Immune Competence Restoration after Therapy with Imidocarb Dipropionate and Doxycycline in Dogs Naturally Infected with *Anaplasma Phagocytophilum*, Agent of Canine Granulocytic Anaplasmosis, in Dubai, United Arab Emirates

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Abstract

Canine granulocytic anaplasmosis is a tick-borne zoonotic disease caused by the obligate intracellular bacterium *Anaplasma phagocytophilum* that infect myeloid cells, primary neutrophils, of the host, including humans, dogs and many wildlife species. Anaplasmosis is often described as an immuno-suppressive condition characterized by leucopenia, neutropenia, lymphopenia, thrombocytopenia and non regenerative anemia that predispose to secondary infections. However, the level of such alterations and the type of associated secondary infections are rarely mentioned in literature. In order to provide numerical data on the condition we evaluated the presence and severity of secondary conditions and the degree of leucopenia, neutropenia, lymphopenia, thrombocytopenia and anemia recognizable in 15 cases of...
canine anaplasmosis observed in Dubai. Cellular immune-suppression was seen in all cases. Specific therapy for anaplasmosis, including 4 weekly injections of imidocarb dipropionate and a daily administration of 10 mg/kg of doxycycline for 21 days, was capable to restore the immune competence within 3 weeks, confirming that immune-suppression was due to anaplasmosis only. Secondary conditions noticed more often were demodicosis, ringworm and Malassezia yeast infection. Therapy for anaplasmosis significantly helped their elimination. In this presentation, we produce numeric data on the immunological defects expressed by canine anaplasmosis by using its specific therapy as a probe.


INTRODUCTION

Canine granulocytic anaplasmosis is a worldwide distributed Ixodid tick-borne disease caused by the Gram negative intracellular obligate zoonotic bacterium Anaplasma phagocytophilum [9]. It is the most widespread tick-borne infection in animals in Europe [27], the 3 rd most common vector-borne infection in humans in the U.S. and Europe [9] and an emerging pathogenic condition of concern in China [34].

Originally recognized as the agent of tick-borne fever (TBF) in cattle, the condition was first described in 1932 in Scotland [33]. Then came the discovery of a similar disease in horses in the USA in 1969, followed by the description of two distinct granulocytic agents causing similar diseases in dogs in the USA in 1971 and 1982, Ehrlichia ewingii and the agent of canine granulocytic ehrlichiosis (CGE), today known as canine granulocytic anaplasmosis (CGA), Anaplasma phagocytophilum [5, 33]. In 1994 the disease was discovered in humans as well [33].

Many species of wild and domesticated animals are susceptible, however clinical manifestations are species-dependant, with humans [9], horses [8], cattle [33] cats [1, 4, 29] and dogs [6, 11, 13, 16, 20, 26, 28, 30] showing mild to severe clinical signs. The incubation period is 1 to 2 weeks in dogs [5, 26]. Many healthy dogs have antibodies to A. phagocytophilum, suggesting that mild or subclinical infections are common [25]. Antibodies to A. phagocytophilum were found in 563 (50.1%) of 1124 dogs in Germany suspected of having anaplasmosis and tested by an indirect
immunofluorescence test (IFT) [3]. In another study from Germany 43% of 522 dogs, 50% healthy and 50% diseased, resulted seropositive for *Anaplasma phagocytophilum* [16].

Epidemiological surveys indicate a high prevalence of *Anaplasma phagocytophilum* in Italian hunting dogs (14.8%) [10], in pet dogs from Tunisia (25.2%) [21] and stray dogs from Jordan (39.5%) [24]. *Anaplasma phagocytophilum* was detected in 7.11% of 253 dogs from Brazil by PCR [25].

Among wildlife, there is molecular and/or serological evidence of infection in deer, moose (*Alces alces*) and other cervids, chamois (*Rupicapra rupicapra*), European bison (*Bison bonasus*), mountain lions (*Puma concolor*), Eurasian lynx (*Lynx lynx*), bears [32], red foxes, wild boar (*Sus scrofa*), raccoons, opossums, striped skunks (*Mephitis mephitis*), hares (*Lepus capensis*), rodents [14] and birds [7, 19]. Livestock and wild ungulates, as well as wild rodents, might be reservoir hosts in Europe [27]. Diagnosis of CGA is based on light-microscopy detection of characteristic clusters of bacteria, named elementary bodies and morulae, within neutrophils in blood smears stained with Wright, Giemsa or Diff-Quick, or the combination of acute and convalescent serology using immune-fluorescent antibody tests (IFA) and/or detection of the DNA of *A. phagocytophilum* using specific polymerase chain reaction assays [5]. When serology and PCR are not readily available, the search for inclusions in neutrophils by microscopy is a quick method that confirms the diagnosis [28-30]. Bacteria appear as stippled dark blue or purple inclusions bodies detectable in 20-80% of clinical cases in the acute phase of the disease [2, 33]. Evocative hematologic abnormalities and a positive response to treatment further support the diagnosis [28, 30]. The main preventive measure is tick control [5]. The drug of choice is doxycycline (10 mg/kg body weight daily) for at least 3 weeks. Usually there is a dramatic clinical improvement within 24-48 hours [11]. The efficacy of treatment is faster in dogs simultaneously treated with imidocarb dipropionate [28] and the healing process is quicker when concurrent secondary conditions are simultaneously eliminated [31]. Previously eradicated infections do not confer lifelong immunity, and dogs can become infected with the same bacterium after re-exposure to infective ticks [22].

Lethargy, fever, poor appetite, poly-arthritis (lameness, joint pain), conjunctivitis, cough, vomiting, diarrhea and hemorrhagic signs are the most frequently reported symptoms in canine granulocytic anaplasmosis, with fatal outcomes apparently being rare [6, 11, 15, 18, 20, 21, 28, 30].
Laboratory abnormalities include anemia, leucopenia, neutropenia, lymphopenia, thrombocytopenia and mildly elevated levels of hepatic enzymes in the serum of some dogs [11, 13, 15, 20, 26]. It is widely accepted that anaplasmosis is an immune-pathologic disease that induce leucopenia, characterized by neutropenia or lymphocytopenia [13, 26, 33] and that some clinical signs are caused by opportunistic infections associated with *A. phagocytophilum*-mediated immune suppression [9].

However, reports on the incidence and severity of the hematological anomalies and on the associated opportunistic infections observed in dogs diagnosed with *Anaplasma phagocytophilum* are hard to find in literature.

Therefore, we thought it was worthy to retrospectively review the medical records of 15 consecutive CGA cases observed between 2010 and 2013 in Dubai, United Arab Emirates, and to report the hematological anomalies, clinical signs, concurrent opportunistic conditions and outcomes of therapy in order to expand the current knowledge of the condition.

**MATERIAL AND METHODS**

Medical records of 15 dogs microscopically diagnosed with *Anaplasma phagocytophilum* between 2010 and 2013 were reviewed retrospectively. Fresh blood smears from each patient were stained with Wright modified technique and examined with a Leica microscope at day 1 and at day 22. Hematology was carried out immediately after blood sampling in EDTA at day 1 and day 22 using an in-house Idexx VetTest Laser Cyte.

**RESULTS**

Signalment, clinical signs and associated conditions are listed in Table 1. Male (10/15) were prevalent over females (5/15). Median age was 4 years. Clinical signs commonly observed were lethargy (6/15), fever (5/15), limping (4/15), vomiting (3/15) and hemorrhagic signs (2/15). Tick infestation was seen in 3/15 cases. Dark blue or purple inclusions bodies were microscopically detected in neutrophils at day 1 but not at day 22. Percentage was not recorded. Associated diseases were adult-onset generalized demodicosis (5/15), ringworm (3/15), pyoderma (2/15) and *Malassezia* yeast dermatitis (2/2).
In Table 2 are showed the laboratory abnormalities, including anemia (10/15, 66%), leucopenia (13/15, 86.6%), neutropenia (15/15, 100%), lymphopenia (10/15, 66%), and thrombocytopenia (13/15, 86.6%), observed at day 1 before therapy (B.T.) and the immune restoration seen at day 22, after therapy (A.T.) when all values were normalized.

**DISCUSSION**

In these authors’ knowledge, reported results are noteworthy for 3 main reasons: the constant detection of neutropenia (Table 2), the complete hematological recovery seen after the end of therapy in all cases (Table 2) and the identification of adult-onset generalized demodicosis, ringworm and *Malassezia* yeast as opportunistic infections in 7/15 cases (Table 1).

It is interesting that neutropenia was present in all CGA cases (15/15, 100%), either in association with lymphopenia (9/15, 60%) or alone, confirming neutropenia as the ‘hallmark of infection with CGA that plausibly reflects direct pathogen-mediated cytolytic injury’…’since *A. phagocytophilum* infects almost exclusively neutrophils in vivo.’ [9].

Traditionally, canine granulocytic anaplasmosis has been more often associated with lymphopenia [5, 6, 18] thrombocytopenia [11, 20, 26] and anemia [9]. Recently, a timber wolf with CGA was diagnosed with thrombocytopenia, lymphopenia and anemia but not neutropenia [17]. The present study does not contradict such observations since 10/15 (66%) patients were lymphopenic and 13/15 were thrombocytopenic (86.6%).

However, the dominant feature in this study was neutropenia, a direct indicator of cellular immune-suppression which is often reported in literature in ruminants with tick-borne fever (TBF) and humans with granulocytic anaplasmosis [33]. Leukopenia with neutropenia but not lymphopenia was observed recently in an infected puppy in the USA [13].

Manifestation of hematological anomalies can vary in tick-borne infections, considering that many strains of *A. phagocytophilum* exist [9].

These authors couldn’t retrieve a series or a collection of CGA cases in literature reporting the severity of neutropenia observed in dogs. It was thus worthy to submit the data obtained from 15 cases examined in Dubai, showing neutropenia ranging
from 40 to 1990 neutrophils per microliter. Remarkably, all hematological defects were eliminated at day 22, concurrently with clinical recovery, confirming restoration of the immune competence in all cases.

Clinical signs observed in this retrospective analysis (table 1) are compatible with those recorded in previous canine studies [6, 11, 15, 18, 20, 21, 28, 30] with lethargy (6/15), fever (5/15), limping (4/15), vomiting (3/15) and hemorrhagic signs (2/15) reported more often.

Curiously, dog n. 10 had 3 epilepsy-like seizures during the month before consultation that disappeared after therapy (6 months follow-up confirmed the complete recovery). Neurological syndromes, fits and seizures are occasionally described in literature on anaplasmosis [5]. However, this is the first case in which seizures reportedly disappeared after therapy for anaplasmosis in our knowledge.

Because of the severe hematological disorders and other adverse effects on the host’s immune functions, animals and humans infected with anaplasmosis are said to be more susceptible to other infections [33].

Opportunistic infections are considered the result of impaired neutrophilic function or leucopenia [9] and are frequently mentioned but rarely tackled in literature on canine granulocytic anaplasmosis.

*Anaplasma phagocytophilum* seems to favor subcutaneous dirofilariasis caused by the nematode *Dirofilaria repens*, for instance [31]. In a review article on dermatitis associated with *Dirofilaria repens* in dogs authored by one of us (W. Tarello) 40/100 cases observed between 1990 and 2010 appeared to be co-infected with granulocytic ehrlichiosis (anaplasmosis) and therapy for anaplamsosis greatly contributed to the clinical resolution of subcutaneous dirofiariasis [31].

In a study from Germany, Pingen and colleagues observed that 12% of dogs imported from Hungary carried *D. repens* microfilariae and 11.6% were infected with *A. phagocytophilum* [23].

It is interesting to observe that, apart from pyoderma, diseases found associated with canine anaplasmosis in this case series are all conditions considered opportunistic in dogs. Adult-onset generalized demodicosis was the most common (5/15, 33%) followed by ringworm (3/15, 20%) and *Malassezia* yeast dermatitis (2/2, 13.3%).
Demodicosis is a severe parasitic cutaneous disease of dogs caused by the uncontrolled proliferation of demodectic mites belonging to the genus *Demodex* sp. (Acari, Prostigmata) in presence of concomitant factors potentially immune-suppressive, including diabetes, hypothyroidism and granulocytic ehrlichiosis (anaplasmosis) in dogs [30].

In a previous study, among the underlying conditions associated with 12 cases of adult-onset generalized demodicosis refractory or incompletely responding to therapy, one of us (W. Tarello) recorded 8 cases of canine granulocytic ehrlichiosis (anaplasmosis) and surprisingly observed that therapy for anaplasmosis helped the remission of clinical signs of demodicosis, apparently confirming, with the use of the specific therapy as a probe, that the elimination of underlying immune suppressive agents can control the proliferation of *Demodex* mites [30]. Therefore, it should not be controversial to include demodicosis among the pathogens triggered by canine granulocytic anaplasmosis and, moreover, take into consideration ringworm and *Malassezia* as suitable candidates for the same position.

Reported cases were diagnosed in Dubai between 2010 and 2013 in both imported and locally raised dogs. No previous study was carried out in the United Arab Emirates on the presence and prevalence of anaplasmosis in dogs and unfortunately PCR and serology testing are not available locally.

We hope that the Central Veterinary Research Laboratory of Dubai will provide soon such an invaluable service. This is apparently the second study on canine anaplasmosis from the Middle East, though.

One epidemiological survey on stray dogs from Jordan reporting 39.5% prevalence was published in 2012 [24]. Canine anaplasmosis seems quite prevalent in the Middle East. Further studies are needed to assess its prevalence among pet dogs in Dubai and the United Arab Emirates.

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## TABLE 1. SIGNALMENT, CLINICAL SIGNS AND CONCURRENT CONDITIONS OBSERVED IN 15 DISEASED DOGS DIAGNOSED WITH CANINE GRANULOCYTIC ANAPLASMOSIS IN DUBAI, BETWEEN 2010 AND 2013

<table>
<thead>
<tr>
<th>N.</th>
<th>Breed</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Clinical Signs</th>
<th>Associated conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>St. Bernard</td>
<td>M</td>
<td>10</td>
<td>Lethargy, alopecia</td>
<td>Benign Papilloma</td>
</tr>
<tr>
<td>2</td>
<td>Pitt Bull</td>
<td>F</td>
<td>6</td>
<td>Lethargy, severe weakness, limping</td>
<td>Adult-onset generalized demodicosis</td>
</tr>
<tr>
<td>3</td>
<td>W-H Terrier</td>
<td>F</td>
<td>7</td>
<td>Blood in the urine, polydipsia, limping</td>
<td>Adult-onset generalized demodicosis</td>
</tr>
<tr>
<td>4</td>
<td>Chihuahua</td>
<td>M</td>
<td>1</td>
<td>Itching chronic dermatitis</td>
<td>A-OG Demodicosis and Malassezia</td>
</tr>
<tr>
<td>5</td>
<td>Chihuahua</td>
<td>M</td>
<td>8</td>
<td>1-year chronic cough, limping</td>
<td>Ringworm</td>
</tr>
<tr>
<td>6</td>
<td>En. Bulldog</td>
<td>M</td>
<td>3</td>
<td>1-month itching dermatitis</td>
<td>Adult-onset generalized demodicosis</td>
</tr>
<tr>
<td>7</td>
<td>Labrador R.</td>
<td>M</td>
<td>4</td>
<td>Fever, recurring dermatitis on abdomen</td>
<td>Pyoderma and Ringworm</td>
</tr>
<tr>
<td>8</td>
<td>Saluki</td>
<td>F</td>
<td>1</td>
<td>Fever, tick infestation, dermatitis</td>
<td>Pyoderma and Ringworm</td>
</tr>
<tr>
<td>9</td>
<td>G. Retriever</td>
<td>M</td>
<td>2</td>
<td>Limping, lethargy</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Shi-Tzu</td>
<td>M</td>
<td>6</td>
<td>Conjunctivitis, 3 seizures in a month</td>
<td>Mast Cell Tumor grade 1 in the paw</td>
</tr>
<tr>
<td>11</td>
<td>Yorkshire</td>
<td>F</td>
<td>2</td>
<td>Fever, lethargy, anorexia, vomiting, ticks infestation</td>
<td>Malassezia yeast dermatitis</td>
</tr>
<tr>
<td>12</td>
<td>Dachshund</td>
<td>F</td>
<td>3</td>
<td>Fever, lethargy, vomiting, skin hyperesthesia</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Dachshund</td>
<td>M</td>
<td>4</td>
<td>Fever, vomiting</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Boxer</td>
<td>M</td>
<td>2</td>
<td>Blood in the urine, lethargy, ticks infestation, pallid mucous membranes</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Fr.Bulldog</td>
<td>M</td>
<td>1</td>
<td>2-months itching dermatitis,</td>
<td>Adult-onset generalized demodicosis</td>
</tr>
</tbody>
</table>
Immune competence restoration

### TABLE 2. ANEMIA AND CELLULAR IMMUNOSUPPRESSION OBSERVED BEFORE (B.F.) BUT NOT AFTER THERAPY (A.T.) AGAINST CANINE GRANULOCYTIC ANAPLASMOSIS IN 15 DOGS EXAMINED IN DUBAI

<table>
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<tr>
<th></th>
<th>PCV</th>
<th>WBC</th>
<th>Neutrophiles</th>
<th>Lymphocites</th>
<th>Thrombocytes</th>
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<tr>
<td></td>
<td>(37.55%)</td>
<td>(5500-16900/µL)</td>
<td>(2000-12000/µL)</td>
<td>(500-4900/µL)</td>
<td>(17500-500000/µL)</td>
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<tr>
<td>1</td>
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<td>45.2</td>
<td>2480</td>
<td>5600</td>
<td>1560</td>
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<tr>
<td>2</td>
<td>32.7</td>
<td>50.1</td>
<td>1740</td>
<td>14610</td>
<td>1114</td>
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<tr>
<td>3</td>
<td>47.3</td>
<td>52.1</td>
<td>4430</td>
<td>9110</td>
<td>940</td>
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<tr>
<td>4</td>
<td>43.4</td>
<td>44.2</td>
<td>280</td>
<td>12020</td>
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<tr>
<td>5</td>
<td>24.9</td>
<td>43.1</td>
<td>6029</td>
<td>15060</td>
<td>1990</td>
</tr>
<tr>
<td>6</td>
<td>37.0</td>
<td>49.7</td>
<td>250</td>
<td>8980</td>
<td>100</td>
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<tr>
<td>7</td>
<td>21.5</td>
<td>46.8</td>
<td>480</td>
<td>5530</td>
<td>180</td>
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<tr>
<td>8</td>
<td>22.4</td>
<td>32.0</td>
<td>300</td>
<td>9840</td>
<td>60</td>
</tr>
<tr>
<td>9</td>
<td>23.2</td>
<td>37.1</td>
<td>340</td>
<td>14320</td>
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<td>52.4</td>
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<td>7900</td>
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<td>44.8</td>
<td>6040</td>
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<td>15</td>
<td>45.1</td>
<td>57.3</td>
<td>5320</td>
<td>9310</td>
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