Short Paper

Naturally occurring ehrlichiosis in Egyptian dogs

Salem, N. Y.[*]; Rakha, G. H. and Baraka, T. A.

Department of Clinical Sciences, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

[*]***Correspondence:*** *N. Y. Salem, Department of Clinical Sciences, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt. E-mail: nemovet2011@gmail.com*

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Summary

Canine ehrlichiosis has emerged as one of the most clinically important tick-borne diseases affecting dogs. Eighty-five naturally infected dogs have been investigated, the most consistent clinical signs recorded were fever, emaciation and lymphoadenopathy, anemia, monocytosis, thrompocytopenia, hypoalbuminemia, elevation in liver enzymes and total bilirubin were the most remarkable changes associated with canine ehrlichiosis in Egyptian dogs; microscopic examination failed to provide definitive diagnosis of canine ehrlichiosis. Season did not greatly influence the disease; the type of ticks involved in the disease transmission in Egypt was *Rhipicephalus sanguineus.*

Key words: Canine ehrlichiosis, Fever, Anemia, Liver enzymes, PCR

Introduction

Canine ehrlichiosis has emerged as one of the most important infectious diseases affecting dogs (Moreira *et al*., 2003); infecting mainly macrophages, monocytes and granulocytes (Jadhav *et al*., 2011), transmitted chiefly by *Rhipicephalus sanguineus* (Troy and Forrester, 1990). The pathogenesis involves an 8-20 day incubation period and 3 clinical aspects: acute, subclinical and chronic phases (Alleman, 2005). Fever, weight loss, hepatosplenomegaly, lymphadenopathy and hemorrhage were described (Troy and Forrester, 1990). Thrompocytopenia, anemia and leucopenia are common hematologic abnormalities (Scorpio *et al*., 2008). Hypoalbuminemia, hyperglobulinemia, elevated liver enzymes, hyperbilirubinemia have been reported (Rungsipipat *et al*., 2009; Stephanie *et al*., 2010). Morulae are uncommonly detected and its absence cannot rule out the infection (Alleman, 2005). PCR has proven to be a more specific, sensitive and reliable tool for definitive diagnosis (Breitschwerdt *et al*., 1998). The aim of this study is to describe naturally occurring ehrlichiosis in Egyptian dogs by assessment of clinical and laboratory findings as well as PCR detection of *Ehrlichia* spp. This study pinpoints the effect of the season and other risk factors on disease epidemiology in this area of the world.

Materials and Methods

Eighty five dogs of different ages, sexes, and breeds were used in this study. The dogs were referred to Small Animal Medicine Teaching Hospital, Faculty of Veterinary Medicine, Cairo University. Signs were recorded; complete physical examination and clinical hematology were done. Serum samples were analysed for AST, ALT, ALP, TP, albumin, total bilirubin, BUN and creatinine with respective kits (Stanbio® Inc., USA), ticks were collected and identified (Nuttal *et al*., 1908). Blood films were examined under oil immersion lens.

DNA was extracted from whole blood using Blood DNA Preparation Kit (Jena Bioscience GmbH, Jena, Germany) according to manufacturer's instructions. Purified DNA was tested with primers that amplify a portion of 16S RNA gene.

ECC (5′AGAACGAACGCTGGCGGCAAGC-3′) and ECB (5′ CGTATT ACCGCGGCTGCTGGCA-3′) primers amplified all *Ehrlichia* spp. (Dawson *et al*., 1996); PCR cycle was performed according to Murphy *et al*. (1998), and a previously confirmed sample was used as a positive control.

Student's t-test (STATISTICA for Windows, version 5.1, StatSoft, Inc.) was used.

Results

Dogs were divided into 4 groups: <1 year (3/85, 3.52%), between 1-<3 years (15/85, 17.64%), between 3-<5 years (49/85, 57.64%) and >5 year (18/85, 21.17%). Male (46/85, 54.11%) and female (39/85, 45.88%) were almost equally infected.

German shepherd dogs were the most affected breed 50/85 (58.82%), Labrador 12/85 (14.11%) and the other breeds 23/85 (27.05%). The seasonal results were: summer (27/85, 31.76%), autumn (21/85, 24.70%), winter (17/85, 20.01%), and spring (20/85, 23.52%). The ticks were identified as *Rhipicephalus sanguineus* according to morphologic characteristics. The most consistent signs (Fig. 1) were pyrexia (67/85, 78.82%), pale mucous membrane (39/85, 45.88%), presence of ticks at time of admission (54/85, 63.52%) Fig. 1A, hepatosplenomegaly (26/85, 30.58%), lymphadenopathy

Fig. 1: 1A) Severe tick infestation in head of Great Dane infected dog. 1B) Severe emaciation in German shepherd infected dog. 1C) Yellowish discoloration in oral mucosa of Rottweiler infected dog. 1D) Analysis of PCR products by 1.5% agarose gel electrophoresis and ethidium bromide staining showing the positive samples after the primary amplification cycle, DNA ladder (100 bp DNA ladder, Jena Bioscience, Jena, Germany) was loaded with the samples simultaneously

Table 1: Hematologic and serum biochemical findings of 85 dogs with ehrlichiosis[1]

Parameter	Patient data	Control data[a2]
RBCs ($\times10^6$/µl)	4.7±0.32*	7.41±1.19
Hemoglobin (g/dl)	12.06±0.6*	15.74±2.17
PCV (%)	35.69±6.35*	44.14±6.14
MCV (fl)	76.62±14.7*	69.14±6.15
MCH (pg)	22.85±2.3	23.10±2.02
MCHC (%)	32±3.77	33.56±0.77
WBCs ($\times10^3$/µl)	8.354±2.69*	11.850±1.16
Neutrophil ($\times10^3$/µl)	3.574±0.393*	6.728±0.19
Lymphocyte ($\times10^3$/µl)	3.612±0.340	3.928±0.27
Monocyte ($\times10^3$/µl)	1.050±0.124*	0.775±0.23
Platelets ($\times10^3$/µl)	103.93±16.67*	270.93±14.12
Total protein (g/dl)	7.75±0.51*	9.66±0.48
Albumin (g/dl)	2.256±0.65*	4.38±0.66
ALT (u/l)	118.84±24.00*	48.87±7.25
AST (u/l)	124.82±13.8*	35.103±6.346
ALP (u/l)	213.21±15.2*	90.159±17.912
BUN (mg/dl)	21.004±8.27*	11.93±3.65
Creatinine (mg/dl)	0.99±0.408	1.07±0.31
Total bilirubin (mg/dl)	0.909±0.4*	0.46±0.15

* Show statistically significant difference at $P<0.05$. [1] From Small Animal Internal Medicine Teaching Hospital, Faculty of Veterinary Medicine, Cairo University, and [2] A control data was taken from healthy dogs in the same locality

(38/85, 44.70%), emaciation (17/85, 20%) Fig. 1B, icterus (2/85, 2.35%) Fig. 1C, epistaxis (2/85, 2.35%), polyarthritis (3/85, 3.52%), peripheral edema (1/85, 1.17%), morulae were detected in 5/85 (5.88%) films.

The mean hematologic and serum biochemistry values are shown in Table 1.

Reduction in RBCs, HB content, PCV percentage with normocytic normochromic anemia, leucocytic count, and neutrophils along with increase in monocytes count were observed and 95% of dogs had thrombocytopenia. Significant increase in ALP, ALT, AST with hypoalbuminemia was observed. 488 bp were amplified as shown in Fig. 1D.

Discussion

Canine ehrlichiosis is a widely known tick-borne disease. The highest occurrence was at 3-5 years followed by dogs over 5 years; Poitout *et al*. (2004) found the highest prevalence in dogs between 4-13 years. German shepherd was the most affected breed; the defective cell-mediated immunity may be associated with the severity of the disease in this breed (Nyindo *et al*., 1980).

Seasonal similarity was found, although Mosallanejad *et al*. (2010) reported the highest prevalence was in summer. The global climatic changes made ticks more adaptive (Leschnik *et al*., 2008); the dynamicity of ticks depends on the climatic condition followed by changes in seasonal patterns (Friedhoff, 1988). The weather in Egypt is warm throughout the year, which may have an impact on the pattern in Egypt.

Ehrlichiosis causes multisystem involvement, the organism multiplies inside mononuclear cells spreading to liver, spleen and lymph nodes (Woody and Hoskins, 1991). These circulating infected MNCs adhere to vascular epithelium inducing vasculitis and subendothelial tissue infection (Iqbal and Rikihisa, 1994). Fever and leucopenia were common in canine ehrlichiosis and should be added as differential diagnosis of pyrexic dogs (Unver *et al*., 2005).

Morulae were detected in 5/85 (5.88%); the detection is ultimate for diagnosis, but it's seldom found and mostly unrewarding (Alleman, 2005).

Reduction in RBCs, HB content, and PCV percentage with normocytic normochromic anemia have been recorded. *Ehrlichia* is thought to cause bone marrow destruction leading to mild to severe non-regenerative anemia (Rungsipipat *et al*., 2009). Davoust *et al*. (1996) believed the anemia was due to immune-mediated destruction of RBCs, as antibodies would be fixed at the membrane causing their lyses by effector cells of the immune system.

Decrease in leucocytes count and neutrophils were observed, and the depletion of granulocytic precursors or damage of myeloid cells may be implicated (Codner and Farris-Smith, 1986). Monocytosis has been documented in ehrlichiosis (De Castro *et al*., 2004); this elevation is expected in inflammatory disease with high demand for macrophages, hemolytic or hemorrhagic and immune-mediated diseases (Raskin *et al*., 2004).

Hypoprotenemia and hypoalbuminemia were detected along with blood loss or decreased protein production due to concurrent mild liver disease. Reardon and Pierce (1981) observed development of many expanding foci in hepatic sinusoids, with the compression leading to necrosis of adjacent hepatocytes with increased enzymes and bilirubin activities.

Elevated BUN levels were observed in patients without similar rise in creatinine levels; catabolic state is likely to occur in anemic patients due to tissue hypoxia, and pyrexia (Michel *et al*., 1997).

PCR detection of *Ehrlichia* is possible; recent studies showed efficacy of PCR in *Ehrlichia* detection (Iqbal and Rikihisa, 1994). The difference between direct microscopic examination and PCR revealed the low capability of microscopic examination in establishing a definitive diagnosis (Lakshmanan *et al*., 2007).

This study showed season did not greatly influence the disease; the type of tick involved was *Rhipicephalus sanguineus*. Pyrexia, anemia, leucopenia, thrombocytopenia, and elevated liver enzymes are the base for tentative diagnosis but for definitive diagnosis, PCR is found to be more efficient.

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