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Diagnostic Performance for Detection of *Helicobacter Pylori* Infection in Gastric Biopsy Specimens with No Gold Test: Non-linear Regression Approach

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Abstract : The selection of a test as a reference with no perfect sensitivity and specificity may lead to bias, yielding distortion of the diagnostic performance. This means it is inappropriate to use imperfect diagnostic tests as a reference method to identify infected patients in clinical environments. In this study, diagnostic performance of rapid urease test, polymerase chain reaction (PCR), and histology of gastric biopsy specimens for diagnosing *Helicobacter pylori* infection separately and in combination was estimated by using non-linear regression. Based on this approach, the sensitivity, specificity and likelihood ratio positive and negative values for each test were as follows: urease test 99.9%, 99.9%, 99.9%, 99.6%, respectively; PCR 88.6%, 99.9%, 99.9%, 70.5%, respectively; histology 78.3%, 97%, 78.3%, 97%, respectively. Predictive values for positive and negative changes with varying prevalences. A positive histology test will be correct at least 90% of the time provided prevalence of *H. pylori* is greater than 35%, whereas a negative test will be correct in more than 90% if the prevalence is less than 25%. Combination of three diagnostic tests employed in the study gives no substantial benefit for practitioners to screen infected patients, and urease test or PCR represents an appropriate single test in clinical environments.

Key words **:** *Helicobacter pylori*, Gold standard, Diagnostic performance

Introduction

Since the first isolation of *Helicobacter pylori* from human beings, a number of Helicobacter species have been identified in domestic and laboratory animals[3,8,10,16,21-23]. The accurate detection of the organism is essential for proper patient management[1], and particularly for the eradication of the bacteria following treatment, although the clinical importance of the *H. pylori* infection in veterinary patients has not been studied well.

The most problematic point for diagnosing *H. pylori* infection is that no single test provides the definitive diagnosis by itself. Culture is specific but it is less sensitive than histology[5]. Some researchers have questioned the use of histology as a gold standard for *H. pylori* detection[18]. Sensitivity and specificity of histological examination, bacterial culture or rapid urease testing are reported to be varied depending on the prevalence of the disease in the population, clinical characteristics of the patient profile, misinterpretation or to the low number of bacteria in each site of gastric mucosa[2,7,12,14,15,27]. Due to this lack of a gold standard, in many studies diagnostic characteristics of culture and histology were assessed with other diagnostic tests as reference methods[4,13,25,26,28]. The selection of test used as a reference may lead to bias.[24] One way avoiding this problem is to use a combination of tests, i.e., without singling out any specific test as the reference method. However, this method also causes bias, because test results are not independent of the reference method that is chosen.[11] Such bias usually leads to distortion of the diagnostic performance. Therefore it is inappropriate to use other imperfect diagnostic tests as a reference method to measure diagnostic performance.

In the present study the authors evaluated diagnostic performance of bacterial culture, histological examination, and polymerase chain reaction (PCR), as a single test and in combination, of gastric biopsies without using a reference standard for diagnosing *H. pylori* infection in canine patients.

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Materials and Methods

Study population and diagnostic procedures

The study population and diagnostic procedures included in the present study were reported in the previous edition of this journal[20]. Briefly, the study population consisted of consecutive patients undergoing routine endoscopy at the three local hospitals, Colorado, USA. About half of the subjects (52.9%) were from sample of the outpatient and the others were selected from the in-patient. They were all did not associated with gastric illness. The median age of dogs was 2.3 years, ranging from 1.5 to 3.2 years. The presence of absence of gastric *Helicobacter spp.* was ascertained by evaluating gastric biopsies for urease activity, histology, and PCR. The detailed test procedures were described elsewhere[19,20].

Likelihood ratio

To express test characteristics in terms of the probability

of patients having a *H. pylori* infection and one that does not vary with the prevalence in the population, the likelihood ratios (LR) were derived from sensitivity and specificity according to the formulae: LR^+ = sensitivity/(1-specificity) and LR^- = (1-sensitivity)/specificity.

The odds are similar to the probability when the event is rare but as the event becomes more common it is necessary to switch back and forth between probabilities and odds. The pre-test odds for *H. pylori* infection was calculated to be unity, as follows: pre-test odds = prevalence of infection/(1-prevalence). The post-diagnostic test odds was obtained by multiplying the pre-test odds by the LR. Finally, the post-test probability of infection was obtained by dividing the post-test odds by (1+post-test odds).

Non-linear regression approach

Estimation of the sensitivity and specificity of bacterial culture, histological examination, and rapid urease testing was estimated from a solution of 8 non-linear equations with 7 unknowns. Assuming λ for true prevalence of *H. pylori* infected patients, α for the false positive rate of the test (1-α = specificity), and β for the false negative rate of the test (1-β = sensitivity), the mathematical expression to calculate the probability of a positive test result (P^+) for one diagnostic test can be derived using the equation: $P^+ = \lambda(1-\beta)+(1-\lambda)\ \alpha$. The seven parameters ($\lambda$, α_1, β_1, α_2, β_2, α_3, β_3) for the present study were estimated by putting the probability equal to the measured number of tests divided by the total number of tests (observed probability) for each combination of test results, using only seven combinations of test result because the sum of probabilities is always one. For a numerical solution, the sum of squares of the differences between the probabilities was minimized.

The NLIN (non-linear regression) procedures from the statistical package SAS (SAS Inst., Cary NC) was used to calculate the mathematical solution of the equations. As the model has two solutions, we assumed that all alpha and betas were between 0.0 and 0.5.

Results

Based on the non-linear regression, the sensitivity, specificity and likelihood ratio positive and negative values for each test were as follows: urease test 0.999, 0.999, 999.0, 0.001, respectively; PCR 0.886, 0.999, 999.0, 0.705, respectively; histology 0.783, 0.970, 26.1, 0.224, respectively (Table 1).

Predictive values for positive and negative were 99.9% and 99.6% for urease test, 99.9% and 70.5% for PCR and 78.3% and 97.0% for histology (Table 1). Predictive values for positive and negative changes with varying prevalences. The PPV and NPV was 100% and 100% for urease test, 100% and 85% for PCR, and 98% and 75% for histology, respectively at the prevalence of 60%, whereas this changes to 100% and 100% for urease test, 100% and 95%, and 92% and 91% for histology, respectively at the prevalence of 30% (Table 2).

A positive histology test will be correct at least 90% of the time provided prevalence of *H. pylori* is >35% (Fig 1), whereas a negative test will be correct in more than 90% if the prevalence is <25% (Fig 2).

Table 1. Sensitivity, specificity, predictive values for positive (PPV) and negative (NPV), likelihood ratio positive (LR^+), and likelihood ratio negative (LR^-) for urease test, PCR and histology as a single test, and in combination

Tests	Sensitivity (PPV)	Specificity (NPV)	LR^+	LR^-
Single test				
Urease test	0.999	0.999	999.0	0.001
	(0.999)	(0.996)		
PCR	0.886	0.999	999.0	0.705
	(0.999)	(0.705)		
Histology	0.783	0.970	26.1	0.224
	(0.940)	(0.820)		
		Parallel testing		
Urease test or histology	0.999	0.969	32.226	0.001
Urease test or PCR	0.999	0.998	499.5	0.001
PCR or Histology	0.975	0.969	31.452	0.026
		Serial testing		
Urease test and histology	0.783	0.999	783.0	0.217
Urease test and PCR	0.886	0.999	886.0	0.114
PCR and Histology	0.694	0.999	694.0	0.306

Table 2. Variations in positive predictive value (PPV, %) and negative predictive value (NPV, %) with prevalence of disease for a test performance obtained from Table 1

Prevalence (%)	Urease test		PCR		Histology	
	PPV	NPV	PPV	NPV	PPV	NPV
30	100	100	100	95	92	91
40	100	100	100	93	95	87
50	100	100	100	90	96	82
60	100	100	100	85	98	75
70	100	100	100	79	98	66
80	100	100	100	69	99	53

Discussion and Conclusion

In the present study the concepts of LR was employed in assessing diagnostic performance of several independent tests. LRs are a more clinically relevant method of expressing

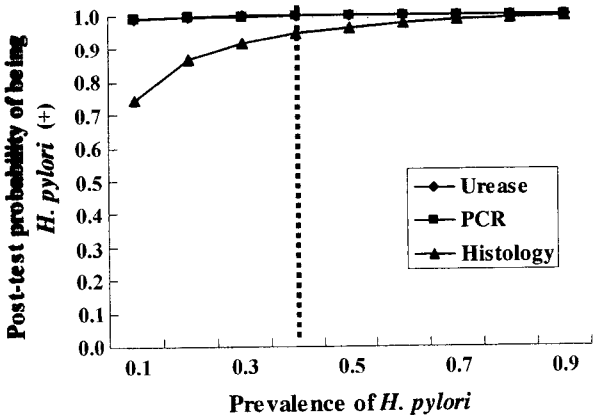

Fig 1. The value of a positive test with varying prevalence of *H. pylori*.

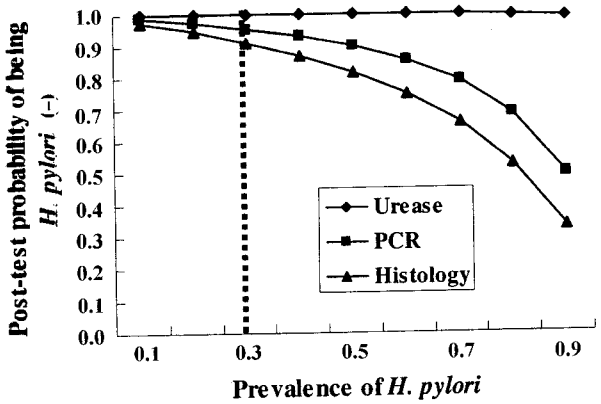

Fig 2. The value of a negative test with varying prevalence of *H pylori*.

the accuracy of an investigation, and yet they are not widely quotec in the veterinary medical literature. The reason for this is that clinicians are usually used to dealing with probabilities, whereas LRs express result in terms of odds. The odds are the probability of an event occurring divided by the probability that it will not occur. A simple nomogram is available that obviates the need to perform any calculations[6].

There have been many invasive and non-invasive methods for the detection of *H. pylori*. Although some of the invasive methods are still accepted for the primary diagnosis in symptomatic patients, noninvasive methods are much easier to perform. The most accurate method for detecting *H. pylori* in tissue is a combination of culture and histologic staining of mucosal biopsy specimens obtained by endoscopy[2,13,27]. When several diagnostic tests for detecting *H. pylori* results give conflicting results the LR can be applied to obtain final probability of infection. For example, if a patient has a positive PCR, a negative urease test and a positive histology, the probability of infection could be calculated as follows: pre-test odds = 1; after a positive PCR, post-test odds = 999 × 1 = 999 and probability of infection = 999/1000 = 99.9% (Table 1); a negative urease test will decrease the odds of infection to 999 × 0.001 = 0.999 and the probability to 0.999/1.999 = 49.98%; finally, a positive histology will increase the odds of *H. pylori* infection to 0.999 × 26.1 = 26.1 and the final probability of infection 26.1/27.1 = 96.3%.

Despite the frequent occurrence of *Helicobacter spp.* in companion animals[9,17,22] with either naturally occurring or experimentally induced, there have been few studies assessing the clinical utility of the diagnostic tests used for detecting *H. pylori* infection, all of which are adaptations of methods used to diagnose human infection; in some of them the organisms were detected histologically, urease tests and electron microscopy. This lack of information makes difficult direct comparison of the results between present study and literatures. Smythies *et al*[21] reported diagnostic effectiveness of culture, histopathology and PCR assays using mouse model experimentally induced. The sensitivity, reported by the authors ranged 15-58% with a mean of 38% for culture, 61-74% with a mean of 66% for histopathology, and 76-89% with a man of 82% for PCR, and all tests were equally specific with 100%. Diagnostic performance of both histopathology and PCR from Smythiess report is quite similar to the present study, although sensitivities tend to lower than this study: 66% vs 78.3% for histology (two-tailed z-test = 1.69; $P < 0.05$) and 82% vs 88.6% for PCR (two-tailed z-test = 0.17; $P > 0.05$). These comparisons were consistent with Happonen *et al*[10] comparing several diagnostic tests using tissue samples in dogs: for urease test, 85.7-87.5% of sensitivity and 100% specificity, and for histopathology, 92.3% of sensitivity and 100% specificity. Direct comparison between this study and Happonens findings is unrewarding owing to different sampling sites (ie, fundus, corpus, antrum of the stomach). In general, however, the sensitivity of urease test in the present study was a bit higher, while that of histopathology was a bit lower. These small discrepancies among studies may be due in part to the difference in experimental designs including different study population and small sample size with different patient profile and as well as genetic differences in host susceptibility to infection or colonization and *H. pylori* isolate used.

In conclusion, although histology can be the method of choice for detecting the organism, this method may not be appropriate screening test in areas of very low *H. pylori* prevalence. The urease test and PCR were also of diagnostic value. Based on the results of post-test probability with varying prevalence of *H. pylori* infection, rapid urease test can be used for general practitioners for initial screening purpose. Combined use of three diagnostic tests employed in the present study seems to give no beneficial at the expense of decreasing performances of the test. Further comprehensive studies are still needed to evaluate the clinical significance of

different diagnostic methods for detecting gastric *H. pylori*-infected individuals.

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위 조직 생검 시료의 *Helicobacter pylori* 균 검출에 사용되는 진단검사의 특성을 추정하기 위한 비선형 모형의 응용

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요 약 : 감염된 환축을 찾는 진단과정에 완벽하지 못한 진단검사를 사용하는 경우 진단검사 결과는 흔히 왜곡되어 나타난다. 본 연구에서 저자는 *Helicobacter pylori* 감염을 진단하는데 사용되는 urease 검사, PCR 검사 및 조직학적인 검사법을 단독으로 사용하는 경우와 병행하여 사용하는 상황으로 구분하여 각각 진단적 특성을 평가하였다. 비선형 회귀모형 분석결과 민감도, 특이도, 양성우도비 및 음성우도비는 urease 검사법의 경우 99.9%, 99.9%, 99.9%, 99.6%, PCR 검사의 경우 88.6%, 99.9%, 99.9%, 70.5%, 조직검사법의 경우 78.3%, 97%, 78.3%, 97%로 나타났다. 예측도는 유병율의 변화에 따라 다양한 값을 보였으며 *Helicobacter pylori* 감염의 유병율이 35% 이상일 때 조직 검사상 양성결과는 90% 이상의 일치도를 보였고, 유병율이 25% 미만일 때 조직 검사상 음성결과는 90% 이상의 일치도를 보였다. 본 연구결과 임상에서 감염된 개체를 스크리닝하는 목적으로 세가지 진단검사를 병행하는 것은 실질적인 이익이 없으며 단독검사로서 urease 검사와 PCR 검사가 가장 적합한 것으로 나타났다.

주요어 : *Helicobacter pylori*, 진단검사, 정확도, 비선형회귀모형