

## 6: VL AND THE VETERINARY SURGEON

### 6.1 How can a differential clinical diagnosis of canine VL be made?

The following table summarises the differential diagnosis of canine VL:

<b>Signs of VL</b>	<b>Other possible causes</b>
bleeding in the muzzle area	trauma
lymphadenopathy	lymphoma; leukaemia
ulcers/nodules on the skin or around the mouth	viruses; other infections or traumas
subcutaneous nodules	sebaceous cysts
dermatitis and loss of hair (depilation)	fleas; other ectoparasites (e.g. scabies); food induced inflammation of the skin (dermatitis); adrenal disease
elongated deformed nails (onychogriposis)	lack of use of claws (e.g. due to confinement in an apartment)
anaemia	poor nutrition; endoparasites; food poisoning; ticks; fleas
emaciation	loss of appetite (anorexia); starvation; endoparasites

### 6.2 How can samples be collected from dogs for serological diagnosis?

(a) *For the direct agglutination test (DAT):*

Puncture the ear margin with a lancet and collect two drops (each making a separate blood spot of 1 cm diameter) on to Whatman No. 3 filter paper.

Label the filter paper with the dog's name, code number and date.

Allow the blood spots to dry at room temperature and then store them dry singly in a plastic envelope or sealed container, or with clean dry paper separating filter paper sheets.

The filter papers may be stored at room temperature for one week, for months in a 4°C fridge, or for years in a -20°C freezer. One blood spot should be used for diagnosis and one saved for future reference or repeat testing.

The blood spot collect method is also sometimes used for other serological tests, such as the indirect immunofluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) (see (b) below).

Serum ((b) below) can also be used for the DAT and may give more accurate results.

- (b) *For the indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA):*

Take about 1 to 2 ml of blood from the vein with a sterile syringe and needle into a sterile container without anticoagulant. Allow the blood to clot, take off the serum, discard the red cells, and store the serum at 4°C in a fridge, or at -20°C if longer storage is needed.

- (c) *For the formol-gel slide test:*

Serum can also be used for this test. It is not specific for VL but detects hyperglobulinaemia, which is often associated with VL.

### 6.3 **How can samples be collected from dogs for parasitological diagnosis?**

Samples can be collected from the following sites for parasitological examination:

- (a) *A lymph node:* any superficial lymph node may be used but the lymph node behind the knee (popliteal lymph node) or the lymph node in front of the scapular (pre-scapular lymph node) are preferred. The lymph node is punctured with a sterile needle (Slide 37). Grasp the lymph node between thumb and fingers, introduce a 21 gauge needle attached to a 5 ml syringe into the lymph node, milk (gently press the lymph node several times) or move the needle backwards and forwards several times, and remove it. The lymph node aspirate should be smeared on to a clean, glass slide and air-dried rapidly, e.g. by rubbing the finger on the back of the slide to produce heat and drive off the moisture in the sample. It is essential that no blood is taken up during the gland puncture, or if it is, that it is not used in preparing the smear.
- (b) *The bone marrow:* preferably by aspiration from the tip of the sternum with a 5 or 10 cc syringe and a 20 - 21 gauge needle. A smear of the aspirate is prepared on a clean glass slide and air-dried (as above for the lymph node aspirate).

- (c) *The skin:* by biopsy using a local anaesthetic. A small sample (e.g. 2 mm x 2 mm and 2 mm deep) is taken with sterile scissors and forceps or with the aid of a sterile skin punch. The skin biopsy is blotted on clean, dry filter paper to remove all excess blood. An impression smear is made by pressing the internal face of the biopsy on to a clean glass slide, and the smear is then air-dried. Biopsies are best taken from any areas of skin that look altered or abnormal (e.g. with hair loss, discolouration, flaking or unusual roughness). The muzzle, the border of the ear and base of the tail are often affected and used for biopsy.
- (d) *The skin by skin scraping:* the skin is shaved if necessary, cleaned with 70 % ethanol and scraped with a sterile scalpel blade until liquid (exudate) emerges. The exudate is smeared on a slide and air-dried.
- (e) *The nose:* by collecting intranasal secretions on a cotton swab. A cotton swab is rubbed firmly on the interior of the nose to obtain exudate from the mucosa, which is then smeared on a slide and air-dried.

#### 6.4 **How is microscopy of aspirates and biopsies performed?**

Dried smears are fixed immediately with absolute (100 %) methanol (methyl alcohol) for 2 minutes and stained with Giemsa at pH 7 - 7.2. The methanol must be stored in tightly closed bottles to prevent absorption of water.

Stain the films in Giemsa as described in Appendix 5.

Examine the Giemsa-stained smears using a 100X oil immersion objective. *Leishmania* amastigotes are very small round or oval organisms about 3  $\mu\text{m}$  x 5  $\mu\text{m}$  found inside or outside phagocytic cells (macrophages). Each amastigote contains a red-mauve nucleus, a smaller more deeply staining red-mauve kinetoplast, and pale blue cytoplasm. The particular diagnostic feature to look for in VL is the presence of nucleus and kinetoplast in these organisms.

In addition if local conditions allow the preparation of appropriate culture medium (as described in appendix 4) material from lymph node or bone marrow aspirates can be placed aseptically into culture. If cultures are not contaminated with bacteria or fungi free-swimming flagellated promastigote forms may be found when the cultures are examined by microscopy 7 - 14 days after they were inoculated with aspirates. Material from skin biopsies is much more difficult to culture. If this is to be attempted skin is swabbed first with 70 % alcohol containing a few flakes of dissolved iodine and then swabbed again with 70 % alcohol before the skin biopsy is taken aseptically.

### 6.5 **How is a serological diagnosis made?**

Useful serological tests are the formol gel test (aldehyde test), in the absence of any other, in tubes or on slides, the direct agglutination test (DAT), the indirect immunofluorescence test (IFAT) and the enzyme-linked immunosorbent assay (ELISA or Dot-ELISA). These tests are described fully in Appendices 6 (formol gel test), 7 (DAT test), 8 (IFAT) and 9 (ELISA).

The formol gel test and DAT (given a reliable standardised source of DAT antigen) can be performed outside laboratories in primary health care centres. The IFAT and ELISA tests require laboratory facilities and more sophisticated equipment, although rapid ELISA or dipstick tests are being developed for use in primary health care centres or in the home.

### 6.6 **How can infected dogs be treated?**

There is no proven way of obtaining a permanent cure in dogs. The following scheme of repetitive treatment is sometimes used in Europe to obtain temporary clinical improvement.

Pentavalent antimony: at 5 mg/kg/day for 14 - 30 days, repeated every 5 - 6 months.

In almost all cases the infection relapses after each course of treatment. This method of treatment may induce resistance of *Leishmania* parasites to pentavalent antimony and dogs may then act as a reservoir of infection for human disease.

*It is recommended therefore that drugs used to treat human infections must not be used to treat dogs. In this way the development of resistant parasites might be avoided.*

### 6.7 **How can infected dogs be prevented from infecting sandflies?**

At present there is no effective way that infected dogs can be prevented from infecting sandflies. Collars, soaps, shampoos and pyrethroids sprayed on dogs have not yet been proven to be effective.

### 6.8 **How can dogs be protected from being infected?**

There are no protection methods for dogs in endemic areas. Removal of the dog from the endemic area appears to be the only way to protect it from VL infection.

6.9 **What are the criteria for destroying an infected dog?**

Note that in some endemic areas of human VL canine VL is not known to occur and dogs are not therefore considered to be relevant as a reservoir of human infection (see appendix 2).

In many endemic areas governments have stated policy and laws which regulate the elimination of VL infected dogs.

In general all dogs proven to be parasitologically positive should be destroyed as they can act as a source of infection for sandflies and source of human disease.

In endemic areas of human VL it is often recommended that all serologically positive dogs are destroyed as they almost certainly carry active infections and may contribute to the spread of human disease.

In Europe dogs are sometimes treated repeatedly with pentavalent antimony but this is not considered appropriate if the same drug is used to treat human infections as treatment of dogs in this way can give rise to drug-resistant parasites (see section 6.6).

In many endemic areas the implementation of governmental policies and the principle that all parasitologically proven positive dogs should be destroyed is limited by financial resources and availability of reagents and equipment for the humane disposal of dogs (section 7).

6.10 **To whom and how should confirmed cases of canine VL be reported?**

Canine VL infections should be reported to appropriate local veterinary and public health authorities as this will provide important epidemiological information on the distribution and potential spread of the disease (see section 8).

6.11 **What follow-up action should the veterinary surgeon consider (to inform and protect the community)?**

Veterinary surgeons should contribute to the devising and distribution of publicity material (leaflets, posters, contributions to the media) to advise owners of infected dogs and their families of the epidemiology of VL, the prognosis of canine VL and potential risks to human health. Other dog owners in the infected area should also be made aware of the potential risk and consequences of canine VL.

Veterinary surgeons should also contribute to health education and community participation activities.

6.12 **What minimum or special equipment and services are required?**

For microscopical examination of stained smears: microscope with (50 X) or 100X oil immersion objective, microscope slides, staining trays, syringes (10 or 20 ml), needles, sodium chloride, disodium hydrogen phosphate, Giemsa stain.

Access is also required to the reagents and equipment for serological diagnosis of canine VL (see appendices 6 - 9).