
Reproductive performance in all breeds surveyed

Breed	Number of Litters	Number of Pups	Average Litter Size	Mean Birth Weight	Range +/- 1 s.d.	Sex M:F	Presentation A : P	Placenta With:Without	Total Mortality	Stillborn Loss	Early Neonatal Mortality	Perinatal Mortality	Percent Caesarean
Golden Retriever	22	116	5.3	468.6	395.6 - 541.6	47:53	73 : 27	88 : 12	11.2%	6.9%	4.3%	11.2%	9.1%
Hungarian Visula	1	8	8.0	414.5	355.5 - 473.5	88:12	63 : 37	100 : 0	37.5%	0.0%	37.5%	37.5%	0.0%
Labrador	21	154	7.3	422.2	347.2-497.2	50:50	74 : 26	87 : 13	9.7%	5.2%	3.2%	8.4%	33.3%
Rhodesian Ridgeback	10	106	10.6	471.1	389.1 - 553.1	42:58	74 : 26	78 : 22	13.2%	1.9%	11.3%	13.2%	10.0%
Rhodesian Ridgeback*									36.8%	1.9%	34.9%	36.8%	
Samoyed	2	15	7.5	402.1	334.1-406.1	27:73	57 : 43	75 : 25	20.0%	20.0%	0.0%	20.0%	50.0%
Tibetan Mastiff	1	12	13.0	461.8	350.8 - 572.8	62:38	100 : 0	92 : 8	8.3%	8.3%	0.0%	8.3%	0.0%
Giant	**24**	**201**	**8.4**			**45:55**	**64 : 36**	**81:19**	**24.4%**	**12.9%**	**9.5%**	**22.4%**	**20.8%**
Great Dane	23	195	8.5	536.1	388.1-684.1	45:55	64 : 36	81: 19	23.1%	13.3%	7.7%	21.0%	21.7%
									27.7%	12.5%	11.6%	24.1%	
St Bernard	1	6	6.0	563.3	503.3 - 623.3	50:50			66.7%	0.0%	66.7%	66.7%	0.0%

* Mortality in breeds with elective euthanasia
** Non-elective caesarean sections

Appendix 20:1

Summary of histopathological changes identified in foetal asphyxia of stillborn pups

Pup 271 Acute cerebral congestion

Pup 401 Congenital (neonatal) atelectasis with *in utero* hypoxia. Acute systemic congestion - myocardium/epicardium, liver, kidney, cerebrum/choroid plexus and leptomeninges.

Pup 548 Premature thymic involution. Congenital (neonatal) atelectasis with *in utero* hypoxia. Mild to moderate visceral congestion - thymus, mediastinum, lungs, myocardium/epicardium kidney, brain/leptomeninges/choroid plexus and pancreatic mesentery with multifocal acute petechiation (brain and (kidney). Mild haemoglobinuria/myoglobinuria

Pup 646 Congenital (neonatal) atelectasis with *in utero* hypoxia. Acute systemic congestion and petechial haemorrhages

Pup 846 Congenital (neonatal) atelectasis with *in utero* hypoxia. Acute epicardial/myocardial and leptomeningeal congestion.

Pup 886 Congenital (neonatal) atelectasis with *in utero* hypoxia.

Pup 888 Congenital (neonatal) atelectasis with *in utero* hypoxia. Acute hepatic congestion

Pup 1502 *In utero* hypoxia. Acute multi systemic congestion - brain/leptomeninges/choroid plexus, lungs, myocardium/epicardium.

Pup 1522 *In utero* hypoxia. Acute multi systemic congestion (brain/leptomeninges/choroid plexus, lung, liver and thymus) with multifocal acute haemorrhage (especially leptomeninges).

Pup 1616 Congenital (neonatal) atelectasis and *in utero* hypoxia.

Pup 1627 Congenital (neonatal) atelectasis and pulmonary haemorrhage associated with *in utero* hypoxia. Acute systemic congestion - epicardium, cerebrum, choroid plexus, third ventricle and leptomeninges.

Pup 2060 Congenital (neonatal) atelectasis with *in utero* hypoxia. Acute systemic congestion - myocardium/epicardium, liver, (numerous petechial haemorrhages particularly at the periphery of the lobe) cerebrum, choroid plexus and leptomeninges.

Pup 2426 Congenital (neonatal) atelectasis with *in utero* hypoxia. Moderate, acute congestion of the epicardial vessels.

Appendix 20:2 Histopathological changes identified in the lungs and pleura in foetal asphyxia of stillborn pups

Pup	Litter	Dystocia	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
271	DU	Absent																			
401	AX	Present	2	2	1	0	0	0	2	0	0	1	2	0	0	0	0	0	0	0	0
548	DJ	Absent	0	1	0	0	0	0	3	1	0	1	0	0	0	0	?	0	0	0	0
646	CA	Present	2	1	0	0	0	0	3	0	0	1	1	0	0	0	0	0	0	0	0
846	KT	Present	2	1	0	0	0	0	2	0	0	3	1	0	0	0	0	0	0	0	0
886	JH	Present	1	2	2	0	0	0	2	0	0	2	0	0	0	0	0	0	0	0	0
888	JH	Present	2	2	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0
1502	KI	Absent	2	1	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
1522	BY	Absent	4	1	0	0	0	0	2	0	0	1	1	0	0	0	0	0	0	0	0
1616	EE	Absent	0	1	0	0	0	0	3	0	0	3	0	0	0	0	0	0	0	0	0
1627	EY	Absent	3	1	* 0	2	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0
1857	DG	Present	1	2	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0
2060	FU	Present	2	1	0	0	0	0	2	0	0	1	1	0	0	0	0	0	0	0	0
2426		Present	2	2	0	0	0	0	2	0	0	2	2	0	0	0	0	0	0	0	0

Legend

1 : None; 2 : Mild/few cells present; 3 : Moderate changes/numbers of cells; 4: Severe changes/large number of cells present

A: Alveolar inflation
B:Parenchymal congestion
C: Alveolar haemorrhage
D: Alveolar oedema
E: Alveolar milk
F: Alveolar emphysema
G: Alveolar macrophages
H: Alveolar neutrophils
I: Alveolar bacteria
J: Alveolar squames
K: Alveolar meconium
L: Hyaline membrane disease
M: Bronchial epithelial hyperplasia
N: Distended bronchioles, terminal
O: Interstitial thickening
P: Focal granuloma
Q: Bronchopneumonia
R: Pleural congestion/haemorrhage
S: Pleural inflammation/fibrin

Appendix 20:3 Histopathological changes identified in the brains in foetal asphyxia of stillborn pups

Pup	Dystocia	1	2	3	4	5	6	7	8	9	10	11	12
271	Absent	2	0	0	0	0	0	0	0	2	0	1	1
401	FD	3	0	0	0	0	0	0	0	2	0	1	1
548	Absent	2	0	0	0	0	0	0	0	2	0	1	1
646	MSUI	3	1	0	0	0	0	0	0	2	0	1	1
846	MPUI	2	0	0	0	0	0	0	0	2	0	1	1
886	MSUI												
888	MSUI												
1502	Absent	2	0	0	0	0	0	0	0	1	0	1	1
1522	Absent	2	0	0	0	0	0	0	0	1	0	1	1
1616	Absent												
1627	Absent	2	0	0	0	0	0	0	0	1	0	1	2
1857	MSUI	0	0	0	0	0	0	0	0	2	0	0	0
2060	FD	2	0	0	0	0	0	0	0	2	0	0	1
2426	FD												

LEGEND

1: None 2: Mild changes 3: Moderate changes 4: Severe changes

A. Leptomeningeal congestion / haemorrhage	G. Meningeal inflammation
B. Brain haemorrhage	H. Ventricular inflammation
C. Brain necrosis (recent)	I. Oedema/congestion of the choroid plexus
D. Brain necrosis (older)	J. Ventricular haemorrhage.
E. Oedema meninges	K. Perivascular ring haemorrhages
F. Brain inflammation	L. Congestion of the cerebral blood vessels

Appendix 21:1

Summary of histopathological changes identified in live distressed pups which died within the first 48 hours after birth.

Pup 249 Pulmonary haemorrhage and oedema (rare squames). Moderate to acute congestion of the cerebrum, choroid plexus and leptomeninges.

Pup 344 Congenital (neonatal) atelectasis with *in utero* hypoxia. Acute systemic congestion - myocardial/epicardial, cerebrum and leptomeninges

Pup 402 Congenital (neonatal) atelectasis with *in utero* hypoxia. Acute systemic congestion - epicardium, liver, kidney, cerebrum and leptomeninges.

Pup 486 Congenital (neonatal) atelectasis. Mild systemic congestion - kidney, leptomeninges and choroid plexus

Pup 958 Severe, acute cerebral, leptomeningeal and choroid plexus congestion, and to lesser extent, leptomeningeal haemorrhage. Limited areas of atelectasis and pulmonary oedema.

Pup 989 Congenital (neonatal) atelectasis with *in utero* hypoxia. Acute systemic congestion - liver, epicardium and cerebral vasculature.

Pup 1051 Congenital (neonatal) atelectasis with *in utero* hypoxia and possibly infected amnion inhalation. Leptomeningeal congestion and traumatic subdural haemorrhage.

Pup 1103 Congenital (neonatal) atelectasis. Acute, severe leptomeningeal, choroid plexus and cerebral vasculature congestion.

Pup 1376 *In utero* hypoxia.

Pup 1481 Congenital (neonatal) atelectasis with *in utero* hypoxia. Moderate leptomeningeal and choroid plexus congestion.

Pup 1530 Congenital (neonatal) atelectasis.

Pup 1629 Congenital (neonatal) atelectasis with *in utero* hypoxia. Mild leptomeningeal and more severe choroid plexus oedema and congestion.

Pup 1745 Congenital (neonatal) atelectasis with *in utero* hypoxia.

Pup 1746 Congenital (neonatal) atelectasis with *in utero* hypoxia and pneumonia possibly from aspiration of infected amniotic fluid.

Pup 1761 Congenital (neonatal) atelectasis with *in utero* hypoxia.

Pup 1821 Congenital (neonatal) atelectasis with *in utero* hypoxia and aspiration of infected amniotic fluid. Acute thymic congestion. Acute leptomeningeal and choroid plexus congestion and haemorrhage as well as cerebral haemorrhage.

Pup 1841 Congenital (neonatal) atelectasis with *in utero* hypoxia. Moderate to severe acute systemic congestion - thymus, liver, kidney, heart, leptomeninges, choroid plexus and cerebrum.

Pup 1842 Congenital (neonatal) atelectasis with *in utero* hypoxia. Moderate to severe acute systemic congestion - liver, kidney, heart, leptomeninges, choroid plexus and cerebrum.

Pup 1852 Congenital (neonatal) atelectasis with *in utero* hypoxia. Mild systemic congestion - liver, kidney, epicardium, leptomeninges and cerebrum.

Pup 1952 Lung congestion and haemorrhage with *in utero* hypoxia. Severe to moderate congestion of the heart.

Pup 2158 Congenital (neonatal) atelectasis, oedema and haemorrhage.

Appendix 21:2 Histopathological changes identified in the lungs and pleura in foetal asphyxia, live distressed pups which died in the first 48 hours after birth

Pup	Litter	Form of	DOD*	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
249	BR	Absent	2	4	2	0	4	3	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0
279	GA	Absent	3	3	1	0	1	0	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0
344	CW	Absent	2	1	4	0	0	0	0	3	0	0	0	1	0	0	1	0	0	0	0	0	0
402	AX	Present	2	1	4	1	0	4	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
486	BU	Present	1	1	2	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
838	KK	Present	3	4	1	0	0	0	0	1	1	0	1	2	0	1	3	1	0	0	0	0	0
958	MA	Present	2	4	1	1	2	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0
989	DI	Present	1	2	3	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
1051	GY	Absent	2	2	0	4	4	0	0	3	3	0	3	0	0	0	2	0	0	2	0	0	0
1103	NV	Present	1	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1376	DX	Present	1	4	2	0	0	0	0	3	0	0	3	0	0	0	0	0	0	0	0	0	0
1481	HW	Present	1	1	2	0	0	0	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0
1530	DC	Present	1	2	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1629	EY	Absent	1	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
1745	JW	Present	2	1	1	0	0	0	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0
1746	JW	Present	2	2	1	0	0	0	0	4	3	0	1	0	0	0	0	0	0	2	0	0	0
1753	KG	Absent	3	4	1	2	2	0	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0
1761	KO	Absent	2	2	2	1	1	0	0	4	0	0	2	0	0	0	0	0	0	0	0	0	0
1821	BA	Absent	1	2	2	0	0	0	0	3	3	0	4	0	0	0	0	0	0	2	0	0	0
1841	CZ	Present	1	2	3	2	0	3	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
1842	CZ	Present	1	1	2	1	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0
1852	DF	Absent	1	0	3	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
1952	JZ	Absent	1	4	4	2	0	3	0	2	0	0	2	2	0	0	0	0	0	0	0	0	0
2158		Present	1	3	3	2	3	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

Legend DOD* : day of death

1 : None; 2 : Mild/few cells present; 3 : Moderate changes/numbers of cells; 4: Severe changes/large number of cells present

A: Alveolar inflation
B: Parenchymal congestion
C: Alveolar haemorrhage
D: Alveolar oedema
E: Alveolar milk
F: Alveolar emphysema
G: Alveolar macrophages
H: Alveolar neutrophils
I: Alveolar bacteria
J: Alveolar squames
K: Alveolar meconium
L: Hyaline membrane disease
M: Bronchial epithelial hyperplasia
N: Distended bronchioles, terminal
O: Interstitial thickening
P: Focal granuloma
Q: Bronchopneumonia
R: Interstitial pneumonia
S: Pleural congestion/haemorrhage
T: Pleural inflammation/fibrin

Appendix 21:3 Histopathological changes identified in the brains of foetal asphyxia, live distressed pups which died in the first 48 hours after birth

Pup	Litter	Dystocia	Form of	DOD	A	B	C	D	E	F	G	H	I	J	K	L
1103	NV	Absent		1	4	0	0	0	0	0	0	0	4	0	2	2
1629	EY	Absent		1	2	0	0	0	0	0	0	0	3	0	0	0
1821	BA	Absent		1	5	2	0	0	0	0	0	0	2	2	0	0
1852	DF	Absent		1	2	0	0	0	0	0	0	0	0	0	0	2
1952	JZ	Absent		1												
486	BU	Present	FD	1	1	0	0	0	0	0	0	0	1	0	0	1
989	DI	Present	M2I	1	1	0	0	0	0	0	0	0	0	0	3	3
1376	DX	Present	FD	1												
1481	HW	Present	FD	1	2	0	0	0	0	0	0	0	3	0	0	0
1530	DC	Present	MPI	1	1	0	0	0	0	0	0	0	0	0	0	0
1841	CZ	Present	MPI	1	1	0	0	0	0	0	0	0	1	0	2	2
1842	CZ	Present	MPI	1	2	0	0	0	0	0	0	0	2	0	2	2
1	Hal	Present	MPI	1	1	0	0	0	0	0	0	0	1	0	0	0
249	BR	Absent		2	3	0	0	0	0	0	0	0	2	0	0	1
344	CW	Absent		2	3	0	0	0	0	0	0	0	0	0	1	2
1051	GY	Absent		2	3	2	0	0	0	0	0	0	0	0	0	0
1761	KO	Absent		2												
402	AX	Present	FD	2	4	0	0	0	0	0	0	0	0	1	1	1
958	MA	Present	MPI	2	3	0	0	0	0	0	0	0	3	0	3	3
1745	JW	Present	M2I	2												
1746	JW	Present	M2I	2												

Legend DOD* : day of death

1 : None; 2 : Mild changes; 3 : Moderate changes ; 4: Severe changes

A: Leptomeningeal congestion / haemorrhage

B: Brain haemorrhage

C: Brain necrosis (recent)

D: Brain necrosis (older)

E: Oedema meninges

F: Brain inflammation

G:. Meningeal inflammation

H: Ventricular inflammation

I: Oedema/congestion of the choroid plexus

J: Ventricular haemorrhage.

K: Perivascular ring haemorrhages

L: Congestion of the cerebral blood vessels

Appendix 22:1

Summary of histopathological changes identified in live distressed pups which died on day three to day ten.

(Day of death in parentheses)

Pup 279 (3) Areas of atelectasis and *in utero* hypoxia. Mild leptomeningeal congestion

Pup 838 (3) Congenital pulmonary overinflation with *in utero* hypoxia. In the infant this is due partial obstruction of the lobular bronchus and explains why the right lung appeared grossly overexpanded with crepitus on palpation. Acute cardiac and mild leptomeningeal and choroid plexus congestion.

Pup 1753 (3) Alveolar oedema and haemorrhage and *in utero* hypoxia. Leptomeningeal and choroid plexus congestion.

Pup 2394 (4) Severe, acute bronchopneumonia, focal granulomas and pleurisy and *in utero* hypoxia.

Pup 1587 (4) Congenital (neonatal) atelectasis.

Pup 1861 (4) Congenital (neonatal) atelectasis. Mild systemic congestion, - liver, heart, leptomeninges and cerebral vasculature.

Pup 1161 (4) Pulmonary oedema with *in utero* anoxia. Fibrinous pleurisy.

Pup 450 (10) Mild acute renal cortical congestion. Incomplete mobilisation of epicardial brown fat stores with mild, acute, epicardial congestion. Peripheral pulmonary hypoplasia. Mild, acute to early subacute interstitial pneumonia. Severe, multifocal, subacute to chronic, periventricular leukoencephalomalacia.

Pup 866 (10) Pleurisy and subpleural pneumonia.

Appendix 22:2 Histopathological changes identified in the lungs and pleura in foetal asphyxia, live distressed pups which died three to ten days after birth

Pup	Litter	Form of	DOD*	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
450	GL	Present	10	4	2	0	2	2	0	3	3	0	0	0	0	0	0	2	0	2	0	1	2
866	JG	Present	10	4	4	2	3	0	0	2	2	3	0	0	0	0	2	0	0	2	0	2	3
1161	HJ	Absent	6	4	4	0	5	0	0	1	0	5	0	2	0	0	1	0	0	0	0	1	3
1587	CM	Absent	4	2	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0
1861	DH	Absent	4	2	4	2	3	3	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0
2394		Present	4	4	2	1	1	0	0	1	5	0	1	2	0	0	0	0	1	4	4	4	4

Legend DOD* : day of death

1 : None; 2 : Mild/few cells present; 3 : Moderate changes/numbers of cells; 4: Severe changes/large number of cells present

A: Alveolar inflation
B: Parenchymal congestion
C: Alveolar haemorrhage
D: Alveolar oedema
E: Alveolar milk
F: Alveolar emphysema
G: Alveolar macrophages
H: Alveolar neutrophils
I: Alveolar bacteria
J: Alveolar squames
K: Alveolar meconium
L: Hyaline membrane disease
M: Bronchial epithelial hyperplasia
N: Distended bronchioles, terminal
O: Interstitial thickening
P: Focal granuloma
Q: Bronchopneumonia
R: Interstitial pneumonia
S: Pleural congestion/haemorrhage
T: Pleural inflammation/fibrin

Appendix 22:3 Histopathological changes identified in the brains of foetal asphyxia, live distressed pups which died three to ten days after birth

Pup	Litter	Dystocia	Form of	DOD	A	B	C	D	E	F	G	H	I	J	K	L
279	GA	Absent		3	1	0	0	0	0	0	0	0	0	0	0	0
1753	KG	Absent		3	1	0	0	0	0	0	0	0	2	0	0	0
838	KK	Present	M2I	3	1	0	0	0	0	0	0	0	1	0	0	0
1587	CM	Absent		4	0	0	0	0	0	0	0	0	0	0	0	0
1861	DH	Absent		4	1	0	0	0	0	0	0	0	0	0	0	1
2395	MO	Present	MPI	4												
1161	HJ	Absent		6	0	0	0	0	0	0	0	0	0	0	0	0
450	GL	Present	MPI	10	0	0	0	4	0	0	0	0	1	0	0	0
866	JG	Present	M2I	10						*						

Legend DOD* : day of death

1 : None; 2 : Mild changes; 3 : Moderate changes ; 4: Severe changes

A: Leptomeningeal congestion / haemorrhage
B: Brain haemorrhage
C: Brain necrosis (recent)
D: Brain necrosis (older)
E: Oedema meninges
F: Brain inflammation
G: Meningeal inflammation
H: Ventricular inflammation
I: Oedema/congestion of the choroid plexus
J: Ventricular haemorrhage
K: Perivascular ring haemorrhages
L: Congestion of the cerebral blood vessels

Appendix 22:4

Histopathological changes identified in pup 450

The tissues show a mild degree of autolytic degeneration, being most advanced in the brain.

The kidney shows mild, acute, diffuse, cortical congestion but is otherwise within histologically normal limits.

There is mild congestion of the epicardium in which there is incomplete mobilisation of brown adipose tissue stores. The myocardium, in both haematoxylin and eosin and PTAH stained sections, appears within normal limits.

There is hypoplasia of the pulmonary parenchyma towards the periphery of the lobular specimen. In this area there is a reduction in the number of alveoli which are lined by cuboidal epithelial cells. The alveoli are separated by loose, mildly oedematous, fibrous connective tissue in which there is distention of numerous capillaries. The peripheral parenchyma has a distinctive foetal appearance and contrasts with the more typical postnatal appearance of the rest of the specimen.

There is a discontinuous, cuboidal to low columnar, reactive swelling of the visceral plural mesothelium. The plural surface is covered by a thin film of unorganised, fibrinosupperative exudate. There are multiple, acute, tiny subplural petechiae and petechiae are also identifiable in oedematous interstitial connective tissue. The lung field is diffusely oedematous and congested to a mild to moderate degree. There is mild atelectasis, particularly towards the periphery of the lobe. Floccular protein lies free within alveolar lumina, accompanied by low numbers of alveolar macrophages, neutrophils and rare erythrocytes. The number of alveolar macrophages and neutrophils within the alveoli and terminal bronchiolar lumina increases towards the periphery of the lobe. Rare squames and exogenous pigment fragments can be identified within infrequent alveoli. There is mild, diffuse hypercellularity of the oedematous and congested alveolar septa as a result of leukocytic sequestration. No infectious agents are identifiable with routine H&E stain.

The brain is autolysed to a mild degree, with post mortem, oedematous distention of the leptomeninges and with mild rarefaction of the subpial neuropil due to imbibition of the cerebrospinal fluid.

There are multiple, large foci of subacute to early chronic malacia of periventricular white matter of the centrum semiovale (corona radiate) of the frontal and anterior parietal lobes, extending irregularly into the adjacent internal capsule. The largest foci extends into the corona radiata dorsolateral to the lateral ventricle at the level of the sections through the thalamus and basal nuclei. A subtle focus of malacia can also be identified in white matter immediately lateral to the lateral ventricle dorsal to the caudal part of the lateral rhinal sulcus. Subtle oedema and gliosis radiates across the corpus callosum and the crus of the fornix. Early, subtle malacic lesions can be identified in the white matter immediately deep and lateral to the third ventricle where it underruns the crus of the fornix.

The white matter lesions are of ischaemic type and the rarefied, ghostlike necrotic tissue at the centre of the larger foci is beginning to cavitate. At the immediate periphery of the necrotic zone are dense banks of Gitter cells (compound granular corpuscles) which are

actively phagocytosing and contain fine lipid vacuoles and often large, bright pink-purple, cytoplasmic inclusions. The vessels in the marginal zone are congested and have swollen endothelial cells with increased nuclear size. Some of the vessels are ringed by haemorrhage. In sites of haemorrhage, microglia transformed to macrophages are engaged in erythrophagocytic activity and some contain haemosiderin granules. There is random opoptosis of Glitter cells and other glial elements in this marginal zone. Coarse, purple granule deposits within the cytoplasm of dying and necrotic glial elements suggest mineralisation. Gemistocytic (reactive) astrocytes can be identified frequently around the borders of the malacic foci. These cells have nuclear eccentricity and voluminous, acidophilic cytoplasm, The gemistocytes suggest a lesion age approching 14 days. Radiating external to the densly cellular areas at the margins of the malacic foci are zones of variable depth of neutropil oedema, mild diffuse gliosis, astrocytic nuclear swelling, vascular congestion and axonal spheroid formation.

Where the leukomalacic foci directly abut the left ventricle, there is locally extensive loss of ependyma. Stroma of the choroid plexus of the lateral ventricle is diffusely oedematous, with rare, haemosiderin-pigmented macrophages and with mild to moderate, diffuse hyperaemia.

The leptomeninges are mildly congested and sparse numbers of macrophages, some with lipid-vacuolated cytoplasm, are loosely distributed throughout the pia and arachnoid.

DIAGNOSIS

1. Mild acute renal cortical congestion.

2. Incomplete mobilisation of epicardial brown fat stores with mild, acute, epicardial congestion.

3. Peripheral pulmonary hypoplasia.

4. Mild, acute to early subacute interstitial pneumonia.

5 . Severe, multifocal, subacute to chronic, periventricular leukoencephalomalacia.

Appendix 23

Summary of histopathological changes identified in fading pups considered growth retarded.

FG 105 Neonatal atelectasis with acute intra-alveolar haemorrhage.

FG 106 Neonatal atelectasis with possible *in utero* hypoxia.

GH 924 Congenital (neonatal) atelectasis and severe congestion.

GH 926 Congenital (neonatal) atelectasis with severe lung congestion.

RR 2402 Congenital (neonatal) atelectasis

RR 2403 Congenital (neonatal) atelectasis with *in utero* anoxia and low grade pneumonia.

RR 2404 Congenital (neonatal) atelectasis.

SP 2530 Congenital (neonatal) atelectasis. Acute, severe systemic congestion.

SP 2531 Congenital (neonatal) atelectasis. Acute, systemic congestion

SP 2532 Congenital (neonatal) atelectasis with *in utero* hypoxia. Acute, systemic congestion.

SP 2534 Congenital (neonatal) atelectasis. Acute, severe systemic congestion.

SP 2135 Congenital (neonatal) atelectasis. Acute cardiac congestion.

SP 2136 Congenital (neonatal) atelectasis.

IT 1710 Congenital (neonatal) atelectasis with *in utero* hypoxia

Appendix 24

Summary of histopathological changes identified in fading pups with primary lung pathology

CW 342 Congenital (neonatal) atelectasis.

CW 343 Congenital (neonatal) atelectasis and moderate alveolar haemorrhage

CW 344 Congenital (neonatal) atelectasis and alveolar haemorrhage with *in utero* hypoxia.

GJ 718 Congenital (neonatal) atelectasis with severe pulmonary congestion.

GJ 721 Congenital (neonatal) atelectasis with alveolar haemorrhage and severe congestion.

GJ 725 *In utero* hypoxia

FY 1116 Congenital (neonatal) atelectasis with alveolar haemorrhage and low grade pneumonia.

KL 1466 *In utero* hypoxia with aspiration of meconium and pulmonary congestion.

KL 1467 Congenital (neonatal) atelectasis with *in utero* anoxia. Moderate to severe systemic congestion.

CY 1536 Congenital (neonatal) atelectasis

BJ 1832 Acute pulmonary haemorrhage. Acute leptomeningeal congestion and haemorrhage with clot formation in fissures, particularly longitudinal fissure.

BJ 1833 Bronchopneumonia, possibly due to milk inhalation. *In utero* hypoxia with inhalation of meconium.

QV 2244 Neonatal atelectasis with evidence of *in utero* anoxia. Low grade bronchopneumonia.

QV 2247 Neonatal atelectasis with evidence of *in utero* anoxia. Low grade bronchopneumonia. Severe, acute leptomeningeal congestion and haemorrhage.

Appendix 25

Summary of histopathological changes identified in fading pups dying form overwhelming sepsis

QA 2150 Severe, acute, gastric venous infarction with gastric perforation and acute, fibrinosuppurative, septic peritonitis. Acute. Segmental, necrotohaemorrhagic enteritis. Mild to moderate, neonatal atelectasis with mild pulmonary oedema and congestion and multifocal, pulmonary interstitial haemorrhage. Moderately severe, acute leptomeningeal and left ventricular choroid plexus congestion with leptomeningeal oedema and multifocal petichiation and with moderate to severe choroid plexal oedema.

QA:2153 Intestinal dysbacteriosis with severe intestinal mesenteric congestion and haemorrhage and focal, acute, fibrinosuppurative, septic peritonitis. Moderate to severe, acute, diffuse, hepatic congestion. Moderately severe, acute, renal congestion with mild interstitial oedema, perirenal oedema, congestion and acute petechial haemorrhage. Mild pulmonary atelectasis with mild pulmonary congestion and mild, multifocal, acute pulmonary haemorrhage. Focal, peripheral, chronic, necrotising and granulomatous hepatitis.

QA:2154 Severe, acute, necrotising and ulcerative gastritis with gastric venous infarction. Severe, acute, transluminal intestinal, gastrointestinal mesenteric and pancreatic congestion, haemorrhage and acute fibrinosupperative serositis/peritonitis. Acute to early subacute, diffuse interstitial pneumonia with mild atelectasis, severe post mortem bacterial invasion, possible in utero hypoxia and possible terminal aspiration of gastrointestinal contents. Moderate to severe, acute, diffuse, leptomeningeal, cerebral and lateral ventricular choroid plexal congestion with multifocal acute haemorrhage and very early neutrophilic and lymphocytic leptomeningitis.

QA:2156 Mild to moderate, chronic, diffuse, interstitial pneumonia. Very mild, chronic, active, tricuspid valvular endocarditis. Moderately severe leptomeningitis, cerebral and lateral ventricular choroid plexus congestion with very mild, neutrophilic/lymphocytic/histocytic leptomeningitis and periventriculitis.

QA:2157 Severe, acute, haemorrhagic and necrotising gastroenterocolitis with gastric and colonic venous infarction. Moderate to severe leptomeningeal, cerebral and lateral ventricular choroid plexus congestion with oedema, multifocal, acute, petechial haemorrhage and mild neutrophilic-lymphocytic-histocytic leptomeningitis. Mild pulmonary congestion, oedema, atelectasis and multifocal, acute, intra-alveolar haemorrhage.

Appendix 26

Summary of histological changes identified in fading pups dying in consecutive litters

BE:95 Chronic, subepicardial oedema and reactive fibroplasia/fibrosis. Mild, multifocal, subendocardial myocardial necrosis. Mild, peracute to acute leptomeningitis with moderate to severe leptomeningeal congestion. Mild to moderate, subacute to chronic, interstitial pneumonia - suspected haematogenous bacterial infection.

BE: 96 Mild, acute, suppurative leptomeningitis with moderate to severe leptomeningeal congestion and multifocal haemorrhage, moderate to sever, lateral ventricle choroid plexal oedema and periventricular congestion, oedema and haemorrhage. Mild to moderate, subacute to early chronic, diffuse interstitial pneumonia with mild atelectasis. Mild, multifocal, peracute, individual myocardial myofiber necrosis.

FG:105 Neonatal atelectasis with acute intra-alveolar haemorrhage.

FG:106 Neonatal atelectasis with possible in utero hypoxia

Appendix 27

Summary of histopathological changes in fading pups where death was attributed to iatrogenic damage

AG 756 Diffuse pneumonia the distribution of which is suggestive of inhalation pneumonia.

AG 757 Pneumonia, the distribution of which is indicative of inhalation pneumonia. Acute, severe leptomeningeal congestion and haemorrhage.

AG 761 Pneumonia, the distribution of which id indicative of inhalation pneumonia. Moderate congestion of the leptomeninges, choroid plexus and epicardium.

AG 762 Localised pneumonia and pulmonary haemorrhage. The gross distribution of the pneumonia is suggestive on inhalation pneumonia.

AG 764 Congenital (neonatal) atelectasis with *in utero* anoxia. Moderate congestion of the leptomeninges, choroid plexus, cerebrum and heart.

AG 765 Bronchopneumonia, the gross distribution of which suggests inhalation pneumonia.

BT 481 There was a generalised pleurisy and free milk in the plural cavity. The oesophagus was ruptured over the base of the heart. There was generalised cerebral vascular congestion of the leptomeninges. There was extensive autolysis of the carcase.

BT 482 There was extensive mucosal haemorrhage of the stomach. Congenital (neonatal) atelectasis. Acute, systemic congestion - epicardium, kidney, cerebrum, choroid plexus and leptomeninges. Leptomeningeal haemorrhage with large clot formation on the dorsal surface of the cerebrum.

BT 483 There was mottled generalised lung consolidation present. The right cerebellum of the brain was covered by a large clot.

BT 484 Congenital (neonatal) atelectasis with *in utero* hypoxia. Pneumonia with bacterial proliferation. (Some of the bacterial proliferation may be post mortem). Acute, systemic congestion - epicardium, kidney, cerebrum, choroid plexus and leptomeninges. Leptomeningeal haemorrhage with large clot formation in the transverse fissure.

BT 485 Early pneumonia with bacterial proliferation with evidence of *in utero* anoxia. Acute, systemic congestion - epicardium, cerebrum, choroid plexus and leptomeninges. Multifocal, acute haemorrhages of the leptomeninges.