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Rapid urease test: A newer perspective of diagnosing *Helicobacter pylori* infection in dogs

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Abstract

Dogs presented with history and signs of chronic, recurrent vomiting with or without anorexia were subjected for detailed examination followed by blood, serum and fecal sample evaluation to rule out the systemic infection. Later x-ray, abdomen ultrasound and endoscopy was performed and biopsy specimens were collected from selective cases. Erythemia, petechiation, erosions, ulcers and mucosal thickening or hypertrophy along the mucus lining of cardiac, fundus and pylorus were the significant endoscopic abnormalities. Biopsy samples changed the colour of test solution from yellow to pink within 3-5 min thus suggesting positive for rapid urease test and hence, confirming the typical *Helicobacter* infection.

Keywords: *Helicobacter spp.*, rapid urease test, vomiting dog

Introduction

Helicobacter pylori, that colonize the gastric mucosa of humans, was identified in 1984 [1] followed by detection and isolation of more than 30 different species of *Helicobacter* from various animal species, including humans [2-5]. Canine gastrointestinal (GIT) complaints particularly, upper GIT disorders are acute and self resolving requiring only supportive and symptomatic therapy, while specific diagnosis is often necessary in chronic type of gastric disorders. Chronic gastritis is a common problem in dogs and cats and is associated with infectious and non-infectious causes. *Helicobacter sps* is the most common cause for bacteria associated chronic gastritis in dogs [6]. Rapid urease test remains a reliable test for diagnosis of *H. pylori* in patients [7]. Though reports on *Helicobacter* associated gastritis and its diagnosis is common in humans, related publications in dogs is scanty and dearth in India.

Materials and Methods

Dogs of different breed and age that were presented with history and signs of chronic, recurrent vomiting with or without anorexia to the Veterinary Clinical Complex, Bhoiguda of College of Veterinary Science, Rajendranagar, Hyderabad were considered for the study. After a thorough clinical examination, whole blood, serum, vomitus and fecal samples were collected for evaluation. Cervico-thoracic x-ray and abdomen ultrasound was also performed to rule out the presence of any foreign body. Further, esophago-gastroscopy was performed for selected patients and endoscopic biopsy specimens were collected by the standard procedure [8], using Karl Storz video-endoscope and were subjected for Rapid urease test [9].

Results

All the dogs were presented with similar signs of chronic gastritis viz., chronic, recurrent vomiting, with mucous / blood / blood clots among few, coffee colored / bile / undigested food, among few, dehydration and fever. Hematologically there was a significantly ($P < 0.05$) increased PCV with neutrophilic leukocytosis and biochemically no significant abnormality was noticed on LFT and KFT values. Few gastritis dogs were shown positive for certain endoparasitic ova. Radio-opaque foreign bodies in the esophagus and stomach along with gas filled intestinal loops, were the radiographic findings and thickened hyperechoic stomach wall layers were the significant ultrasonographic findings. Whereas, endoscopic evaluation revealed partially digested food through esophagus to the stomach. Further, reddened gastric mucosa with petechiation, increased granularity or friability, erosions, ulcers and mucosal thickening or hypertrophy along the mucus lining of cardiac, fundus and pylorus were the significant abnormalities recorded in the present study. Later, biopsy samples were collected (from those dogs that were clear from gastro-intestinal foreign body and endoparasitic load) from 10 cases, using biopsy forceps to perform rapid urease test and thereby to confirm

Helicobacter spp. induced gastritis. Biopsy samples from 6/10 chronic vomiting dogs revealed a change in colour of test solution from yellow to pink within 3-5 min which correlates with the decrease in pH of the test solution containing the test biopsy sample thus suggesting positive for rapid urease test and hence, confirming the typical *Helicobacter* infection (fig.1).



Fig 1: Test sample negative (A); positive sample (B)

Discussion

Helicobacter pylori is a curved, gram-negative, motile rod urease producing organism with a characteristic “gull-wing” appearance that lies in the interface between the gastric epithelial cell surface and overlying mucus gel. It is often difficult to routine detection of these bacteria and culture because of its fastidious micro aerophilic growth requirements. Moreover, *Helicobacter spp.* are urease-positive [10], and hence, a urease test is often used to distinguish *Helicobacter pylori* like organism (HPLO) from other bacteria [11]. In humans, *H. pylori* is known to cause type B gastritis followed by gastro-duodenal mucosal ulcers, which can progress to gastric carcinoma [12]. The condition is more commonly reported in humans when compared to canine population probably due to lack of appropriate diagnostic procedures [13]. In human population, the prevalence of the same ranges from 8.9 to 72.8% among children from developed and developing countries, respectively, with the re-infection rate also being significantly higher in the latter [7]. Chronic vomiting with or without blood, bile and undigested food material were the common manifestation [13].

In the present investigation 60% of dogs were found positive for helicobacter gastritis infection based on the rapid urease test owing to the decrease in pH of the test solution containing the test biopsy sample which is indicated by a change in colour within 1-5 min. The customary reagent formulation for the detection of urease-producing organisms is 2% urea (w/v) and the indicator phenol red, which changes from yellow-orange to pink-red. Bacterial urease hydrolyzes urea, resulting in ammonia production and a subsequent rise in the micro environmental pH, which is indirectly detected by a urease test reagent that contains urea and a pH indicator. In general, gastric mucosa do not contain urease, but increases when there is an overload of *Helicobacter* like organisms, which are well known to produce them. A negative result was indicated when there was no change in the colour appreciated after hours of observation [14]. When a comparative study was carried out using rapid urease test, gram's staining, culture and serology for diagnosing helicobacter infection in humans, the rapid urease test was found to be more sensitive (72.17 percent)

with a specificity of 92.94 percent and positive predictive value 93.25 percent [9]. These findings have corresponded the findings of the present study. Evidence is conflicting on the results obtained by [15] who carried out a study to record the stomach pathologies in dogs and determine the presence of *Helicobacter* in associated gastric injuries and reported 100% positivity to urease was observed. The lower incidence for urease test in the present study could be attributed to well food practice among the dog population in the area of study or collection of the biopsy sample from the stomach mucosa where the *Helicobacter* organism didn't reside. Apart from rapid urease test method, histopathology and direct tissue examinations were also recommended among the chronic vomiting patients that were suspected for *Helicobacter spp.* but, practically there are several limitations with these tests [13]. In the present study it was found that the rapid urease test not only overcomes these limitations, but at the same time was recorded to have high sensitivity and specificity rates as compared to the culture and staining procedures in confirming the diagnosis of *Helicobacter* induced chronic gastritis among dogs. Regular use of endoscopy in chronic vomiting patients along with rapid urease test can bring a revolutionary movement in the awareness of current updates in *Helicobacter* associated gastritis, its diagnosis and treatment in dogs [16, 17]. As per the name (rapid urease test), results are immediate (<5 min to 2 hr, max), allowing confident treatment and better chances of recovery in the patient. These findings confirmed the evidences obtained by previous authors [18, 19], who also opined that the test is inexpensive and easy to carryout.

Conclusion

This rapid urease test can be applied to obtain a presumptive diagnosis of *Helicobacter pilori* infection in cases of suspected chronic gastritis and gastroesophageal ulceration in dogs. At the same time it is most important to remember and reiterate that the test will also detect other urease-producing bacteria.

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