Detection of Gastric *Helicobacter* Species from Broiler Chickens by Rapid Urease Test and Brush Cytology

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Abstract

Laboratory diagnosis of Helicobacter species is problematic. A cross-sectional study was conducted to detect Helicobacter species in proventriculus of chickens sold at different meat stalls in Catarman, Northern Samar and assess the degree of agreement between rapid urease test (RUT) and brush cytology. A total of 30 dressed chickens were randomly collected to obtain the proventriculus. The gastric mucosa was subjected to both RUT and brush cytology. Positive percent agreement to the two tests was 48% while the negative percent agreement was 100%. No visible lesions were observed in the gastric mucosa. Results suggest that the RUT is a more appropriate screening test for gastric helicobacters. The presence of gastric Helicobacter species could be a potential hazard to public health.

Key words: *Brush cytology, chickens; Helicobacter spp., proventriculus, Philippines; rapid urease test*

Introduction

The *Helicobacter* species is a group of gram-negative, slightly curved, spiral, slender rods and microaerophilic bacteria that belong to Order Campylobacterales, Family Helicobacteraceae, many of which cause disease in humans (Fox, 2002; Goldstein, 2003). It is found in the gastrointestinal and biliary tracts of mammals and birds (Fox and Lee, 1997). Gastric *Helicobacter* species are widely distributed in mammals and in many cases, cause an inflammatory response similar to that seen with *Helicobacter pylori* in humans. *H. pylori* is a urease-producing, gastric pathogen implicated in a wide spectrum of chronic gastrointestinal orders in humans such as gastric and peptic ulcers, chronic gastritis, duodenitis, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric carcinoma (Boyanova, 2011; Ramis *et al.*, 2012). It is the second most common cause of cancer morbidity and mortality worldwide, and gastric non-Hodgkins lymphoma (Personnet *et al.*, 1994; Zucca *et al.*, 1998). Gastric *Helicobacter* species from food animals, including *H. pylori* can be transmitted to humans to cause infections.

Diagnosis of *Helicobacter* species using routine methods can be difficult. It includes rapid urease test (RUT), brush cytology, histopathology, and polymerase chain reaction (PCR) assay. RUT is highly specific

for *H. pylori* and is commonly used for the detection of *H. pylori* infection at endoscopy (Ramis et al., 2012).

There are only a few studies on *Helicobacter* species in animals in the Philippines. Using urease test and brush cytology, Ronato, n.d. and Lagrimas, n.d. reported the presence of *Helicobacter species* in the fundic and pyloric regions of the stomach of pigs and the abomasum of cattle, respectively. Camer *et al.* (2005) employed three tests, namely RUT, cytological examination and PCR assay to detect *H. pylori* in the stomach of pigs in Northern Samar. To the authors' knowledge, this is the first published report of gastric *Helicobacter* species in broiler chickens in the Philippines. In the present study, *Helicobacter* species was detected using two different tests and the level of agreement between the two tests was assessed for *Helicobacter* diagnosis.

Materials And Methods

Sample Collection and Processing

A total of 30 broiler chickens were randomly selected to collect the proventriculus from various meat stalls in a wet market in Catarman, Northern Samar. The proventriculus was packed individually into sterile plastic bags for transport on ice to the Microbiology laboratory of the College of Veterinary Medicine, University of Eastern Philippines, Catarman, Northern Samar and examined within 4 h after sampling. The proventriculus was opened and washed with tap water to expose the gastric regions. Samples were examined macroscopically for abnormal changes and lesions prior to collection of mucus for RUT and brush cytology.

Laboratory Tests

For the RUT, gastric mucosal samples 0.25 mm in size, were taken using punch biopsy instrument from each of the three sites of the proventriculus; namely, cardiac, fundic and pyloric regions. The samples were embedded into commercially prepared urea agar slant and incubated for 8- 48 h at 37°C. A positive RUT was indicated by a color change from light orange to bright pink. In addition, a sterile applicator stick was rolled over each region of the proventriculus to collect the gastric mucus. Gastric brushings were immediately smeared onto clean microscope glass slides, stained with Giemsa and Gram and then examined microscopically under the oil immersion objective for the presence of curved, spiral bacterium.

Data Analysis

The Chi square test was performed to compare difference in proportions. The two tests were compared by calculating positive percent agreement, negative percent agreement and the overall percent agreement. The 95% confidence intervals (CI) for pairwise comparison of the two tests were performed using an online calculator (http://www.Graphpad.com / quickcales/contingency 2.cfm.).

Results

Gross Pathology

No gross pathologic lesions were seen in the proventriculus of all sampled chickens.

Laboratory Tests

The percentage distribution of *Helicobacter*-positive samples by RUT and cytological examination from three different sampling sites in the proventriculus of chickens is shown in Table 1. For either test, there was no difference in the percentage positive samples among the sampling sites ($P > 0.05$). The Cytologic examination revealed the characteristic 2-4 µm slightly curved, slender bacilli embedded in mucus in one of the samples taken from the pyloric region (Figure 1).

Table 1. Percentage Distribution of Positive Samples By Rapid Urease Test and Brush Cytology According to Sampling Site in the Proventriculus

	Rapid Urease Test	Brush Cytology
Parts of Proventriculus	*n*=30	*n*=30
Cardiac	83%	40%
Fundic	80%	46%
Pyloric	80%	43%
Total	**81%**	**40%**

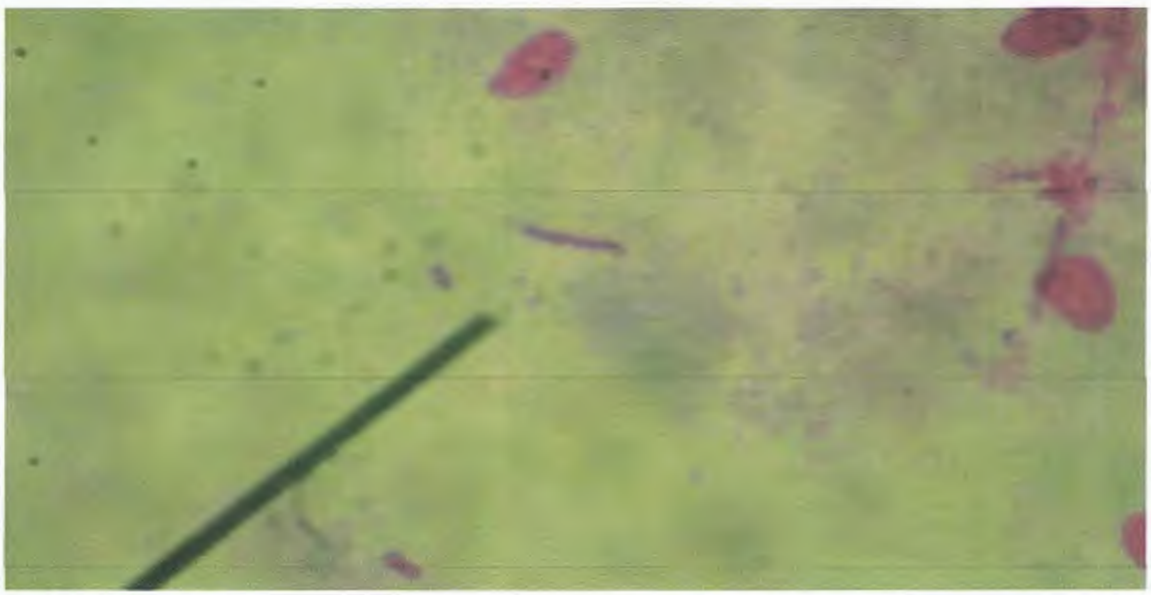

Figure 1. Giemsa-positive stain of gastric mucus positive for slightly curved, slender rods suggestive of *Helicobacter* (1000X).

Comparison between RUT and Brush Cytology

Using overall predictive agreement as a measure, the brush cytology shows less than 58% agreement with RUT (Table 2). Positive percent agreement and negative percent agreement were 48% (95% CI: 0.30, 0.66) and 100% (95% CI: 0.51, 1), respectively.

Table 2. Paired Comparison of Results from Two Different Tests

	Rapid urease test		
Brush Cytology	Positive	Negative	Total
Positive	12	0	12
Negative	13	5	18
Total	25	5	30
Positive percent agreement : 48% (95% CI:0.30, 0.66)			
Negative percent agreement: 100% (95% CI: 0.51, 1)			
Overall percent agreement: 58%			

Discussion

The association of *Helicobacter* with gastric disorders in humans is becoming a global health problem (Malaty, 2010). A wide range of these organisms had been isolated and recognized in humans and animals. Transmission of the disease, although unclear, is through oral-oral route, fecal-oral route, and so on (Kist *et al.*, 2005). The occurrence of *Helicobacter* species in food animals such as broiler chickens, therefore, cannot be overlooked.

No gross gastric pathology in chickens was observed in the present study. In humans, helicobacters colonizing the gastric tissue is nearly always associated with inflammation that may develop into more severe gastric diseases (Kist *et al.*, 2005; Kusters *et al.*, 2006). It is possible that the chronic, persistent *Helicobacter* infection seen in humans does not occur in chickens because they are killed far short of their potential life span.

Comparing the results from the two tests without knowing the true prevalence is difficult. No validated gold standard exists for the detection of *Helicobacter* species (Saurabh *et al.*, 2014). Each test has its own advantages and drawbacks. The RUT is a simple and cost-effective screening method that can provide reliable results in relatively short time (Hazell *et al.*, 1987; Megraud and Lehours, 2007). It indirectly determines the presence of helicobacters based on the detection of urease in the gastric muchosa. All known gastric *Helicobacter* species expresses urease that allows their short-term protection from the action of gastric acid (Yoshiyama and Nakazawa, 2000; Solnick and Schauer, 2001). Brush cytology, on the other hand, yields results that are faster than the RUT; results are within minutes compared to a minimum of 24 h for the RUT (Ramis *et al.*, 2012). It is an inexpensive and easy-to-use

screening test for rapid detection of *Helicobacter* infection (Lee, 2005). According to Mostaghni *et al.* (2008), brush cytology can be performed when a RUT returns a negative result.

Based on the results of the study, the RUT is likely the more appropriate test than cytological examination for the diagnosis of gastric helicobacters. However, because of the possibility that other urease-producing bacteria are present in the proventriculus (Megraud and Lehours, 2007), the test result should be interpreted carefully. Moreover, the use of antimicrobial agents and bismuth-containing compounds can result in false negatives (Datta *et al.,* 2005). Additionally, RUT is affected by the intensity of inflammation (Datta *et al.,* 2005). As RUT can miss a low-level infection, a negative test should not be the sole criterion for absence of *Helicobacter* (Schnell *et al.*, 1998).

In the detection of *H. pylori* in humans, the antrum and body of the stomach have been found to yield more positive results in the RUT (Bermejo *et al.* 2002). This was not observed in the present study, suggesting that sample collection for diagnosis can be done at any site of the proventriculus.

Conclusion

The results of the study showed the presence of Gram-negative urease-positive spiral organism highly suggestive of *Helicobacter* species and that the bacteria are relatively common in the broiler chickens used in the study. The consumption of *Helicobacter*-positive chickens presents a potential risk to the health of the human population. RUT could be a more appropriate test than brush cytology as a screening test for gastric helicobacters. The Giemsa-stained gastric brushings are a useful complement to RUTs. For future studies, then, the use of PCR to detect *Helicobacter* species is recommended.

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