Diagnostic Performance of Plasma Gastrin Concentration for the Diagnosis of Type 1 Abomasal Ulcer in Water Buffalo: A Preliminary Case Control Study

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Short Report

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Abstract

The type 1 abomasal ulcer (AU1) does not have specific clinical signs, and there is a need to identify some early biochemical markers for diagnosis of AU1 in cattle and buffaloes. Plasma gastrin is reported to reflect the gastric mucosa damage but its utility for diagnosis of AU1 in buffaloes has not been evaluated. The objective of this study was to study the test performance of plasma gastrin to distinguish healthy buffaloes and buffaloes with AU1. Twenty-three buffaloes with AU1 and six buffaloes without abomasal ulcer were used. Blood samples were collected from the buffaloes, slaughtered in a buffalo specific slaughter house, for estimation of plasma gastrin. After slaughter abomasa were examined for presence of AU1 and were confirmed by histology. The mean plasma gastrin concentration of ulcer positive buffaloes was significantly ($p<0.05$) higher than the ulcer negative buffaloes. The ROC curve analysis suggested optimal value of plasma gastrin for diagnosis of AU1 was 106.2 pg/ml. Since the abomasal ulcer negative animals were established to be ulcer negative on histopathological examination we consider the values of gastrin valid for detection of abomasal ulceration in buffaloes. The sensitivity, specificity, positive predictive value and negative predictive value of plasma gastrin to diagnose AU1 in buffalo were 78.3, 100, 100 and 69.9, respectively.

Introduction

Abomasal ulcers occur in several forms and produce different clinical signs (Smith et al. 1983). Type 1 abomasal ulcer (AU1) is a non-perforated ulcer of the abomasal mucosa associated with minimal haemorrhage and non-specific clinical signs (Braun 2020). The currently available literature advocates the use of faecal occult blood test for the diagnosis of type 1 abomasal ulcers in both cattle and buffaloes (Hussain et al. 2015). Unlike humans, the use of gastroscopy for visualization of the gastric mucosa in bovids it is difficult and has not been attempted so far. Due to high prevalence of type 1 abomasal ulcer (AU1) in water buffaloes (Hussain et al. 2019; Tajik et al. 2012), there is need to identify some biomarker for diagnosis and management of abomasal ulcer in buffalo.

Gastrin is secreted from gastrin cells of pyloric region of abomasum into the blood circulation, reaches the parietal cells and is an important stimulator of acid and pepsinogen secretion (Argenzio 2005). The plasma gastrin is reported to reflect the damage to abomasal mucosa especially due to parasites diseases like haemonchus and Ostertagia (Fox et al. 1993; Kataria et al. 2008) but its utility for diagnosis of AU1 in bovids has not been evaluated. This case control study was designed to investigate plasma gastrin in ulcer negative and ulcer positive buffaloes and to evaluate the possible role of plasma gastrin in identification of abomasal mucosa damage due to AU1 in buffalo.

Materials And Methods

The study protocol was approved by the Institutional Animal Ethics Committee of Guru Angad Dev Veterinary and Animal Sciences University. This study was conducted on blood samples collected from a mixed population of buffaloes, slaughtered in a buffalo specific abattoir. The buffaloes were selected

randomly as has been discussed in our published findings (Hussain et al. 2019). Plasma was separated with centrifugation and frozen at -20°C until analysis. The abomasum examined for presence or absence of ulcers and the ulcers were confirmed by histopathological examination, and the findings are discussed in detail (Hussain et al. 2019). Out of the 134 examined abomasa, 89 had type 1 abomasal ulcers (AU1) and 45 were ulcer negative. For this study 30 plasma samples were used, representing 24 samples of AU1 and seven ulcer negative animals. The plasma samples from each group were selected randomly using lottery method of randomization. The plasma gastrin was analyzed by IMMULITE 1000 Immunoassay System (Siemens Healthineers, Germany) by using Immulite® Gastrin Kit. The procedure for gastrin estimation was standardized during the preliminary study of the PhD dissertation of the first author. The procedure is fully automated except the sample loading in the sample cups. In brief the principle of the procedure is: IMMUITE/IMMULITE 1000 gastrin is a chemiluminescent, enzyme labeled immunometric assay based on ligand labeled murine monoclonal capture antibody specific for gastrin and separation by anti-ligand coated solid phase. The sample along with the ligand labeled anti gastrin monoclonal antibody, an alkaline phosphatase-conjugated rabbit polyclonal antigastrin antibody and an alkaline phosphatase-conjugated murine antibody are simultaneously incubated in presence of the immobilized anti-ligand bead within an IMMULITE Test Unit. During the 60 minute incubation, gastrin molecule in the sample form antibody sandwich complexes which in turn, bind to anti-ligand on the solid phase. Unbound conjugate is then removed by a centrifugal wash, after which luminogenic substrate is added and the Test unit is incubated for a further ten minutes. The chemiluminescent substrate, a phosphate ester of adamantly dioxetane, undergoes hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate. The continuous production of these intermediate results in the sustained emission of light. The bound output, as measured by the luminometer is proportional to concentration of gastrin in the sample. Incubation cycle was of 60 minutes.

Statistical analysis

Data were analyzed using SPSS for Windows (Version19.0, SPSS Inc. 83 Munich, Germany) and MedCalc (Version 19.6.1, MedCalc Software, Free trial). Normal distribution was tested by Shapiro-Wilk's test and visual examination of the histograms, normal Q-Q plots and box plots. Two gastrin values (one each of the ulcer negative and ulcer positive group) were outliers, as indicated by box plots, so these two values were not used in the subsequent statistical analysis. Unpaired t-test was used for comparison of the plasma gastrin concentration between the ulcer negative and the ulcer positive buffaloes.

The sensitivity, specificity, positive predictive value and negative predictive value were calculated with a receiver operating characteristic (ROC) curve analysis using MedCalc. The continuous variable was plasma gastrin and the classification variable was the presence or absence of type 1 abomasal ulcer. The 95% confidence interval was calculated for all the test characteristics. The predictive values calculated were based on the apparent prevalence (89/134 = 66.42%) of AU1 in the present study population. The optimal test criterion was determined using the Youden index giving equal weight to the sensitivity and specificity.

Results

The Shapiro-wilk's test ($p > 0.05$), visual examination histograms, normal Q-Q plots and box plots showed that the plasma gastrin concentrations were approximately normally distributed for both the ulcer negative and ulcer positive buffaloes. The prevalence of AU1 was 66.42% and the results are discussed in detail in our already published research findings (Hussain et al. 2019). The plasma gastrin concentrations of the ulcer negative and the ulcer positive buffaloes (Fig. 1) are presented in Table 1. The mean plasma gastrin concentration of the ulcer negative buffaloes was significantly ($p < 0.05$) lower than the ulcer positive buffaloes.

Table 1
Plasma gastrin concentration in buffaloes with or without type 1 abomasal ulcer

	Plasma gastrin concentration (pg/ml)		
	N	Mean ± S.E	Minimum, maximum
Ulcer negative	6	91.43 ± 4.79	76.6, 106.2
Ulcer positive	23	148.59 ± 8.52**	98.6, 248.9
**differ significantly from ulcer negative group at p < 0.05*			

For the evaluation of a predictive test, information on test performance needs to be available (i.e. sensitivity, specificity, positive and negative predictive values). The ROC analysis allows the calculation of sensitivity and specificity for different thresholds and plots sensitivity on the y-axis against 100-specificity on the x-axis. The closer the ROC curve is to the upper left corner, the greater the accuracy of differentiation between buffaloes with and without AU1. The area under the ROC curve (AUC) is greater when the ROC curve is closer to the upper left corner. If the test could distinguish perfectly between buffaloes with and without AU1, the AUC would be 1 whereas an AUC of 0.5 demonstrates that the test cannot distinguish between the two groups at all. Moreover, an AUC of 0.80 means that in 80% of cases a buffalo suffering from AU1 will have greater plasma gastrin than a buffalo without AU1 (Zweig and Campbell, 1993). An AUC between 0.5 and 0.7 means that the test distinguishes between two groups less accurately. An AUC between 0.7 and 0.9 indicates a moderately accurate and > 0.9 a highly accurate test (Greiner et al. 2000).

Figure 2 *illustrates the Receiver operating characteristic (ROC) curve of gastrin for diagnosis of AU1 in buffaloes. The area under the ROC curve (AUC) was 0.942 with standard error of 0.045.*The green line represents the null hypothesis i.e the curve that has absolutely no value or represents the test with zero sensitivity and zero specificity. The curve related to our data is the blue line and the curve was statistically significant ($p < 0.001$).

Table 2 *presents test performance of plasma gastrin to diagnose AU1 in buffaloes, for the optimal threshold determined with the Youden index.* As suggested by the AUC and the corresponding P-value,

plasma gastrin had the ability todistinguish between the healthy buffaloes and the buffaloes with AU1 and was highly accurate test (AUC > 0.9).

Table 2
Test performance of the plasma gastrin concentration to diagnose type 1 abomasal ulcer in buffaloes

Plasma gastrin concentration (pg/ml)	Test performance (95% Confidence interval)				
	Sensitivity	Specificity	Positive predictive value	Negative predictive value	AUC
> 106.2	78.26 (56.3–92.5)	100 (54.1–100)	100 (-)	69.9 (51.7–83.5)	0.942 (0.79–0.995)*
*P < 0.001					

Discussion

The present case control study was conducted on buffaloes with confirmed diagnosis of AU1 along with a control group. The changes in plasma gastrin concentration are reported to be associated with neoplastic and ulcerogenic gastric diseases in cattle (Smith et al. 1983) and have been studied in cattle and sheep with parasitic diseases, bleeding abomasal ulcers and abomasal dysfunction (Fox et al. 1993; Ok et al. 2001; Kataria et al. 2008). However, plasma gastrin levels in cattle or buffaloes with AU1 have not been evaluated so far. *In fact, plasma gastrin concentration has not been previously evaluated in water buffalo. This is the first study to document the relationship between the plasma gastrin concentration and the abomasal disease in buffaloes.* There was a significant difference in the plasma gastrin between the healthy buffaloes and the buffaloes with AU1. The results of the present could not be compared to previous studies due to lack of available literature in the buffaloes. However, the values could be compared to the studies conducted on cattle and sheep. The plasma gastrin has been evaluated in haemonchus affected sheep and reported to be 489.61 ± 12.23 pg/ml (Kataria et al. 2008). The mean plasma gastrin concentration in healthy cattle (103.2 pg/ml) and sheep (103.45 ± 10.41pg/ml) is reported to be similar to our suggested cutoff value in buffaloes (Ok et al. 2001; Kataria et al. 2008).

This is the first study where a ROC-analysis was performed to determine the optimal thresholds of the plasma gastrin for the diagnosis of AU1 in buffaloes. The ROC- analysis helped to determine the optimal threshold of plasma gastrin with the Youden index. As this study aimed to suggest a diagnostic test for AU1 in the buffaloes, it was decided to include the buffaloes with best available definition of AU1 (Braun et al. 1991; Hussain et al. 2019). Both the ulcer positive and ulcer negative animals were established to be so on histopathological examination we consider the thresh hold value of the plasma gastrin to be valid for detection of AU1 in the buffaloes.

With regard to test performance it is evident that the *plasma gastrinconcentration > 106.2 pg/ml was the most acceptable combination of sensitivity and specificity. The AUC value of 0.942 indicated that plasma*

gastrin was a highly accurate test to distinguish between AU1 positive and negative buffaloes (Greiner et al. 2000) and *justifies its use for the diagnosis of AU1 in clinical setting*. In sheep and cattle, it is reported that the plasma gastrin concentration of about 103 pg/ml is not associated with any significant damage to the abomasal mucosa, and mean values of 489.61 ± 12.23 pg/ml and 213.6 pg/ml have been observed in haemonchosis and bleeding abomasal ulcers, respectively (Ok et al. 2001; Kataria et al. 2008)

This preliminary study, the affected abomasa were not grouped on the basis of age or parity of the buffaloes, as was done in the previous prevalence study (Hussain et al. 2019). Also, the correlation between the plasma gastrin concentration and the number of ulcers in the abomasum was not evaluated in this study. Further, the plasma gastrin dynamics was not evaluated with respect to severity of type 1 abomasal ulcers.

Conclusions

This is the first study that determined the test characteristics of the plasma gastrin to diagnose the AU1 in buffaloes. The overall test performance of the plasma gastrin was highly accurate. So, we suggest that plasma gastrin can be used as diagnostic markers for abomasal mucosa damage due to type 1 abomasal ulcers in buffaloes. However, the information on the effect of number and severity of abomasal ulcers on plasma gastrin concentration is still not available and requires further studies.

Declarations

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Compliance with ethical standards

This study is a part of PhD dissertation of the first author and the ethical clearance was granted by Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India, vide no. VMC/13/2187-2215, dated 02/05/2013.

Conflict of interest: The authors declare that there is no conflict of interest to publish this manuscript.

Availability of data: All the data are present in the manuscript. However, the rough data may be provided on request.

Code availability: Not applicable

Consent to participate: All the authors consented to participate in this study.

Consent for publication: All the authors consent to publication of this article.h.

Author Contributions: SKU and NKS conceived the research. SKU and SAH designed the research. SAH and NKS collected the samples and did laboratory evaluation. SAH wrote the manuscript. SAH and SKU analyzed data and critical reviewed the manuscript. All authors read and approved the manuscript.

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Figures

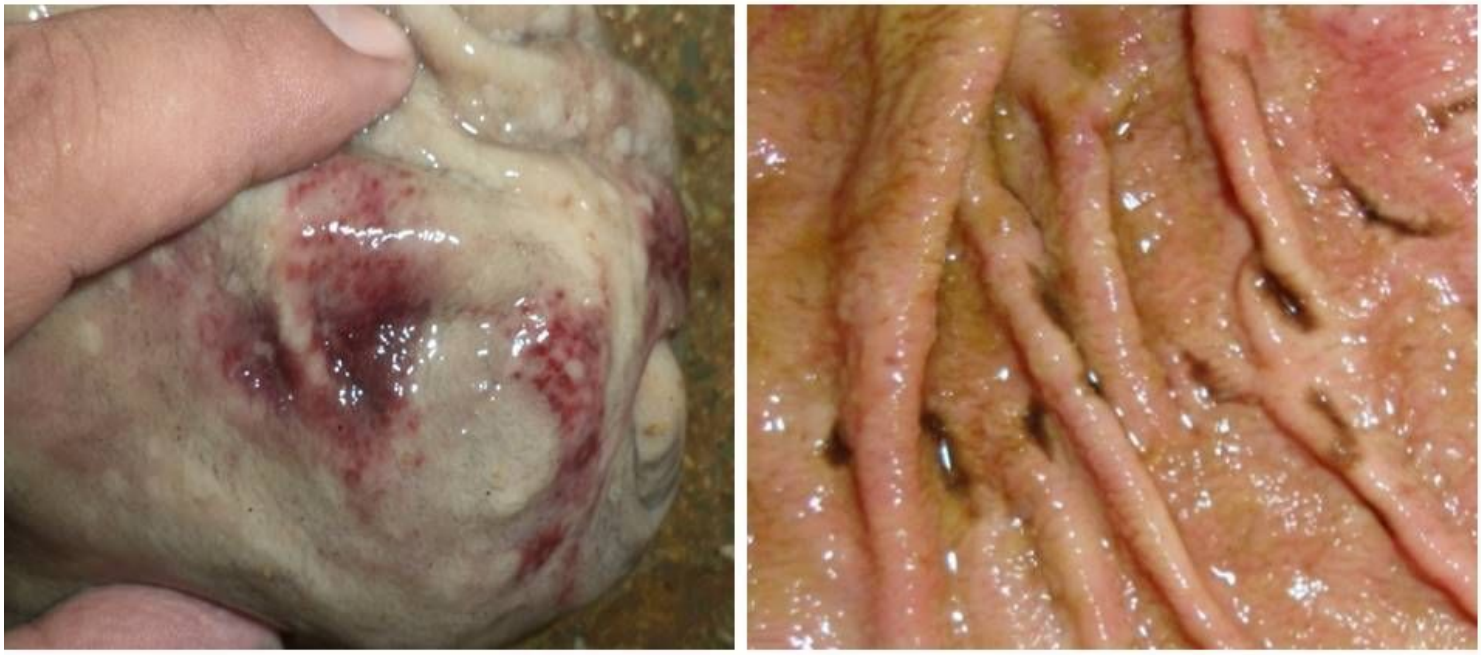

Figure 1

Gross appearance of different degrees of type 1 abomasal ulcers

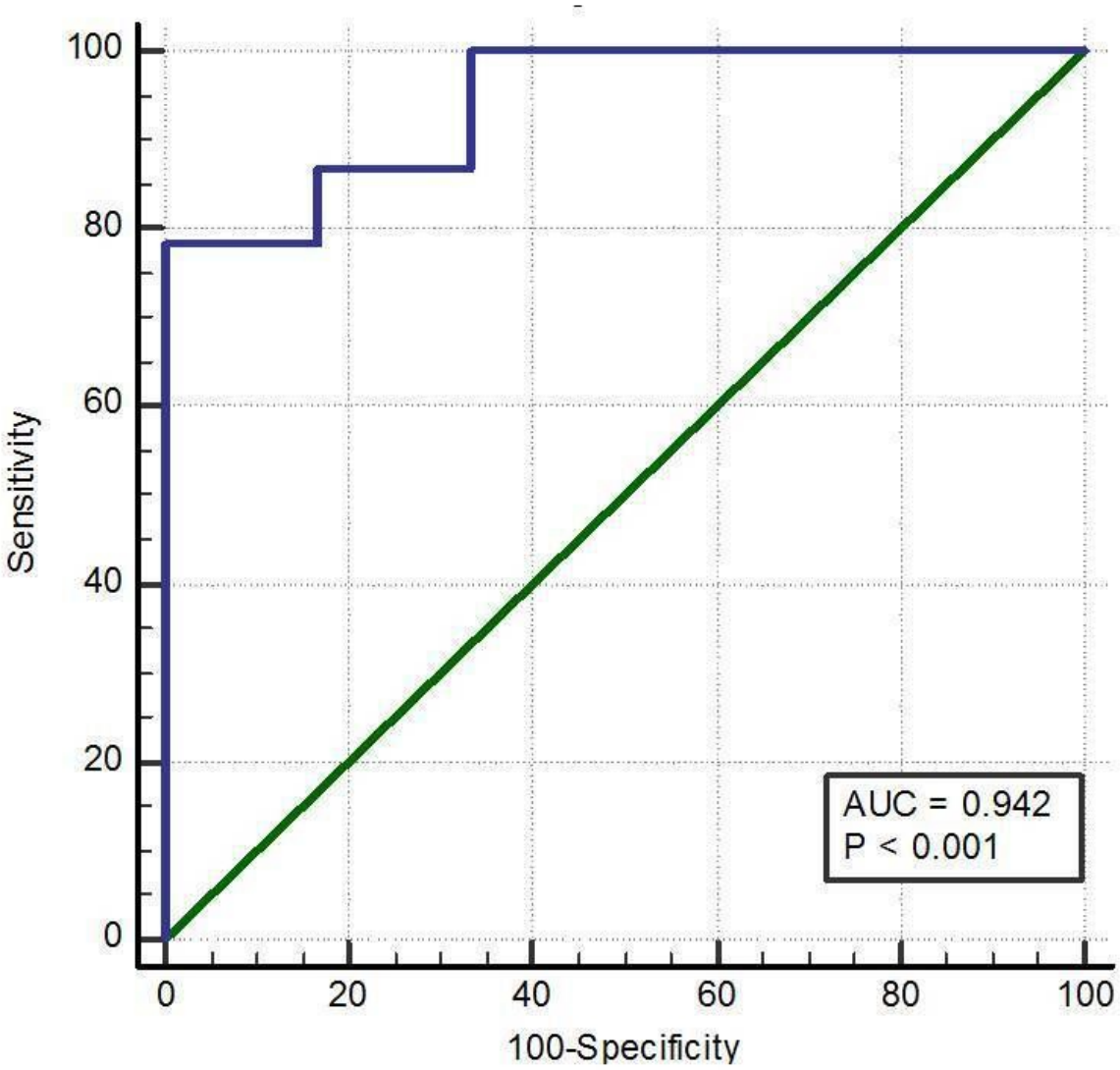

Figure 2

Receiver-operating characteristic (ROC) curve of plasma gastrin concentration in the diagnosis of abomasal ulcer in buffaloes