Hypertrophic Osteopathy: a Retrospective Case Control Study of 30 Dogs

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ABSTRACT

Hypertrophic osteopathy (HO), mostly described in dogs with thoracic neoplasia, is characterized by pathologic periosteal new bone formation (PNBF). This study compared 30 dogs with HO with 101 age- and primary lesion-matched negative controls. Boxers were overrepresented in the HO group compared to mixed breeds. HO-related clinical signs preceded or appeared concurrently with the primary disease signs in 85% of the dogs. Pyrexia, regional lymphadenopathy, warm and swollen limbs and joints and nasal hyperkeratosis were significantly ($P \leq 0.015$) more frequent in the HO group compared to the controls. Mean platelets count and frequency of thrombocytosis and schistocytosis were also significantly ($P \leq 0.012$) higher in the former. Anemia and leukocytosis were common in both groups. All HO-dogs showed increased alkaline phosphatase (ALP) activity, which was higher ($P=0.042$) compared to the controls. The HO-related PNBF involved the metacarpi or metatarsi (76%), distal and proximal long bones (66% and 38%, respectively), carpi or tarsi (31%) and phalanges (21%).The HO-associated primary lesions included esophageal mass (33%), likely due to the high prevalence of spirocercosis in Israel, metastatic lung disease (27%), primary pulmonary mass (20%) and thoracic wall masses (13%). The median survival time of the HO dogs was 24 days (range 1-117). Presence of macroplatelets, thrombocytosis and schistocytosis in HO dogs support the hypothesized pathophysiology of human HO. This is the first case-control study of canine HO, and the second to describe its clinical and laboratory findings in a relatively large number of dogs.

Keywords: Canine, neoplasia, *Spirocerca lupi*, esophageal sarcoma, hematology.

INTRODUCTION

Hypertrophic osteopathy (HO) is characterized by pathologic periosteal new bone formation (PNBF) (1). It occurs most commonly in humans and dogs, and has been described in other species (2, 3). It develops mostly secondary to various underlying conditions, classified as pulmonary, non-pulmonary intra-thoracic and extra-thoracic. Pulmonary pathology was recorded in 57/60 dogs with HO, commonly with metastatic lung disease (MLD) (61%), and primary pulmonary neoplasia (31%), and is more frequent in dogs with primary pulmonary neoplasia or osteosarcoma compared to those with other secondary MLD (2, 3, 4). HO was also found to be associated with other, non-neoplastic pulmonary lesions, including tuberculosis, abscesses, fibrosis, granuloma and chronic bronchopneumonia (2). It was also was described in 38% of dogs with spirocercosis-associated esophageal sarcoma, but not in benign spirocercosis (2, 3, 5). Other non-pulmonary, HO-associated intra-thoracic diseases include esophageal (i.e., chronic megaeosophgus and foreign bodies) and cardiac diseases (i.e., bacterial endocarditis and right to left shunt patent ductus arteriosus), dirofilariasis and rib tumors. Extra-thoracic HO-associated diseases include American hepatozoonosis, hepatic failure, hepatic adenocarcinoma and primary urinary bladder neoplasia (3, 4, 6-10).

Since HO is secondary to a variety of diseases and mostly neoplastic conditions, data of breed, sex, and age distribution may have limited value, however, it was recorded more frequently in large breeds, likely due to their propensity to develop primary bone tumors, often presenting with MLD, and in Boxers, due to their propensity for primary bone and lung tumors (2, 3). Most dogs with HO are middle-aged to old (mean age 8.7 years), while dogs with non-neoplastic thoracic disease or urinary bladder neoplasia are younger than dogs with thoracic neoplasia (2, 3). Although HO has no sex predisposition, the occurrence of females was higher in a previous survey, probably due to the fact that 12 of the 60 dogs were bitches with metastatic pulmonary mammary neoplasia (2).

The pathogenesis of HO is obscure; however, increased limb blood flow is a consistent early change (4). The "neurologic" theory suggests that vagal nerve stimulation induces limb vasculature vasodilatation, with subsequent periosteal changes. This is supported by the observation that bone lesions regress following vagotomy (11). Another hypothesis suggests that peripheral hypoxemia, resulting from congenital or acquired arteriovenous shunts, stimulate periosteal osteophyte production (4, 12), which possibly occurs due to vascular endothelial growth factor (VEGF) induction, promoting angiogenesis and osteoblast differentiation (13, 14). Angiogenesis may also occur due to tissue hypoxia or decreased inactivation by the lungs leading to increased peripheral concentration of circulating vasodilators and growth factors. Constitutive production and release of VEGF by megakaryocytes and platelets has been documented (15). Lastly, macroplatelets, normally fragmented within the pulmonary microvasculature, are hypothesized to shunt through arteriovenous anastomoses within the primary lesion and circulate to the distal limbs, where they fragment, resulting in release of several growth factors, including VEGF (16-19).

HO is confirmed using radiography, and classically manifests as bilateral symmetrical digital and limb long bone PNBF. Initially, only soft tissue swelling occurs. The earliest changes are observed in the metacarpi and metatarsi, often abaxially, in the medial, second, and lateral, fifth digits. With progression, PNBF occurs on the radial, ulnar and tibial diaphyses, infrequently involving the femur, humerus, scapula, ribs and pelvis (2, 3, 20, 21). It tends to occur initially, most severely, where periosteum is free of tendinous insertions or adjacent bones (4). Articular surfaces are spared, and joint swelling in dogs results from periarticular vascularized connective tissue proliferation (22).

The main treatment of HO aims to eliminate the primary lesion. Once successfully treated, bony lesions rapidly regress (4, 21). Lobectomy, in cases of primary focal pulmonary lesions, may lead to remission of pain, swelling and lameness within weeks, while bony lesions regress progressively, within months (3, 20). Metastatectomy is controversial, since extensive lung tissue resection is required, while its long-term benefit has never been proven. However, in four dogs with HO and MLD, lameness and reluctance to move resolved within 24 hours post metastatectomy, recurring in only one dog, suggesting that palliative large lesion resection can be considered even in cases with multiple lesions (10, 13). Improvement in HO signs occurred also after vagotomy (18, 23). Complete resection of *Spirocerca lupi*-induced esophageal sarcomas in cases with HO is difficult due to their local infiltration (3), although successful partial esophagectomy of such masses has been described (24).

Early diagnosis of HO is important, especially when its signs precede those of the underlying disease, in order to promote early detection and treatment of primary lesions. Therefore, better understanding of the risk factors, underlying diseases, and clinical and laboratory abnormalities of HO is warranted. Studies of canine HO are limited, not case-controlled, and mostly consist of case reports (2, 6-9, 23, 25-29). Very recently, a study of 30 dogs with HO has been published, but was uncontrolled (30).

This retrospective case-control study was aimed to characterize the findings in dogs with HO, compared to negative controls presenting similar underlying diseases.

MATERIALS AND METHODS

The medical records of all dogs presented to the Hebrew University Veterinary Teaching Hospital (HUVTH) between 1989 and 2009 were reviewed retrospectively. Dogs diagnosed with HO, based on limb radiography, were included in the study group. Thoracic radiography was performed in all the study group dogs. The cases were divided into four subgroups, based on their thoracic radiography findings and the underlying primary condition that presumably induced HO: a) caudal esophageal or mediastinal mass, b) solitary pulmonary mass, c) pulmonary metastases and d) thoracic wall mass. Dogs presenting more than one of the above le-

sions were included only in one of these subgroups, based on the condition that was present when HO was diagnosed. In all dogs with HO, a wide diagnostic workup (i.e., laboratory tests and imaging) was made to identify the primary condition leading to formation of HO. The controls presented no clinical signs of HO, and were selected based on similar underlying pathologies to those of the HO group. For each HO dog with a certain underlying lesion, three to four time-matched controls were selected, using the Department of Radiology, HUVTH database. In most of the control dogs, radiography of the limbs was not performed.

The signalment, history, duration of clinical signs prior to presentation, clinical, clinicopathologic and radiographic findings and the outcome were retrieved from the medical records. Non-survivors included dogs that died naturally or were euthanized due to the primary disease within 30 days from the diagnosis of HO (study group) or from their first presentation (controls).

Blood samples for complete blood count, serum chemistry and electrolyte analysis were collected at presentation in potassium-EDTA tubes, and red-top tubes with gel separators, respectively. Modified-Wright's stained blood smears were used for manual differential leukocyte counts and blood cell morphological evaluation.

The distribution pattern of quantitative variables was assessed using the Kolmogorov-Smirnov test. Quantitative variables were compared between groups using Student's *t*-or Mann-Whitney *U*-tests, depending on data distribution. The latter was also used to compare small groups. Categorical variables were compared between groups using chi-square and Fisher's exact tests. All tests were two-tailed and a $P < 0.05$ was considered significant. Odds ratios (OR) with their 95% confidence interval ($CI_{95\%}$) were calculated to compare breed proportions between the HO group and the general HUVTH dog population. Statistical analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL).

RESULTS

The HO group included 30 dogs, with 20 females (67%; 11 spayed, 55%) and 10 males (33%; one neutered, 10%). The controls included 101 dogs, with 56 females (55%, 37 spayed, 66%) and 45 males (45%, 10 neutered, 22%). There were no sex proportional group differences, and no differences compared to the general population, expected as 50% sex proportion. There was no mean age difference between the HO and control groups (8.5 ± 2.8 *vs.* 8.8 ± 3.5 years, respectively; P=0.712). Most dogs in both groups were purebred (23 study dogs, 77% *vs.* 63 controls, 62%). There were significant breed distribution differences between the HO and control groups (P=0.005), and between the HO group and the general HUVTH population (P=0.00003). Boxers were overrepresented in the HO group (OR 10.8, $CI_{95\%}$ 1.65-71.4) compared to mixed-breed dogs (designated OR of 1), and tended to be overrepresented in the HO group compared to the general HUVTH dog population (13.3% and 5.5%, respectively; P=0.06).

The median duration of HO-related clinical signs (i.e., lameness and limb weakness and swelling), prior to presentation was 30 days (range 1 to 90 days). Data regarding the appearance of clinical signs of HO in relation to signs of the primary disease were available in 27/30 dogs. Clinical signs of HO and of the primary disease (i.e., respiratory system-or esophageal mass-related signs) were noted concurrently in 17 dogs (63%). In six dogs (22%), HO-related signs preceded those of the primary disease, and in four dogs (15%),thoracic disease-related signs preceded those of HO. Overall, in 23/27 dogs (85%) HO-related clinical signs preceded or occurred concurrently with primary disease signs.

Dogs with HO had significantly higher rectal temperature than controls (39.3 ± 0.7*vs.* 38.7 ± 0.9˚C, respectively; P=0.012). Pyrexia (rectal temperature > 39.4 ˚C) was significantly (P=0.015) more frequent in the HO group (11 dogs, 50%) compared to the controls (16, 19%). Musculoskeletal signs were significantly (P=0.0001) more frequent in the HO dogs compared to the controls (Table 1). In the HO group, 23/30 dogs (77%) had swollen limbs or joints at presentation, compared to 3/101 controls (3%) (P=0.0001). In 27% HO dogs, but not in the controls, warm limbs or pain upon palpation accompanied this swelling. Additionally, HO dogs presented significantly more frequently popliteal lymphadenopathy (P =0.01), prescapular lymphadenopathy (P =0.049) and nasal hyperkeratosis ($P < 0.0001$).

Most HO dogs had mean corpuscular volume (MCV) within reference interval (RI), however, their MCV was significantly (P=0.007) lower compared to the controls (Table 2). The mean platelets count was significantly (P=0.002) higher, and thrombocytosis was significantly (P=0.012) more frequent in the HO dogs compared to the controls. Both the HO and control dogs had mildly low mean hematocrit,

Table 1: Musculoskeletal and selected* clinical signs in 30 dogs with hypertrophic osteopathy and 101 negative controls.

Clinical sign	HO[a] n (%)	Controls n (%)	All dogs n (%)	*P* value
All musculoskeletal signs[b]	27 (90%)	27 (27)	54 (41%)	0.0001
Panting	2 (9%)	28 (33%)	30 (28%)	0.033
Limb weakness	15 (50%)	18 (18%)	33 (25%)	0.0001
Lameness	15 (50%)	13 (12.9%)	28 (21%)	0.0001
Pyrexia	11(50%)	16(19%)	27 (25%)	0.015
Swollen limbs or joints	23 (77%)	3 (3%)	26 (20%)	0.0001
Conjunctivitis and ocular discharge	10 (33%)	16 (16%)	26 (20%)	0.065
Lymphadenopathy[c]	10 (33%)	16 (16%)	26 (208%)	0.065
Submandibular lymphadenopathy	6 (20%)	9 (9%)	15 (12%)	0.108
Popliteal lymphadenopathy	6 (20%)	4 (4%)	10 (8%)	0.01
Prescapular lymphadenopathy	5 (17%)	5 (5%)	10 (8%)	0.049
Swollen, warm limbs or joints	8 (27%)	0 (0%)	8 (6%)	0.0001
Restricted joint movement	6 (20%)	0 (0%)	6 (5%)	0.0001
Head muscle atrophy	4 (13%)	1 (1%)	5 (4%)	0.01
Nasal hyperkeratosis	5 (17%)	0 (0%)	5 (4%)	0.0001

* including clinical signs in which *P* was below 0.11.
[a] hypertrophic osteopathy.
[b] including all cases that presented one or more of the following: limb weakness, lameness, swollen limbs or joints, swollen warm limbs or joints and restricted joint movement, combined.
[c] all cases of peripheral lymphadenopathy combined.

and over 50% of both groups showed anemia. The median white blood cell count (WCC) was above RI, and leukocytosis was frequent in both groups. Morphological blood smear assessment was available in 11 HO dogs and 17 controls. Compared to controls, dogs with HO had significantly higher proportions of schistocytosis (1/17 dogs, 6% *vs*. 6/11 dogs, 55%, respectively; *P*=0.007) and anisocytosis (11/17 dogs, 65% *vs*. 10/11 dogs, 91%, respectively; *P*=0.039). The serum biochemistry results in which significant group differences were noted are presented in Table 3. In both groups median alkaline phosphatase (ALP) activity was above RI, but it was significantly (*P*=0.042) higher in the HO group, as was the proportion of dogs with increased ALP activity (*P*=0.003) (Table 3).

There were no group differences in proportions of the primary thoracic lesion location, based on thoracic radiographs (Table 4). The caudal mediastinum was most common thoracic lesion location in the HO group (11/30 dogs, 37%). Eight HO dogs (27%) had multiple lesions or metastases. When this study was conducted, 29/30 limb radiographs of the HO dogs were available and re-examined. The anatomic locations of the periosteal reaction typical of HO included metacarpi or metatarsi (22/29 dogs, 76%), distal long bones (i.e., radius and ulna or tibia, 19, 66%), proximal long bones (i.e., humerus or femur, 11, 38%), carpi or tarsi (9, 31%), phalanges (6, 21%) and pelvis, ribs and scapula (1 each, 3%). There were no group differences in the proportions of dogs among subgroups (Table 4). The HO-associated pathologies included esophageal mass (10/30 dogs, 33%), MLD (8/30, 27%), primary pulmonary mass (6/30, 20%) and intrathoracic-extrapulmonary (thoracic wall) mass (4/30, 13%). Two dogs had both caudal esophageal masses and MLD, but were included in the latter subgroup, because HO-related clinical signs were observed when metastases were already present. Two dogs (7%) were unclassified, because no definitive diagnoses were made. One had spleno-hepatomegaly and unremarkable thoracic radiography. The other had left caudal lung lobe atelectasis and mediastinal lymphadenopathy with tracheal elevation.

Outcome data of the dogs in this study were limited. The median survival time from diagnosis to death or euthanasia of the HO dogs (recorded in 21/30 dogs, 70%) was 24 days (range 1 to 117), and was significantly (*P*=0.003) higher compared to the controls (median survival time 1 day, range 1-233; recorded in 41/101 dogs). The 30-day survival rates of the HO and control groups were 52% and 20%, respectively (*P*=0.01).

DISCUSSION

This is the first case-control study of HO in dogs, and the second to describe their clinicopathologic findings and the frequency of clinical signs compared with negative controls with similar primary conditions. A very recent retrospective study of HO in dogs was uncontrolled (30).

The mean age of the HO group (8.5 years) is in agreement with previous findings, probably reflecting the association of HO with neoplasia, which occurs more commonly in older dogs (2, 30), and was similar to that of the controls. Unlike some previous results (2), but in agreement with a recent study (30), there was no sex proportion difference between groups, probably because mammary gland neoplasia was uncommon, in contrast with the previous study (2).

Boxers tended to be overrepresented in the HO group compared to general HUVTH population, and had significantly

Table 2: Complete blood count results of 30 dogs with hypertrophic osteopathy and 101 negative controls.

Analyte (Units)	Hypertrophic osteopathy				Controls				Reference interval	*P* value^	*P* value†
	n [a]	Median (range)	%≤RI [b]	%>RI [b]	n [a]	Median (range)	%≤RI [b]	%>RI [b]			
Leukocytes ($\times 10^9$/L) [$]	21	20.3 (7.7-59.5)	0.0	61.9	88	18.4 (5.9-109.0)	0.0	56.0	5.0-17.0	0.82	0.63
Red blood cells ($\times 10^{12}$/L) [#]	21	6.05 (3.14-7.52)	28.6	0.0	88	5.62 (1.59-9.79)	51.1	4.5	5.50-8.0	0.21	0.08
Hemoglobin (g/dL) [#]	20	130 (880-171)	35.0	0.0	88	125 (34-224)	43.2	10.2	120-175	0.50	0.23
Hematocrit (L/L) [#]	21	0.38 (0.20-0.47)	33.3	0.0	88	0.37 (0.12-0.62)	42.0	12.5	0.35-0.50	0.73	0.13
MCV [c] (fL) [#*]	21	63 (56-74)	14.3	0.0	88	67 (51-79)	4.5	3.4	60.0-77.0	0.007	0.21
MCHC [d] (g/L) [#]	20	342 (264-450)	10.0	15.0	87	337 (181-408)	14.9	11.5	320-.365	0.30	0.85
RDW [e] (%) [#]	13	18.6 (16.1-22.2)	0.0	84.6	62	17.7 (13.5-21.7)	0.0	64.5	13.0-17.0	0.16	0.20
Platelets ($\times 10^9$/L) [#⊥†]	20	445 (94-688)	5.0	30.0	85	290 (2-750)	35.3	16.5	200-500	0.002	0.01
MPV [f] (fL) [#]	19	8.2 (6.8-11.5)	NA	NA	77	9.4 (5.8-14.0)	NA	NA	NA	0.08	NA
PDW [g] (%) [$]	19	37.2 (15.2-41.9)	NA	NA	63	37.4 (15.2-46.4)	NA	NA	NA	0.15	NA

[a] number of dogs in which the test was available.
[b] reference interval.
[c] mean corpuscular volume.
[d] mean corpuscular hemoglobin concentration.
[e] red blood cell distribution width.
[f] mean platelet volume.
[g] platelet distribution width.
NA not assessed.
[#] normal data distribution.
[$] non-normal data distribution.
[†] significant ($P < 0.05$) group difference in proportion of abnormality.
[^] significant ($P < 0.05$) group difference in group means or medians (for normally or non-normally distributed, respectively).

high OR to present HO compared to mixed-breed dogs. This has been previously hypothesized to occur due Boxers' predisposition to primary bone and lung tumors, and to neoplasia in general (3). However, this hypothesis cannot be the sole explanation of this overrepresentation, since the HO and control groups both included similar, mostly neoplastic, underlying diseases, and among the controls, Boxers were neither overrepresented compared to the general HUVTH population, nor was their OR increased compared to mixed-breed dogs. Thus, the present findings suggest that Boxers might be predisposed to HO. However, this interpretation should be made cautiously, since this study group included only four Boxers, and in a recent study of HO in dogs, Boxers were not overrepresented (30).

Dogs with HO had a significantly higher rectal temperatures and proportion of pyrexia compared to the controls. The prevalence of fever in canine neoplasia is unknown; however, neoplasia accounts for over a third of human fever cases of unknown origin (31). Paraneoplastic fever is induced by tumor-associated or immune-mediated pyrogenic cytokines, particularly interleukins 1 and 6 and tumor necrosis factor, affecting the hypothalamic thermoregulatory center (32). Since both study groups had similar underlying diseases, including neoplasia, the higher body temperature and proportion of pyrexia in the HO dogs likely were not direct tumor-associated effects, but rather resulted from the ongoing HO-related bony lesion inflammation, its presence supported by the observed limb warmness, swelling and pain in a third of the HO dogs. Pyrexia was previously recorded in 11/30 dogs with HO (30). Swollen limbs, lameness and limb weakness were presently the most common clinical signs in the HO dogs, while pain upon limb palpation was infrequent, observed in a third of these, in agreement with previous findings (2, 3, 10, 20, 30). In contrast with previous findings (2, 3, 10), limb warmness occurred in only a third of our HO dogs, in agreement with a previous study of HO, in which this was observed only in 6/30 dogs (30).

The median time-lag from onset of HO-related clinical signs to diagnosis was approximately 1 month, in agreement with previous findings (up to 4 weeks in 40/60 dogs, and

Table 3: Selected* serum chemistry results of dogs with hypertrophic osteopathy and negative controls.

Analyte	Hypertrophic osteopathy				Controls				Reference interval	*P* value^	*P* value†
	n[a]	Median (range)	%≤RI[b]	%>RI[b]	n[a]	Median (range)	%≤RI[b]	%>RI[b]			
ALP [c] (U/L) $⊥†	17	324 (156-1111)	NR	100	54	204 (31-1650)	NR	64.8	4.0-140	0.04	0.003
AST [d] (U/L) $⊥	18	27 (16-62)	NR	16.7	53	39 (15-228)	NR	34	9-47	0.004	0.24
Cholesterol (mmol/L) #⊥	16	4.56 (3.19-6.58)	0.0	0.0	53	5.72 (2.41-12.69)	3.8	18.9	3.05-8.00	<0.0001	0.15
Creatinine (μmmol/L) $⊥	19	44.2 (30.5-114.4)	0.0	5.3	75	62.5 (16.8-808.4)	2.7	12	27.5 -103.7	0.019	0.80
TG [e] (mmol/L) #⊥†	12	0.70 (0.45-0.94)	0.0	0.0	40	1.05 (0.23-4.48)	0.0	40	0.17-1.13	0.001	0.01
Urea (mmol/L) $⊥	17	6.68 (2.46-18.71)	11.8	0.0	58	11.64 (0.43-130.67)	5.2	8.6	3.93-24.99	0.0002	0.40

* including only analytes in which group differences were noted.
[a] number of dogs in which the analyte was available.
[b] reference interval.
[c] alanine aminotransferase.
[d] aspartate aminotransferase.
[e] triglycerides.
NR clinically irrelevant and not assessed.
# normal data distribution.
$ non-normal data distribution.
† significant ($P < 0.05$) group difference in the proportion of abnormality.
^ significant ($P < 0.05$) difference between group means or medians (for data distributed normally or non-normally, respectively).

within 3 weeks prior to presentation in 21/30 dogs) (2, 30). The majority (63%) of our HO dogs showed signs of both HO and the underlying disease; however, HO-related signs preceded those of the primary condition in 22% of the cases, in agreement with previous findings (2). Thus, because HO is most commonly secondary to pulmonary neoplasia (2), and is common in spirocercosis-induced esophageal sarcoma (5), the presence of HO-related clinical signs potentially serve as a clinical diagnostic marker of such occult diseases, warranting appropriate investigation.

Prescapular and popliteal lymphadenopathy were significantly more frequent in the HO group. The popliteal lymph nodes drain the distal hind limbs, while the forelimb is drained by the prescapular or axillary lymph nodes (33). Such lymphadenopathy was never associated with canine HO and is probably secondary to the HO-related inflammation and PNB formation; however, lymph node cytology, required to confirm this hypothesis, was mostly unavailable.

Because the underlying diseases in both groups were similar, comparison of clinical pathological findings could be made, in order to examine their association with HO. Mild normocytic normochromic anemia was present in over 30% of both groups. Anemia is a common paraneoplastic syndrome, recorded in 25% of human cancer patients, resulting from inflammation, immune-mediated and microangiopathic hemolysis, blood loss or myelophtisis (31, 32). Anemia of inflammation is normocytic normochromic and non-regenerative. It is extremely common in human and veterinary oncological patients with disseminated or metastatic tumors (31). It was described in HO dogs diagnosed with pulmonary metastatic osteosarcoma, hepatic necrosis, renal carcinoma, metastatic mammary neoplasia, chronic pulmonary abscess (7, 23, 25-27, 29, 30) and in a cat with HO and pulmonary sarcoma (34). Schistocytosis results from erythrocyte fragmentation, and occurs due to the presence of intravascular fibrin filaments, aberrant blood vessels or turbulent blood flow. It is associated with disseminated intravascular coagulation (DIC), hemangiosarcoma, glomerulonephritis, congestive heart failure and vasculitis (35). Schistocytosis was never associated with HO in dogs, but was significantly more common in our HO dogs compared to the controls. Erythrocyte shearing, resulting in schistocyte formation and mild microangiopathic hemolytic anemia potentially occurs due to increased distal limb peripheral blood flow, aberrant angiogenesis, arteriovenous shunts and platelets clumping, all of which are hypothesized to occur in HO. Erythrocyte fragmentation potentially contributed to the lower MCV of the HO group. Microcytosis might account for the higher red blood cell distribution width (RDW) of the HO group. It takes place due to chronic blood loss leading to iron deficiency, and in severe anemia of chronic disease (35). If iron deficiency and chronic disease were indeed the mechanisms leading to the lower MCV in the HO group, these were more dominant in this group. Microcytic hypochromic anemia and melena are common in dogs with esophageal sarcomas, due to chronic blood loss caused by such ulcerated tumors, resulting in iron deficiency (24). Leukocytosis was commonly recorded in both groups, and was previously described in canine HO (8, 25, 27-30). Neutrophilic leukocytosis occurs in various canine tumors, including pulmonary carcinoma (32), and was recorded in 11/20 dogs with HO (30).

The mean platelet count and proportion of thrombocytosis were both significantly higher in the HO group compared to

Table 4: Primary radiographic diagnoses in 30 dogs with hypertrophic osteopathy and 101 negative controls

Primary radiographic abnormality	HO[a] n (%)	Control n (%)	All dogs n (%)	*P* value
Esophageal mass	10 (33)	32 (32%)	42 (32)	0.236
Pulmonary mass	6 (20)	23 (23%)	29 (22)	
Pulmonary metastases	8 (27)	32 (32%)	40 (31)	
Intra-thoracic extra-pulmonary mass	4 (13)	14 (14%)	18 (14)	
Lung lobe atelectasis	1 (3)	0 (0.0)	1 (1)	
No thoracic abnormalities	1 (3)	0 (0.0)	1 (1)	

[a] hypertrophic osteopathy.

the controls. Thrombocytosis was previously reported in canine and human HO (10, 28, 30, 36, 37). It is hypothesized to occur due to pulmonary arteriovenous shunting, decreasing pulmonary megakaryocyte fragmentation, and thereby increasing peripheral platelet numbers and fragmentation. The latter induces platelet derived growth factor (PDGF) release, resulting in increased capillary permeability and connective tissue hypertrophy, the hallmark of "clubbing" (i.e., PNBF) in human HO (38). Histopathologically, numerous intravascular platelet clusters were observed in clubbed, but not in control, human samples (39). Both PDGF and VEGF are released when platelets aggregate. Their stromal expression, as well as that of hypoxia inducible factor, is increased in clubbed, but not in control, human tissue samples, supporting their role in the pathogenesis of human, and possibly canine HO (40). PDGF and VEDF expression was documented in spirocercosis-induced esophageal nodules and sarcomas and were proposed as diagnostic markers (41). The higher proportion of thrombocytosis and the higher platelet numbers in the present HO dogs support the hypothesis of decreased pulmonary platelet clump fragmentation in canine HO, suggesting that the platelet number in the affected limbs is possibly increased. Reactive, inflammatory thrombocytosis in canine HO is another putative mechanism accounting for our findings, however, it would be expected to occur in the controls as well, but was not recorded presently in this group.

Increased serum ALP activity was recorded in both groups, however, its activity was significantly higher in the HO dogs, all of which were above RI. The latter was reported previously in canine HO (23, 25, 30). This most probably resulted from increased bone ALP isoform activity. Increased serum ALP activity has been reported in 23% of dogs with spirocercosis (42). In another study, ALP activity was increased in 5/7 dogs with neoplasms secondary to spirocercosis (24). Both of these studies did not compare ALP activity between dogs with benign and malignant lesions and with presence of HO, or determined if the high frequency of osteosarcoma reported to occur secondary to spirocercosis could have been a contributing factor to the increased ALP activity. In a more recent study, serum ALP was compared between dogs with benign (88 dogs) and malignant (37 dogs) esophageal spirocercosis (43). Although ALP activity was increased in 17% of the dogs, there were no differences between groups. That study also did not investigate the association between occurrence of HO and serum ALP activity (43). Because increased ALP was presently observed also in the controls, the possibility that activity of other ALP isoforms was increased cannot be ruled out, such as corticosteroid-induced, if dogs were exposed to excess endogenous or exogenous corticosteroids (44) or the liver isoform, in cases of cholestasis due to hepatobiliary metastases, and pancreatitis (45).

As previously described, PNB in the HO dogs was most commonly noted in the metacarpi and metatarsi (3). Conflicting results of phalangeal involvement were reported in HO. While some consider this the earliest PNB site, other studies reported that these were rarely affected (2, 4, 22). Phalangeal PNB was observed in only 21% of our HO dogs, supporting its relatively lower involvement. In agreement with previous studies (3, 4), the radius, ulna or tibia were commonly effected. Humeral or femoral involvement was reported as variable (3, 4), and was observed in approximately one-third of our dogs.

The most commonly reported underlying primary pathologies in canine HO include pulmonary metastases and primary pulmonary neoplasia (2, 30) and were the second and third most common thoracic lesions in this study. In the present study, caudal mediastinal or esophageal masses were the most common underlying thoracic lesions, probably due to the high prevalence of spirocercosis in Israel (46), often resulting in esophageal sarcoma (47).

The 30-day survival rate of the HO dogs was higher compared to the controls, possibly because their underlying diseases were diagnosed relatively earlier, because preceding or concurrent HO-related signs made the owners more aware of the disease.

This study has several limitations. First, it is retrospective, and included cases recorded over a 20-year period and therefore, a standardized questionnaire was not used. Some data were missing, limiting the power of statistical analyses. Second, the primary diagnoses were based mostly on thoracic

radiography, and some were not confirmed by additional tests (e.g., computed tomography, cytology, biopsy or necropsy) .In addition, due to the clinical nature of this study, cases could not be completely and entirely matched based on the final diagnosis and its severity (i.e., identical final diagnosis and exact size and location of lesions). It is therefore difficult to definitely conclude if differences found between the study and the control groups resulted from the presence of HO or from differences in the underlying disease. This warrants caution in the conclusions made regarding group comparisons. Third, limb radiography was mostly not performed in the controls. Thus, the presence of mild HO lesions could not be completely excluded. Fourth, since HO is a relatively rare disorder, this survey included a small population, with limited data, thereby reducing the power of statistical analyses, especially when subgroups were compared between study groups. Fifth, although the location of radiographic thoracic lesions were similar between study groups, it is noteworthy to point that HO was frequently observed in association with caudal mediastinal and esophageal masses, reflecting the high prevalence of spirocercosis in Israel (35, 48), in contrast with its frequent association with pulmonary metastases in previous reports from other geographical areas. This likely influenced our results, and thus, their general application to canine HO should be made cautiously.

In conclusion, this is the first case-control study of canine HO, and the first to describe laboratory findings in a relatively large number of dogs. HO dogs showed higher mean platelet counts, cases of thrombocytosis and peripheral schistocytosis compared to negative controls. These findings potentially support the hypothesized pathophysiology of human HO. All HO dogs showed increased ALP activity. Caudal esophageal or mediastinal masses were commonly associated with HO, likely due to the high prevalence of spirocercosis in Israel. The clinical HO-related signs appeared prior to, or concurrently with, signs of the primary disorders in 85% of the cases. Therefore the presence of HO is a marker of an ongoing, underlying severe disease, and its recognition might facilitate its early diagnosis and treatment, thereby potentially prolonging survival.

ETHICAL STATEMENT

None of the dogs of this study was subjected to any experimental procedure.

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