FACTORIAL ANALYSIS OF MALIGNANT HISTIOCYTIC TUMOURS IN BERNESE MOUNTAIN DOGS:

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Introduction:

Studies of the genetic background of histiocytic sarcoma / malignant histiocytosis (HS/MH) complex are conducted in the framework of the collaboration between NIH, University of Rennes, North Carolina State University and Utrecht Faculty of Veterinary Medicine (UFVM). Progress is good, and at least one chromosomal location appears to differ between healthy veterans and dogs suffering from HS/MH.

Some areas of research are not (completely) addressed in the above study and need special attention:
A. Optimalization of pathological and immunohistochemical analysis of malignant round cell and mesenchymal tumours, including HS/MH.
B. Clarification of possible causes of the variation in clinical manifestation of HS/MH.
C. Are dogs carrying gene(s) predisposing for HS/MH predisposed to develop other types of tumour?

Detailed description of fields of investigation:

A. An earlier analysis of archives at UVFM learned that a significant proportion of BMD considered (by veterinarians in practice) to be affected by HS/MH suffered from other conditions (Rutteman et al, Burgdorf symposium, 2007). For some cases it is likely that even the immunohistochemical (IHC) assay for CD18 is not always able to discriminate between HS/MH and other round cell or mesenchymal tumours. Differential diagnoses may include plasmacytoma/myeloma, amelanotic melanoma, malignant lymphoma, undifferentiated sarcoma, atypical synovial cell sarcoma and malignant fibrous histiocytoma (MFH). Possibly, MFH and HS/MH resemble different phenotypes of the same disease.

B. HS / MH is manifested as (I) localized disease (HS) – sometimes already with signs of distant metastases, (II) generalized disease ("MH") and (III) forms that cannot be classified as either (I) or II). There is the possibility that the type of manifestation (I versus II) is determined by a genetic variation between animals. Furthermore, it might be possible that joint disease (LCD, immune-mediated arthritis) predisposes for HS.
In addition, there appears to be two forms of manifestation in relation to age: (I) early onset (EO: 2 – 7 years), late onset (LO: 7-11 years). It is possible that a genetic variation between individuals determines the age of manifestation.

C. In families that contain dogs developing HS/MH, some members develop other tumours, that stem from the same primitive hematological precursor stem cell. It is possible that the gene predisposing for HS/MH, in some animals predisposes for a different type of tumour. Examples may be myeloma and hemangiosarcoma. If such cases are kept outside the study for

genes predisposing for HS/MH, this might lead to an underestimation of the true incidence of such predisposing gene(s) in the BMD population.

Materials and Methods:
The current collection of BMD with HS/MH at our institute includes approximately 250 cases. With the search through earlier years in the archive plus a search through other laboratories in NL, we will attempt to compose complete clinical – pathological records of at least 350 cases. In addition, cases classified earlier as 'malignant round cell tumour', sarcoma, myeloma and T-cell lymphoma, will be retrieved from the archives.

In order to improve tumour classification **<A>**, all cases mentioned above will be re-examined with a panel of markers by IHC, compared to the classical clinic-pathological classification, in order to improve the classification. It is expected that with the re-analysis, several of the latter tumours will be reclassified as HS/MH.

<B> Upon this complete classification, all cases will divided amongst (I) localized disease, (II) generalized disease and (III) cases that cannot be separated. Also, all HS/MH cases will be grouped in two age classes (EO: below or LO: above median age).
Based on pedigree data, the groups I versus II will be compared for a possible variation of the degree of familial relationship. The same will be done for EO and LO. Grouping will be done based upon results of a stepwise statistical analysis.
Data from the history and clinical examination will be examined for a possible predisposing effect of joint disease in the development of HS. It should – however – be recognized that a detailed diagnosis of the type of joint disease often may not be able, by the lack of proper testing (radiographs, arthroscopy, cytology of synovial fluid) in the veterinary practices.

<C> Cases that are classified in the ongoing study plus the archival study (after re-examination) as malignant lymphoma, myeloma, (hemangio)sarcoma, will be prepared for genomic testing at the moment the gene(s) predisposing for HS/MH is/are recognized. At that point in time, it will be important to examine whether testing for HS/MH could also reduce the number of individual animals that might develop other types of tumours. Similarly, whether testing for HS/MH predisposing gene(s) should be extended to family members of cases with the above mentioned other types of tumours.

Aims of the study
<A> Improvement of classification of hematological / mesenchymal / malignant round cell tumours; validation of criteria to diagnose tumours as HS/MH
<B> To see whether genetic or other causes are influential upon the type (localization; age of onset) of HS/MH
<C> To prepare a series of BMD with tumours, currently kept outside the ongoing genomic analysis of HS/MH, for a second round of testing: once gene(s) predisposing for HS/MH are known, to examine these cases for the same gene defect(s).

Length of the study:
The study to retrieve additional cases from pathology archives will start September 1, 2008. The cases will be added to those already present or collected in 2008-2009.
From veterinary practices full clinical (radiological, ultrasound examination, hematology) data will be collected, together with data from the history provided by the owners. From laboratories outside UFVM tissue blocks will be retrieved or re-examination, including

immunohistochemistry. (the ongoing collaborative study includes about 125 cases fully classified, for another 125 cases IHC is pending).
Together, at least 225 cases at the end classified as HS/MH will be examined by IHC, plus approximately 75 cases of other mesenchymal / malignant round cell tumour.
It total, this accounts to 300 tumours for which IHC needs to be done (average 2-3 IHC assays).
With the results of the complete classification and typing of manifestation of disease, pedigree analyses will be performed, to assess the variance of degree of familial relationship between groups. The outcome of this test may have an effect upon the current (US/F/NL) genomic study. At the end of the study period (December 31, 2009), a second group of cases, at the present time excluded from the genome analysis for the gene(s) predisposing for HS/MH, will be ready to study any shared predisposing gene.

Funding:
From the Committee for Prevention of diseases in Companion Animals, funding to pay a veterinarian for the period September 2008 – December 2009 has been obtained.
There is need to pay for the logistics of data retrieval and tissue retrieval from practices and outside laboratories, plus the immunohistochemical assays.
With 350 tumours to be analyzed with IHC (averaging 50 Euro per case) there is need for 17,500 Euro. We expect to obtain approximately 5000 Euro from the Dutch BMD societies, and are thus in need for 12,500 Euro.

It would highly be appreciated if the 2008 Alberto Vittone Award could help us with 12,000 Euro

Addendum:
Description of immune markers

vimentin (mesenchym)	Glial protein
desmin (myo)	smooth muscle actin
cytokeratins	
S-100 (/enolase?)	VWF
Lysozym	MHCII (all antigen presenting cells)
CD3, CD79(a)	CD18
kappa, lambda (or Fc portion IgG/IgA/IgM)	

Literature pertinent to the study:

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Experience of the Department related to the subject of the study.

The Departments at the UFVM have major experience in advanced clinico-histopathological characterization of neoplastic diseases, and in epidemiological / hereditary analyses as evidenced in the literature

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