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Canine Abortion / Stillbirth Investigation

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Introduction

In canines, foetal asphyxia / hypoxia / dystocia account for the majority of abortion / stillbirths, with bacterial infection identified as the second most common cause of neonatal losses. Viral infection, protozoal infection and in-utero growth restriction are other documented causes of abortion and stillbirth.

The investigation processes

To obtain the maximum benefit out of your post-mortem examination it is extremely important that you follow a very formal structured procedure to ensure that you evaluate all aspects of the investigative process which include

1. Examination of the bitch.
2. External examination of the foetus.
3. External examination of the placenta.
4. Morphometric measurements.
5. Dissection and sample collection.

1. **Examination of the bitch**

The bitch should be examined as soon as possible after abortion and this should include a complete physical examination (general health, nutritional status, possibility of endocrine abnormalities or other disease processes) plus abdominal palpation / diagnostic imaging to determine if any further foetuses / foetal membranes remain in the uterus or whether there is evidence of uterine pathology (uterine torsion, retained placenta etc).

Laboratory analyses on the bitch

- Clinical pathology: Blood should be collected into EDTA, serum and glucose tube for a full haematology, blood smear examination, serum chemistry profile (ALP, ALT, urea, creatinine, CK, AST) and blood glucose.
- Microbiology: Vaginal swabs collected into charcoal transport medium for bacterial and fungal culture and cytology.
- Serology: Serum should be submitted together with foetal heart blood / thoracic fluid to enable comparative antibody analysis (canine herpesvirus, canine adenovirus, canine parvovirus-1, canine distemper virus, canine brucellosis).
- Endocrinology: Serum submitted for selected endocrine assays if indicated.

2. **External examination of the foetus.**

Take time to stand back and look, palpate and observe for the following

- Obvious congenital abnormalities (anasarca, hydrocephalus, umbilical hernia, cleft palate, hair lip etc.).
- Meconium staining of pups is the sign of a distressed foetus and strong indicator lesion of foetal hypoxia.
- Examine mucous membranes for evidence of anaemia or icterus, suggestive of foetal infection with haemoparasites, and with icterus, certain viral agents (herpes virus, infectious canine hepatitis).
- Observe for foetal oversize. Foetal oversize is a common problem with overnutrition in pregnant bitches.
- Examine the umbilicus for evidence of haemorrhage or infection.
- Age-appropriate features (see table 1). External characteristics of the foetuses are matched to foetal age and this can be used to determine if they are appropriate for the actual age of the foetus being examined. If features observed are inappropriate for the actual age of the foetus, this would indicate that some in-utero incident, whether that be infectious or non-infectious, has occurred which has affected foetal growth.

Figure 1: meconium staining of the tail, rump and hindlimbs of an aborted foetus.

3. **External examination of the placenta**

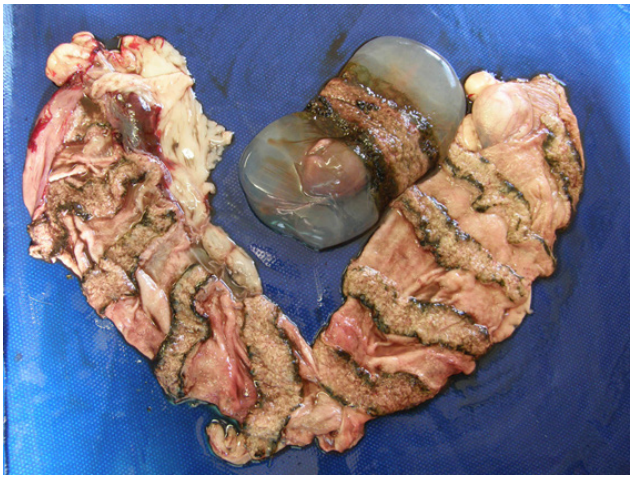

Figure 2: uterus and placenta exhibiting the zonal placentation of the canine placenta.

- Determine if all placenta's have been passed, remember each foetus has its own placenta.
- Examine if all the placentas are complete.
- Are there any signs of premature placental separation or retained placenta, as indicated by green staining of placentas and/or foetuses with bilevirdin / uterovirdin?

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- Examine for evidence of placental necrosis.
- Examine for evidence of placentitis.

4. **Morphometric measurements**

A complete examination should be performed on each foetus submitted irrespective of the degree of autolysis. Determine the sex, measure and record the weight, crown rump length, bi-parietal diameter and evaluate body condition (emaciated / normal / over-sized).

Body weight: Weights depressed more than 25% below the average, for that particular breed, are considered low and indicative of in-utero growth restriction. Such low birth weights are associated with increased neonatal mortality. As a general guide to birth weight the following can be applied.

- Toy Breeds: 100-200g
- Medium: 250-400g
- Large: 400-600g

Crown-Rump Length + External Foetal Characteristics: These are further measures to estimate gestational age.

Figure 2: crown-rump length measurement.

Age (days)	C:R Length (mm)	External Foetal Characteristics
20	8	First and second brachial arches present
30	20	Eyelids forming, grooves between digits, pinnae partly cover external acoustic meatus, five pairs of mammary primordia present, intestines herniated into umbilical cord.
35	35	Eyelids partly cover eyes; pinnae cover acoustic meatus; digits separated distally; external genitalia differentiated.
38	55	Tactile hairs appear on upper lip and above eyes.
40	60	Eyelids fused; intestines returned to abdominal cavity; claws formed.
45	80	Body hair forming; colour markings appear; digits widely separated.
53	140	Hair covering complete; digital pads present.
57-63	160-185	Term foetus / stillbirth

Table 1: External foetal characteristics.

Figure 3: Bi-parietal diameter measurement.

Bi-parietal diameter of the foetal skull in late gestation is highly correlated to gestational age. With the parietal bones orientated in parallel, the distance between the parietal aspects of the skull are measured. Measurements used in the calculation should be expressed as centimetres.

Gestational Age = (15 x head diameter) + 20

Measurements should be carried out on all foetuses from the same litter submitted.
In small breed dogs (< 9 kg), add 1 day to the calculated gestational age.
In large breed dogs (> 40 kg), subtract 2 days from the calculated gestational age.

Body Condition Score: In the foetus body condition scored as emaciated / normal / oversize

Sex: Male / Female / Intersex.

*Low foetal body weight and bi-parietal diameter measurements are strong indicators of in-utero growth restriction.

5. **Dissection and sample collection**

The primary focus of the dissection is to evaluate the foetus and placenta for congenital abnormalities, visible pathology particularly lesions consistent with perinatal asphyxia / hypoxia. Always follow the same routine of foetal examination. This imprints the normal and facilitates recognition of abnormal. Below is a guideline procedure to follow.

- Foetuses are placed in right lateral recumbency.
- Skin is cut along the midline with left fore and hind legs reflected and left aspect of the carcass skinned.
- Subcutaneous tissues are examined for evidence of bruising, haemorrhage and oedema, plus check for rib-fractures (dystocia).
- The abdomen and chest are then opened and thoracic and abdominal viscera plus diaphragm are examined for normality.
- Rectum examined for the presence or absence of meconium.
- If peritoneal haemorrhage evident, examine the internal viscera and umbilicus closely for evidence of injury.
- Cut through temporomandibular area to evaluate the shape and length of soft palate and for peri-pharyngeal strictures.
- Examine pharynx, oesophagus, larynx and trachea for any lesions which may impair respiratory or suckling function.

- Open the skull and examine the brain (hydrocephalus, hydranencephaly etc.).

Lesions indicative of possible foetal hypoxia or asphyxia include meconium staining, pulmonary congestion with atelectasis and cerebral congestion with or without haemorrhage.

Sample collection.

- Collect samples from MULTIPLE foetuses.
- Pooling is discouraged as not all foetuses are equally affected by infectious agents.
- Submit fresh tissues from at least 2 pups. Freeze away excess fresh tissues and store. Can be used later to further identify or better characterise infectious agents.
- Pooling of histopathology samples is acceptable as multiple tissues can be placed in a single tissue cassette. Looking for indicator pathology not individual pathology.

Microbiological culture.

Samples collected for microbiological culture should include stomach fluid, placenta, lung and liver. Stomach fluid should be placed in a screw-top tube and sealed, to prevent leakage in transit to the laboratory. Samples in syringes with needles and hub frequently auto-express during transit with no material available for culture on arrival at the laboratory. Placenta, lung and liver should be placed in separate sterile containers to prevent cross-contamination.

- The target bacterial organisms which have been associated with abortion in canine include *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus pseudintermedius*, *Streptococcus canis*, *Streptococcus dysgalactiae* subsp *equisimilis*, *Streptococcus equi* subsp *zooepidemicus*, *Brucella canis*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.
- *Mycoplasma* sp and *Ureaplasma* sp are evaluated by molecular PCR analyses.
- Fungal abortion considered extremely rare in dogs.

Protozoal Examination. Spleen smears are prepared and stained with routine Diff-Quick® stains. Smears are examined for blood parasites *Babesia canis* and *Ehrlichia canis*.

Histopathology.

Once the internal examination is complete samples are collected into 10% buffered formalin for histopathology. The routine set of tissue samples should always include brain, thyroid, heart, lung, thymus, liver, spleen, kidney, lymphnode, adrenal and intestine.

- Only use buffered formalin as un-buffered formalin labels haematin pigment which interferes with IHC stains, which are used for definitive diagnosis of many infectious agents.
- Do not overfill containers with organs as this leads to poor fixation.
- Many foetal organs are blood-tinged and have high water content which can significantly lower the fixation strength of the formalin. Change formalin at least once in the first 12 hours to ensure adequate fixation.

Immunohistochemical stains for use on formalin fixed tissue sections are available for some important abortifacients in dogs and including

- Viral agents (canine herpesvirus, canine adenovirus, canine distemper virus)
- Protozoal agents (*Toxoplasma gondii*, *Neospora caninum*) associated with abortion and neonatal disease.

Virus Examination

Fresh tissue pool (liver, lung, spleen, kidney, placenta) + thoracic fluid / heart blood).

- Fresh tissue is utilised for PCR analysis for target viruses (canine herpes virus, canine adenovirus, canine minute virus, canine distemper virus).
- Thoracic fluid / heart blood is submitted for serological analysis as part of comparative serology in conjunction with the dam serum.

Serology

Serum from the bitch and thoracic fluid or heart blood from the pups are used for comparative serology (canine herpesvirus, canine adenovirus, canine parvovirus-1, canine distemper virus, canine brucellosis).

Drawbacks of serology include

- Serology from a single bleed foetal heart blood / thoracic fluid should be interpreted with caution.
- There is no specific titre which distinguishes between infection / vaccination or mere exposure.
- The titre at the time of abortion may have reached its maximum in both bitch and foetus and so convalescent antibody titres may be mis-leading.
- A four-fold or higher increase in convalescent antibody tires suggests possible infection.
- Serology must always be supported by ancillary laboratory tests.

Genetic testing

Dry tissue swabs or FTA cards are the preferred sample types for karyotyping and the testing testing for a range of breed associated inherited conditions.

Toxicology

Exposure of the bitch to coumarin derivative rodenticides has been associated with abortion / neonatal deaths due to haemorrhagic diathesis in foetuses / neonates.

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QUESTION 1

Which of the following factors are considered to account for the MAJORITY of abortions and stillbirths in domestic dogs?

a. Fetal asphyxia / dystocia
b. Bacterial infection
c. Viral infection
d. Protozoal infection
e. In-utero growth restriction

QUESTION 2

Bitches which are OVERWEIGHT, due to overnutrition, at the time of abortion, run the risk of developing which of the following fetal abnormalities?

a. Hydrocephalus
b. Foetal oversize
c. Cleft palate
d. In utero growth restriction
e. Anasarca

QUESTION 3

MECONIUM staining of the foetuses is indicative of which pathological process?

a. Congenital abnormality
b. Bacterial infection
c. Viral infection
d. Fetal hypoxia
e. In-utero growth restriction

QUESTION 4

Which of the following statements about performing morphometric measurements on foetuses is INCORRECT?

a. The weight of each foetus is recorded
b. Measurements are only taken from fresh foetuses; autolysed foetuses are not measured
c. Crown-rump length of each foetus is measured
d. Biparietal measurements of each foetus are recorded
e. Body condition score of foetuses is recorded

QUESTION 5

By what gestational AGE are canine foetuses completely covered with hair?

a. 35 days
b. 38 days
c. 40 days
d. 45 days
e. 53 days

QUESTION 6

The preferred set of tissues for microbiological culture DOES NOT INCLUDE which of the following?

a. Stomach fluid
b. Placenta
c. Lung
d. Liver
e. Pancreas

QUESTION 7

Of the following organisms associated with infectious abortion in canines, which is considered an extremely RARE cause of abortion?

a. *Brucella canis*
b. *Streptococcus canis*
c. Fungi
d. *Babesia*
e. *Klebsiella pneumonia*

QUESTION 8

Which of the following statements about the use of serology in abortion investigation is CORRECT?

a. Single bleed serology can be reliably interpreted
b. There are clear specific titres which enable distinction between infection and vaccination
c. The titre at the time of abortion may have reached its maximum in both bitch and foetus and so convalescent antibody titres may be mis-leading
d. A two-fold increase in convalescent antibody titres confirms infection
e. Serology is a reliable stand-alone test

QUESTION 9

The PREFERRED sample type for genetic testing is which of the following?

a. Transport medium swab
b. Serum blood sample
c. Formalin fixed tissue
d. Dry tissue swab
e. Air dried tissue smears

QUESTION 10

Which of the following TOXIC agents have been associated with abortion in dogs due to haemorrhagic diathesis in the foetuses?

a. Coumarin derivative rodenticides
b. Paraquat
c. Organophosphates
d. Strychnine
e. Heavy metals

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