ISSN NO. 2320-5407

*Journal homepage: http://www.journalijar.com*

*INTERNATIONAL JOURNAL OF ADVANCED RESEARCH*

**RESEARCH ARTICLE**

# Secondary subclinical diabetes mellitus in dogs infected with *Ehrlichia canis*

***Deepa.P.M.[1], Dimri, U[1], Ricky Jhambh[1], Ramees, T.P[2], Vijaykumar H[1], Devi Gopinath ,[1] Mahendran, K[1] and Mondal, D.B.[1]**

**1.** Division of Medicine, Indian Veterinary Research Institute, Izatnagar, Bareilly, UP, India.
**2.** Division of Public Health, Indian Veterinary Research Institute, Izatnagar, Bareilly, UP, India.

## *Manuscript Info*

***Manuscript History:***

Received: 11 November 2013
Final Accepted: 25 December 2013
Published Online: January 2014

***Key words:***
subclinical, diabetes, dogs, Ehrlichia canis

***Corresponding Author***

**Deepa. P.M.**

## *Abstract*

Ehrlichiosis is an important and potentially fatal disease of dogs caused by the rickettsia *Ehrlichia canis.* This disease has a worldwide distribution and pathogenic mechanism involves haematological alteration that may occur due to immune mediated inflammatory changes and hormonal alterations. Diabetes mellitus is a frequently occurring endocrinopathy in dogs. Non insulin dependent diabetes mellitus is very rarely reported in dogs. The present study aims at evaluation of diabetic biomarkers like fasting blood glucose, glycated haemoglobin,serumfructosamine,serum insulin and oral glucose tolerance test in *E. canis* infected dogs. Out of 67 dogs screened, 26.8% showed antibodies to *E.canis* by SNAP 3DX test.But only 8.9% of dogs demonstrated morulae in the buffy coat smear indicating antibody test is more sensitive compared to buffy coat smear examination. Out of 18 dogs infected with *E.canis*, 77.7 % of dogs exhibited subclinical diabetes with biomarkers above the range in healthy animals, but below the level in clinical diabetes. These animals also showed higher insulin level signifying insulin resistance. Future research is warranted to identify the factors contribute to insulin resistance in *E.canis* infection in dogs.



## Introduction

Canine monocytic ehrlichiosis caused by *Ehrlichia canis* is a tick-borne rickettisial disease, manifested by a variety of clinical signs and mortality in the chronic severe form. Chronic severe ehrlichiosis is a serious health problem in dogs in India as well as other parts of the world. Decreased concentrations of thyroid hormones and cortisol in dogs with chronic severe ehrlichiosis was reported by Kumar et al. (2006) and suggested that the pathogenesis of ehrlichiosis in dogs involves disruption of endocrine function. T3 and insulin have a synergistic role in glucose homeostasis as these hormones possess similar action sites in the regulation of glucose metabolism, at both cellular and molecular levels (Kim et al, 2002). Hypothyroidism may result in disturbance in glucose metabolism by post receptor defect in insulin action, possibly with a concurrent decrease in insulin secretion and receptor affinity in dogs ([Handisuryet al,2008) and can predispose to insulin resistant state by decreasing the ability of insulin for glucose utilisation in peripheral tissues like muscle (Dimitriadi et al,2006; Rochon et al,2003; Cettour-Rose et al,2005). Maratou et al. (2009) suggested the impairment in Insulin-stimulated rates of glucose disposal in muscle and adipose tissue in hypothyroidism could be due to impaired translocation of GLUT4 transporters on the cell surface. Changes in the lipid composition and microviscosity of the islet membranes induced by hypothyroidism results in decreased amount and velocity of H islets in the release of insulin in response to glucose (Diaz et al, 1993).

Though the positive correlation between human Type-1 disease and canine diabetes is well established, yet, there is little convincing evidence that dogs suffer from Type-2 diabetes (German et al, 2010). Incidence of obesity in dogs is increasing as human obesity increases overall population. Obesity in dogs is associated with the development of insulin resistance, altered lipid profiles, and mild hypertension, which are ameliorated by weight loss (German et al, 2009; Yamka et al, 2006). Overweight dogs are more likely to suffer from diabetes mellitus

(Lund et al, 2006). Diagnosis of diabetes in animals is difficult because often apparent clinical manifestations are not clear indicators of type of problem; moreover, detection in advanced stage of this disease is of little value because sometimes conditions may become irreversible in nature. Studies aimed at detecting early stages of diabetes are critical. Detection of diabetes in dogs as well as taking corrective measures at its subclinical stage is essential to appropriate health care, and increases the quality of life for pets. Subclinical diabetic animals often appear healthy with persistent fasting blood glucose (BG) concentrations above the reference range but below the concentration that results in glycosuria (Rucinsky et al, 2010). Biomarkers reflect the presence and severity of hyperglycemia. Glycated haemoglobin (HbA1c) is considered as a valuable predictive biomarker of diabetes (Sacks et al, 2011; Lyons and Basu, 2012; Mared et al, 2012). Serum fructosamine retorts much more quickly than the HbA1c to a change in glucose situation and reflects diabetes control over the previous 2-3 weeks (Kostolanska et al, 2009; Mittman et al, 2010). Nathan et al. (2007) revealed the most efficient sequence of testing for diabetes is fasting plasma glucose concentration first followed by the oral glucose tolerance test on a subsequent day to demonstrate the presence of impaired glucose tolerance .Present study aims at assessing the biomarkers related to diabetes in dogs affected with *Ehrlichia canis* infection.

## Materials and Methods

### Animals and sample collection

Dogs irrespective of sex and breed, above 5 years of age, presented to Referral Polyclinic of the Indian Veterinary Research Institute, with various disease manifestations like anorexia, vomiting, weakness, anemia, emaciation were screened for diabetes. The blood samples collected from these animals were subjected to tests like Complete blood count (haemoglobin, total erythrocyte count, Total leukocyte count, platelet count, differential leukocyte count), blood glucose and glycated haemoglobin. Serum from these animals was tested for serum fructosamine, insulin and antibodies to *Ehrlichia canis* using SNAP 3DX test (IDEXX laboratories). Blood sample was centrifuged and the smear prepared from buffy coat smear was used for screening hemoprotozoa by Giemsa staining. Urine sample was collected and checked for glucose.

### Estimation of glucose and glycated hemoglobin

Glucose level in blood was measured by glucose oxidase perxidase method (Kaplan.1984). Urine glucose was measured by Benedict'stest (Benedict, 1908). Glycosylated haemoglobin was estimated by ion exchange chromatography method of Trivelli et al. (1971). Initially hemolysate was prepared by mixing 100µl of blood sample to 500 µl of 10 mm potassium cyanide surfactant (lysing reagent) solution. Glycohemoglobin was prepared by adding 100 µl of hemolysate to 3 ml of cation exchange resin buffered at pH 6.9. Glycohemoglobin fraction was determined by measuring the absorbance of supernatant at 415 nm. Total haemoglobin fraction was determined by adding 20 µl of the hemolysate to 5 ml of deionized water and absorbance was measured at 415nm. The result was expressed as percentage of glycosylated haemoglobin of the total haemoglobin fraction.

### Serum fructosamine

Serum fructosamine was estimated by NBT reduction method (Sahu and sarkar, 2008). 200µl of serum was added to 1ml of 9gm/l sodium chloride and incubated at 37$^{o}$c for 10 min. 1ml of pre warmed NBT reagent prepared in carbonate buffer (0.2mol/l, pH 10.8) was added and absorbance was measured at 530nm at interval of 5 min(A1) and 10 min (A2). The difference was calculated and expressed as AA/min. 40 mmol/l stock solution of 1deoxy- 1 mopholino-D fructose (DMF) prepared in bovine serum albumin solution (40 g/l in 155 mmol/l saline). This stock solution was diluted with bovine serum albumin solution to prepare DMF standard containing 4 mmol/l. Absorbance was measured at 530 nm at interval of 5 min and 10 min. The result was expressed in mmol/l.

Detection of *Ehrlichia canis* antibody

A commercially available diagnostic kit (canine SNAP 3DXtest, IDEXX laboratories) was used for detection of *Ehrlichia canis* antibody. This ELISA test uses asynthetic peptide (C6) derived from invariable region as a diagnostic antigen. Two drops of serum was taken in the sample tube and 5 drops of conjugate which contains HRP labelled *E. Canis* peptide conjugate was added. The resultant mixture was dropped in to sample well in the device. Development of colour in the designated reaction area indicates sample positive for *E. Canis* antibody.

### Oral glucose tolerance test (OGTT)

OGTT, as described for dogs by Kaneko et al. (2008) consisted of an oral glucose bolus of 4 g/kg body weight given as a 50% w/v solution. The blood glucose was estimated just before dosing and 2hrs after administering glucose.

**Estimation of Insulin**

Immunoreactive insulin (IRI) concentrations were measured by immune radiometric assay using poly clonal antibodies.

**Statistical analysis:** The data were expressed as mean ± standard error mean (S.E.M). The test followed by student's test p values less than 0.05 were considered as significance.

**Results**

A total of 67dogs with various clinical manifestations of anorexia, vomiting, lymphadenopathy, anemia, weakness were subjected to the study. Out of these, 18dogs (26.8%) showed antibody to *E. canis* by SNAP 3DX test. Of these, six dogs (8.9%) demonstrated morulae (inclusion bodies) in the cytoplasm of monocytes. The haematological values of affected dogs are depicted in Table1.hemoglobin, PCV, Total erythrocyte count, and platelet counts were significantly lowered in E.canis affected dogs. Among the 18 dogs, fourteen (77.7%) had fasting blood glucose level above 110 mg/dl, but below 200 mg/dl, with a mean value of 147.77±16.32 mg/dl. Urine samples showed negative reaction to glucose in all the dogs tested. The mean glycated haemoglobin and serum fructosamine values are given in Table 2. In oral glucose tolerance test, all the fourteen dogs exhibited elevated level of blood glucose two hrs following glucose load. Blood glucose level increased from mean fasting glucose level of 147.77±16.32 mg/dl to 188.89±18.74mg/dl after 2hrs of glucose load. Twelve dogs showed serum insulin level above the higher range of insulin in healthy dogs (above 20 µU/ml).

**Positive for E. canis antibody**

**SNAP test sowing antibody positive to E canis**

**Discussion**

Detection of morulae in circulating monocytes is a routine diagnostic method for ehrlichiosis (Moreira et al, 2005). The present study showed an incidence of 8.9% Ehrlichiosis in dogs by buffy coat smear examination. 26.8% dogs were infected with *Ehrlichia canis* by SNAP antibody test. *E.canis* can be detected for a short period of time in monocytes, but cannot be found during chronic phase of infection. Altas et al (2013) demonstrated *E. canis* antibodies in 62% of stray dogs. SNAP test for ehrlichiosis had a specificity of 98.2% (Belanger et al, 2002; Tzipory et al, 2010). The present study revealed an impaired glucose tolerance in 77.7% of *E. Canis* infected dogs with fasting blood glucose, glycated haemoglobin and serum fructosamine values above the normal range. This is in agreement with findings of Kumar et al (2006) showed endocrine dysfunction in *E canis* infected dogs, particularly affect thyroid glands resulting in decreased concentration of thyroid hormones. Hypothyroidism contributes to type 2 diabetes mellitus by impairing glucose utilisation and disposal in muscles, overproduction of hepatic glucose output and enhanced absorption of splanchnic glucose (Wang, 2013). According to Kaneko et al (2008) a normal glucose profile should reach a peak between 30 and 60 min and return to baseline by 120 min .The failure of glucose to return to baseline levels within 2 h in this study indicates a prediabetic glucose-intolerant state. The elevated serum insulin levels in this study also suggest insulin resistance. This is in agreement with Shanik et al. (2008) suggested hyperinsulinemia is both a result and driver of insulin resistance. Hypothyroidism can cause hyperinsulinemia and thyroid hormone treatment was shown to be highly effective in eliminating hyperinsulinemia in obese zucker rats (Torrance et al, 1997).

**Table1. Hematological indices of dogs affected with *E.canis* (Mean±SE values)**

| Parameters | E.canis infected | Healthy dogs |
|---|---|---|
| Haemoglobin(g/dl) | 7.69±0.86[a] | 12.63±0.67 |
| PCV (%) | 25.7±1.43[a] | 39.1±0.54 |
| Total erythrocyte count ($10^6$/µl) | 3.74±0.52[a] | 5.97±0.33 |
| Total leukocyte count($10^3$/ µl) | 9.13±1.6 | 9.62±1.21 |
| Neutrophil(%) | 73.12±2.75 | 69.7±0.85 |
| Lymphocyte (%) | 18.53±2.73 | 17.85±0.72 |
| Monocyte (%) | 5.83±1.12 | 7.83±0.84 |
| Eosinophil(%) | 2.1±0.63 | 1.2±0.47 |
| Platelet ($10^3$/ µl) | 39.16±3.21[a] | 203.21±18.6 |

Values with superscript a differ significantly (P< 0.05) in the row

**Table 2. The fasting blood glucose, glycated haemoglobin serum fructosamine and insulin values in *E. canis* affected dogs (Mean ±SE)**

| Parameters | E.canis infected | Healthy dogs |
|---|---|---|
| Fasting blood glucose(mg/dl) | 147.77± 16.32[a] | 94.21±14.23 |
| Glycated haemoglobin (HbA1c %) | 7.08±1.6 [a] | 5.4±1.02 |
| Serum fructosamine (µmol/l) | 342.53±17.62[a] | 212.66±17.89 |
| Insulin (µU/ml) | 26.41±4.9[a] | 13.67±3.2 |

Values with superscript a differ significantly (P<0.05) in the row

**Conclusion**

*Ehrlichia canis* infected dogs exhibited an impaired glucose tolerance and higher level of serum insulin which indicates subclinical diabetic stage. It is evident from existing literature that E canis infection in dogs result in decrease concentration of thyroid hormones and hypothyroidism can contribute to insulin resistance. Further research is needed to unveil the mechanism involved in insulin resistance.

**References**

Altas, M.G., Ipek ,D.N., Sevgili, M and Icen, H. (2013). Prevalence of Dirofilaria immitis, Ehrlichia canis, Borrelia burgdorferi infection in stray dogs from sanliurfa in Turkey. Veterinary Research., 6(3 ) :48-53.

Belanger, M., Sorenson, H.L., France, M.K., Bovvie, M.V., Barbet, A.F., Breitschvverdt and Alleman, A.R.(2002). Comparison of serological detection methods for diagnosis of *Ehrlichia canis* infection in dogs. J.Clin. Microbiol.,40 : 3506-308.

Benedict, S.R. (1908). A reagent for detection of reducing sugars. J.Biol.Chem., 5( 6) :485-487.

Cettour-Rose ,P., Theander-Carrillo, C., Asensio, C., Klein, M., Visser, T.J., Burger ,A.G., Meier, C.A and Rohner-Jeanrenaud, F.(2005). Hypothyroidism in rats decreases peripheral glucose utilisation, a defect partially corrected by central leptin infusion. Diabetologia. 48: 624–633.

Diaz, G.B., Paladini, A.A., Garcia, M.E and Gagliardino, J.J. (1993). Changes induced by hypothyroidism in insulin secretion and in the properties of islet plasma membranes. Arch Int Physiol Biochim Biophys., 101( 5): 263-269.

Dimitriadis, G., Mitrou, P., Lambadiari, V., Boutati, E., Maratou, E., Panagiotakos, D.B., Koukkou, E., Tzanela, M., Thalassinos, N and Raptis, S.A.(2006). Insulin action in adipose tissue and muscle in hypothyroidism. Journal of Clinical Endocrinology and Metabolism, 91:4930–4937

German, A. J., Hervera, M., Hunter, L., Holden, S.L., Morris, P.J., Biourge, V and Trayhurn, P.(2009b). Improvement in insulin resistance and reduction in plasma inflammatory adipokines after weight loss in obese dogs. Domestic Animal Endocrinology. ,37: 214–226.

German , A. J., Ryan, V.H., German, A.C., Wood, I.S and Trayhurn, P.(2010). Obesity, its associated disorders and the role of inflammatory adipokines in companion animals. The Veterinary Journal. 185:4-9.

Handisury, A., Pacini, G., Tura, A., Gessl, A and Kautzky-Willer A. (2008). Effects of T4 replacement therapy on glucose metabolism in subjects with subclinical (SH) and overt hypothyroidism (OH). Clinical Endocrinology, 69(6): 963–969.

Kaneko, J.J., Harvey, J.W and Bruss, M.L. (2008). Clinical Biochemistry of Domestic animals. Academic Press, Elsevier's publication . 6th edn.

Kaplan, L.A.(1984). Carbohydrates and metabolites, In: Clinical chemistry: Theory, Analysis and correlation. Kaplan. L.A and Pesce A.J (Eds) C.V. Mosby,Tronto; 1032-1040

Kim, S.R., Tull, E.S., Talbott, E.O.,Vogt, M.T and Kuller, L.H.(2002). A hypothesis of Synergism: the interrelationship of T3 and insulin to disturbances in metabolic homeostasis. Medical Hypotheses. 59: 660–666.

Kostolanska, J., Jakus, V and Barak, L.(2009). Monitoring of Early and Advanced Glycation in Relation to the occurrence of Microvascular Complications in children and adolescents with Type 1 Diabetes Mellitus. Physiol. Res., 58: 553-561.

Kumar, A., Varshney, J.P and Varshney, V.P.(2006). Endocrine dysfunction in chronic severe Ehrlichiosis with or without babesiosis in dogs. Veterinary Research Communications. 30(8): 911-916.

Lund, E.M., Armstrong, P.J., Kirk, C.A and Klausner, J.S. (2006). Prevalence and Risk Factors for Obesity in Adult Dogs from Private US Veterinary Practices. Intern J Appl Res Vet Med., 4, 2: 177-186.

Lyons, T.J and Basu, A.(2012). Biomarkers in diabetes: hemoglobin A1c, vascular and tissue markers**.** Transl. Res., 159(4):303-312.

Mared, M., Catchpole, B., Kampe ,O and Fall, T. (2012). Evaluation of circulating concentrations of glucose homeostasis biomarkers, progesterone, and growth hormone in healthy Elkhounds during anestrus and diestrus. Am. J.Vet. Res. , 73(2):242-247.

Marotou, E., Hadjidakis, D.J. Kollias, A., Tsegka, K., Peppa, M., Alevizaki, M et al. (2009). Studies of insulin resistance in patients with clinical and subclinical hypothyroidism. European Journal of Endocrinology. 160: 785-790.

Mittman, N., Desiraju, B.,Fazil, I., Kapupara, H., Chattopadhayay , J., Jani , C.M and Avram, M..M. (2010). Serum fructosamine versus glycosylated hemoglobin as an index of glycemic control, hospitalization, and infection in diabetic hemodialysis patients. Kidney Int Suppl., 117:541-545

Moreira, S.M., Machado, R and Passos, L.F. (2005). Detection of *Ehrlichia canis* in bone marrow aspirates of experimentally infected dogs. Cieno Rural., 35, 958-960.

Nathan ,D.M.., Davidson, M.B, DeFronzo, R.A., Heine, R.J, Henry, R.R, Pratley, R and Zinman, B.(2007). Impaired Fasting Glucose and Impaired Glucose Tolerance Implications for care. Diabetes Care. 30 (3): 753-759.

Rochon, C., Tauveron, .I, Dejax, C., Benoit ,P., Capitan, P., Fabricio, A., Berry, C., Champredon, C., Thieblot, P and Grizard, J.(2003). Response of glucose disposal to hyperinsulinaemia in human hypothyroidismand hyperthyroidism. Clinical Science. 1047–1152.

Rucinsky, R., Cook, A., Haley, S., Nelson, R., Zoran, D.L and Poundstone, M.(2010). AAHA diabetes management guidelines for dogs and cats. J. Am. Anim. Hos. Assoc. 46:215-224.

Sacks, D.B. (2011). A1C versus Glucose Testing: A Comparison. Diabetes Care. 34 (2):518-523.

Sahu, A and Sarkar, P.D. (2008). Comparative study of NBT reduction method for estimation of glycated protein (serum fructosamine) with glycated HbA1c estimated on DCA 2000 analyzer. Ind. J. Physiol. Pharmacol., 52 (4):408-412.

Shanik,M.H., Xu, Y., Skrha, J., Danker, R., Zick, Y and Roth,J (2008). .Insulin Resistance and Hyperinsulinemia. Is hyperinsulinemia the cart or the horse? Diabetes Care,3: 5262-5268.

Torrance, C.J., de Vente, J.E., Jones , J.P and Dohm, G.L(1997). Effects of Thyroid Hormone on GLUT4 Glucose Transporter Gene Expression and NIDDM in Rats. Endocrinology, 138(3): 1204-1214.

Trivelli , L.A., Ranney, H.A and Lai, H.T.(1971). Haemoglobin components in patients with diabetes mellitus. N. Engl. J. Med., 284: 353-357.

Tzipory, N., Crawford, P.C and Levy, J.K. (2010). Prevalence of Dirofilaria immitis,Ehrlichia canis and Borrelia burgdorferi in pet dogs, racing greyhounds and shelter dogs in Florida. Vet. Parasitol. 171 : 136-139

Wang, C.(2013). The Relationship between Type 2 Diabetes Mellitus and Related Thyroid Diseases. Journal of Diabetes Research, http://dx.doi.org/10.1155/2013/390534

Yamka, R.M., Friesen, K.G and Frantz, N.Z.(2006). Identification of canine markers related to obesity and the effects of weight loss on the markers of interest. *International Journal of Applied Research in Veterinary Medicine*. **4:**282-292.