

**BIOMARKERS OF NEOPLASTIC TRANSFORMATION
IN CANINE SPIROCERCOSIS**

by

Eran Dvir

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Faculty of Veterinary Science
University of Pretoria**

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Biomarkers of neoplastic transformation in canine spirocercosis

By : Prof. E Dvir
Department of Companion Animal Clinical Studies
Faculty of Veterinary Science
University of Pretoria
South Africa

Supervisor : Prof. J P Schoeman
Department of Companion Animal Clinical Studies
Faculty of Veterinary Science
University of Pretoria
South Africa

Co-supervisor : Dr R J Mellanby
Division of Veterinary Clinical Studies
Royal (Dick) School of Veterinary Studies
University of Edinburgh
United Kingdom

I, Eran Dvir, hereby declare that the work on which this thesis is based, is original and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree at this or any other University, Tertiary Education Institution, or Examining Body.

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SUMMARY

Spirocerca lupi is a nematode that infects the dog's oesophagus and promotes the formation of an inflammatory fibroblastic nodule that progresses to sarcoma in approximately 25% of cases. Differentiating neoplastic from non-neoplastic cases ante-mortally is challenging and has major therapeutic and prognostic implications. More importantly, spirocercosis-associated oesophageal sarcoma is an excellent and under-utilized spontaneous model of parasite-associated malignancy and the pathogenesis of the neoplastic transformation is poorly understood.

The current study objective was to investigate potential clinical, clinicopathological, radiological and tissue biomarkers for the malignant transformation and an attempt to use these biomarkers to gain a deeper understanding of the pathogenesis of the neoplastic transformation. Our central hypothesis was that the parasite produces excretory product(s) which diverts the immune response from a T helper 1 (Th1) to Th2 cell response, typical of many nematode infections, and further to an immunoregulatory (immunosuppressive), FoxP3+ regulatory T cell-predominated response which then facilitates neoplastic transformation.

The following parameters were studied and compared between cases with non-neoplastic and neoplastic spirocercosis: clinical presentation, haematology, serum albumin and globulin, thoracic radiology, haematoxylin-eosin (H&E) histology, Immunohistochemistry for expression of vascular endothelial growth factor (VEGF)-A, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), MAC387 (myeloid cells), CD3 (T cells), Pax5 (B cells) and FoxP3 (T regulatory cells) and plasma cytokine concentrations including IL-2, IL-4, IL-6, IL-8, IL-10, IL-18, GM-CSF and MCP-1.

Hypertrophic osteopathy showed 100% specificity for neoplastic transformation but relatively poor sensitivity (40%). Female gender, anaemia, leukocytosis, thrombocytosis, spondylitis and bronchial displacement were significantly more common in neoplastic cases, but appeared in non-neoplastic cases as well. The H&E study revealed 2 stages in the non-neoplastic nodules: early inflammation, characterized by fibrocytes and abundant collagen, and a pre-neoplastic stage, characterized by activated fibroblasts and reduced collagen. The neoplastic cases were all sarcomas, primarily osteosarcoma with very aggressive features comparable to other appendicular osteosarcoma in the dog. The inflammation in spirocercosis is characterized by pockets of pus (MAC387+ cells) surrounded by organized lymphoid foci (CD3+ and to a lesser degree Pax5+ cells). There was no evidence of a local accumulation of FoxP3+ cells, unlike many previous studies which have reported an increase in Foxp3+ T cells in both malignancies and parasite infections. Interleukin-8 plasma concentration was higher in the neoplastic group compared to the non-neoplastic and the control groups. Interleukin-18 concentration was higher in the non-neoplastic group followed by the control group and finally the neoplastic group.

As with most similar studies, no ideal biomarker with high sensitivity and specificity was identified. However, if examined together, a panel of the biomarkers that were identified more commonly in the neoplastic cases should substantially increase the index of suspicion for neoplastic transformation in a diagnosed spirocercosis case. The inflammatory response showed features of increased myeloid (innate) response and lymphocytic response with pro-inflammatory cytokines. This was not our initial hypothesis and the question remains whether the response is secondary to the worm infection, or to a symbiotic bacterium that is carried by the worm. The role of such a reaction in neoplastic transformation remains to be elucidated.

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ABBREVIATIONS

S. lupi – *Spirocerca lupi*

µg – microgram

µl – microlitre

BSA - bovine serum albumin

BVSc – Bachelor of Veterinary Science

C – Celsius

C – Collagen

CD - Cluster of differentiation

CRP- C-reactive protein

dl – decilitre

DL – detection limit

DN - degenerate neutrophils

DNA - deoxyribonucleic acid

DVM – Doctor of Veterinary Medicine

Dipl. ECVIM-CA - Diplomate of the European College of Veterinary Internal Medicine
– Companion Animals

ED – Eran Dvir

EDTA - Ethylenediaminetetraacetic acid

F – fibroblast

FGF - fibroblast growth factor

Fig. – figure

FoxP3 - Forkhead box P3

GM-CSF - granulocyte-macrophages colony-stimulated factor

H& E / HE - Haematoxylin-eosin

HO - Hypertrophic osteopathy

Hons - Honours

Ht - Haematocrit

IHC – immunohistochemistry

IL - Interleukin

kg – kilogram

L1-5 – Larvae life cycle stage number

LP – lymphocytic-plasmacytic

LSAB - labelled streptavidin-biotin

MAC 387 - Macrophage marker

MCP - monocyte chemotactic protein

Martius, Scarlet and Blue – MSB

MCV - mean corpuscular volume

MCW – Mark C Williams

NF-κB - nuclear factor kappa-light-chain-enhancer of activated B cells

NGS – normal goat serum

Mi – mitoses

ml – millilitre
mm – milimeter
MMedVet – Master in Veterinary Medicine
MN – multinucleated
MVD- Microvessel density
O – osteoblasts
Osm – osteoid matrix
OVAH – Onderstepoort Veterinary Academic Hospital
p – probability
Pax5 - Paired box protein 5
PBS - phosphate buffer
PBST80 - phosphate-buffered saline (PBS) containing 0.5% Tween 80
PDGF - platelet-derived growth factor
pH - power of hydrogen
pg – picogram
RK – Robert Kirberger
RT – room temperature
S. lupi – *Spirocerca lupi*
SC – subcutaneous
SJC – Sarah J Clift
T – temperature
T1-12 – Thoracic vertebrae number
TGF - Transforming growth factor
Th1 - T helper type
Tregs - T regulatory cells
VEGF - vascular endothelial growth factor
UK – United Kingdom
vs – versus
WBC - White blood cell count
 μg – *microgram*
 μl – *microlitre*
 χ^2 - Chi-square

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