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## Role of *Clostridium perfringens* in Causing Abomasal Ulcers in Buffalo

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**Abstract:** In this study, the correlation between abomasal ulcers and presence of *Clostridium perfringens* (*C. perfringens*) was evaluated in 80 (50 affected and 30 non affected) randomly slaughtered buffaloes in Ahvaz slaughterhouse. Immediately after the slaughter, the abomasums was isolated and an incision was made on the wall of it. Then the abomasums were emptied and its interior was washed with water. The inner surface was examined for presence of abnormal lesion. Ulcers from affected and piece of abomasa from non affected buffaloes were cultured. Cultures were also made from contents of all samples and smears were also prepared from affected and non affected tissues. Cultures from content samples (12%) of 50 ulcerated abomasa were positive for *C. perfringens* while the agents were isolated from 1 content (3.3%) of non ulcerated abomasa. There was no statistical difference between presence of *C. perfringens* in contents and abomasal ulcers. Totally *C. perfringens* were isolated from ulcers of 6(12%) ulcerated and tissues of 3(10%) non ulcerated cases. Statistical analysis showed no correlation between presences of *C. perfringens* and abomasal ulcers. There was no statistical difference between sex and age of the affected animals. In conclusion *C. perfringens* seems not to be solely, a cause of abomasal ulcers in buffaloes.

**Key words:** Ruminant, bacteria, *Clostridium perfringens*, abomasal ulcers

### INTRODUCTION

Abomasal ulcers are an important cause of indigestion in dairy cattle (Braun *et al.*, 1991). It affects ruminants of all ages. There are several forms of ulcers which produce different clinical signs (Braun *et al.*, 1991; Guand, 2002; Radostits *et al.*, 2007; Roger *et al.*, 1991). According to Whitlock (1980), abomasal ulcers can be divided into four types. Ulcers with clinical signs are usually type 2 (bleeding ulcer), but occasionally may be type 3 (perforated ulcer with local peritonitis), or type 4 (perforated ulcer with generalized peritonitis). These ulcers produce distinct clinical signs and their diagnosis is therefore straightforward. Type 1 ulcers (erosions and non-perforating lesions of the abomasal mucosa) are usually not diagnosed until slaughter as affected animals frequently have only mild or no clinical signs (Whitlock, 1980). Although in Animals with Type 1 Abomasal ulcer (erosions and non-perforating mucosa) clinical signs are often absent or nonspecific, it is interesting that clinical signs were recorded in cows with Type 1 ulcers as well (Smith *et al.*, 1983). Although, the abomasal ulcer has most often been studied in cattle, buffaloes also suffer as

confirmed during our preliminary observations at the slaughter house of Ahvaz town in South-western Iran, where out of a lot of 200 clinically healthy buffaloes, 127 animals (63/5%) were found to have ulcers in their abomasa. Most of the lesions belonged to Type 1 category. Although in Animals with Type 1 Abomasal ulcers is unknown production of abomasal ulcers is usually considered to be associated with increased abomasal acidity, stress, mechanical attrition of abomasums, use of drug, mineral deficiency (e.g., copper deficiency) and bacterial infection (Mills *et al.*, 1990). *Clostridium perfringens* (*C. perfringens*) being one of the several probable causes suspected in cattle (Radostits *et al.*, 2007), the possibility of its relationship with abomasal ulcers in buffalo was investigated in this study.

### MATERIALS AND METHODS

Eighty randomly collected abomasa from the buffaloes slaughtered at the Ahvaz slaughter house during the period March 2006 to January 2007 was the material of this study. Immediately after the slaughter, the

abomasum was isolated and both its ends (including a small portion of omasum and duodenum) were ligated before transporting it to the autopsy laboratory of the University.

After cleaning the external surface, an incision was made on the wall of the abomasum and samples of the contents were taken with a loop for cultivation on Blood Agar (BA) and Egg Yolk Agar (containing neomycin) (EYA). The abomasum was then emptied and its interior was washed with water under mild pressure. The inner surface was examined for the presence of abnormal lesions (petechia, erosion and ulcers etc.). Of the 80 abomasa, 50 had abomasal ulcers. The remaining 30 which had no ulcers were treated as controls. In the abomasa with ulcers, samples from the ulcer were cultured on B.A and E.Y.A media aseptically. Impression smears were also made and subsequently stained with Grams stain to recognize the lamellae with large Gram positive bacilli.

The cultured plates were incubated at 37°C for 48 h in anaerobic condition. Suspected colonies with double hemolysis in B.A or positive lecitinase in E.Y.A and with negative catalase and oxidase tests were purified in E.Y.A. identification of suspected isolates were made biochemically. The results were analyzed by SPSS 11 and Chi square.

## RESULTS AND DISCUSSION

The results of bacteriological examination of abomasal contents and abomasal tissues are presented in Table 1. *C. perferingens* was isolated from the abomasal contents as well as from the abomasal tissues of six out of 50 (12%) abomasa with ulcers. In the case of 30 abomasa without ulcers, *C. perferingens* was isolated from the abomasal contents of only one (3.3%) and abomasal tissues of three (10%). Statistical test did not show any significant difference between ulcered and unulcered abomasa ( $p>5\%$ ). Similarly, upon tabulation of results according to age groups and according to sex (Table 2, 3), no significant difference due to age or sex was found ( $p>5\%$ ).

The attention to the probable role of bacteria in causing abomasal ulcer grew when *Helicobacter* was found to be a significant cause of human gastric ulcer. Upon this finding, several studies have been conducted in cattle and sheep to evaluate the role of infectious agents (Jelinski *et al.*, 1995; Mills *et al.*, 1990; Roeder *et al.*, 1987, 1988; Vatn *et al.*, 2000a; Welchman and Baust, 1987). Contradictory results were reported, as some studies reported the positive role of *C. perferingens* in pathogenesis of abomasal ulcer

Table 1: The result of culture of contents and tissue of studied abomasa

Abomasums	Contents		Tissue	
	Positive	Negative	Positive	Negative
Ulcerated	6 (12)	44 (88)	6 (12)	44 (88)
Nonulcerated	1 (3.3)	29 (96.7)	3 (10)	27 (90)
Total	7 (8.75)	73 (91.25)	9 (11.25)	71 (88.75)

Values in bracket indicate percentage

Table 2: The result of culture of contents and tissue of studied abomasa according to age

Age	Contents		Tissue	
	Positive	Negative	Positive	Negative
Under 2.5 years old	3 (11.5)	23 (88.5)	3 (11.5)	23 (88.5)
Above 2.5 years old	3 (12.5)	21 (87.5)	3 (12.5)	21 (87.5)
Total	6 (12)	44 (88)	6 (12)	44 (88)

Values in bracket indicate percentage

Table 3: The result of culture of contents and tissue of studied abomasa according to sex

Sex	Contents		Tissue	
	Positive	Negative	Positive	Negative
Male	4 (13.3)	26 (86.7)	4 (13.3)	26 (86.7)
Female	2 (10)	18 (90)	2 (10)	18 (90)
Total	6 (12)	44 (88)	6 (12)	44 (88)

Values in bracket indicate percentage

(Jelinski *et al.*, 1995; Roeder *et al.*, 1987, 1988), while others denied this role (Mills *et al.*, 1990; Vatn *et al.*, 2000a, b).

In the present study, there was no significant differences between isolation of *C. perferingens* in the contents and tissues of ulcered and unulcered abomasa. This was in agreement with some studies (Mills *et al.*, 1990; Vatn *et al.*, 2000a, b) and differed from others (Jelinski *et al.*, 1995; Roeder *et al.*, 1987, 1988).

It may of worth mentioning some other causative factors. In Ahvaz region of Iran, inclusion of concentrates like flour, bran and bread in the rations are often believed to cause abomasal ulcers. Feeding of rations high in carbohydrate materials may cause increased fermentation and volatile fatty acids in the rumen. The fatty acids with short chains have detergent activity which destroys the mucosal tissue (thereby the hydrochloric acid penetrates into the mucosa) leading to production of abomasal ulcers (Anderson, 1992; Braun *et al.*, 1991).

It is also believed that feeding of seeds increases the levels of lactic acid and histamine in the rumen which intensifies rumenal and abomasal stasis. Histamine stimulates abomasal secretion, leading to accumulation of hydrochloric acid and pepsin in the large flexure (ventral part) of abomasum. The mechanical action of these materials for a long time leads to destruction of mucosal layer and focal cell necrosis (Jensen *et al.*, 1976).

In present study there was also no significant difference due to age and sex of animals, although some sources (Radostits *et al.*, 2007; Vatn *et al.*, 2000b) point to

age differences in the incidence of abomasal ulcers. The incidence may also be expected to be higher in female animals because of the stress due pregnancy and parturition.

### CONCLUSIONS

Since *C. perfringens* could be isolated from abomasal contents and tissues of the healthy buffaloes in this study we can not conclude that *C. perfringens* was the only factor responsible for causing abomasal ulcers in buffaloes.

It is proposed to make further studies on the role of infectious agents by PCR, since the latter is more sensitive in comparison with the culture. Probable role of other infectious agents such as *Helicobacter pylori*, *C. sordelli* etc. in causing abomasal ulcer in buffaloes may also be investigated. The recommended studies should be carried out under controlled environment.

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