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# The prevalence, frequency and topographic distribution of Type 1 abomasal ulcers in water buffalo (*Bubalus bubalis*): a case control study

**Syed A. Hussain[1]*, Sanjeev K. Uppal[1], and Naresh K. Sood[2]**

[1]*Department of Veterinary Medicine, Guru Angad Dev Veterinary and Animal Sciences University, Ludiana, India*

[2]*Department of Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University, Ludiana, India*

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**ABSTRACT**

Although abomasal ulcers in cattle have been recognized in the past, an epidemiological study on buffaloes and data on frequency and topographic location of ulcers in abomasum is still lacking. This case control study evaluated the prevalence of type 1 abomasal ulcers in slaughtered buffaloes of India. The sample size was calculated by a standard epidemiological method. The location, number and type of ulcers were recorded photographically and on a predesigned sketch diagram, representing all anatomical parts of the abomasum and both surfaces. On the basis of their macroscopic and microscopic appearance, the type 1 ulcers were classified into four subtypes, viz. type 1a, 1b, 1c and 1d. Out of 134 examined abomasa, 66.42% had type 1 ulcers. The prevalence was significantly higher during the summer than in winter. Buffaloes studied during the summer had 3.39 times greater odds of having a higher prevalence of type 1 ulcers than buffaloes studied during the winter. Although the prevalence did not differ significantly between different age groups, older animals had greater odds of having abomasal ulcers. Type 1b and 1c were the most frequently observed ulcers. Although the abomasal body had 12.32 times and 6.12 times greater odds of having abomasal ulcer than fundus and pylorus, respectively, the ulcer location did not change with increasing age. All ulcers, except type 1a were more concentrated in the caudal third of the parietal surface of the abomasal body, along the greater curvature, and were usually multiple. We believe that abomasal ulcers may be an important cause of the decreased production of buffaloes in this region due to the high prevalence and multiple etiology.

**Key words:** buffalo; abomasal ulcer; prevalence; odds

*Corresponding author:

Syed Ashaq Hussain, M.V.Sc, PhD, Division of Clinical Veterinary Medicine, Ethics and Jurisprudence, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar-India-190006, Phone: +91 941 962 7472; E-mail: draashiqhussain@gmail.com

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## Introduction

An abomasal ulcer is the result of patho-physiological conditions in which the balance between the protective and destructive processes of the abomasal mucosa is altered and as such cannot oppose the destructive action of the gastric juice. Historically, abomasal ulcers have been divided into four types: type 1 being a superficial erosion of the mucous membrane, type 2 a deeper lesion eroding the larger blood vessels leading to substantial haemorrhage, and types 3 and 4 perforating the abomasal wall leading to local or general peritonitis, respectively (WHITLOCK, 1980; SMITH et al., 1983). Later, BRAUN et al. (1991) introduced a more detailed subtyping of type 1 ulcers, ranging from a discoloration of the epithelium (type 1a) to distinct craters (type 1b, c and d).

Buffalos play an important socioeconomic role in many developed and developing countries. In India, Punjab is one of the important buffalo rearing areas of the country. Despite the probable importance of abomasal ulcers (especially type 1) in water buffalo in India in general and in Punjab in particular, there is no published report about the prevalence of abomasal ulcers in this species. Throughout the world no extensive work has been carried out on the patho-morphological features and frequency of type 1 abomasal ulcers in buffalo. Little work has been carried out to correlate gross observations with the histological findings. Lesions that appear alike and receive similar numerical scores may have dissimilar histological appearance and underlying pathogenesis. Further, in earlier studies the population size and sample size calculation has not been taken into consideration. Since abattoir and histopathological studies are more accurate and reliable than faecal occult blood tests to diagnose the abomasal lesions, the aim of this case control study was to determine the prevalence, frequency and patho-morphological features of type 1 abomasal ulcers in water buffalo in Punjab, India.

## Materials and methods

This study was conducted on a mixed population of buffaloes, slaughtered in a buffalo specific abattoir during winter (December to January) and summer months (April to May). The time (day in a season) of the survey was non-random but during one day the buffaloes were selected randomly. The age of the animals was estimated using dental characteristics, and the animals were categorized into three groups. Animals with two, three and four pairs of permanent incisors constituted Group I, Group II and Group III, respectively (the corresponding age was approximately taken as 3 years, 4-6 and >6 years). The study protocol was approved by the Institutional Animal Ethics Committee of Guru Angad Dev Veterinary and Animal Sciences University.

*Sample size calculation.* As per the abattoir practice, 40 ± 3 female buffaloes were slaughtered per day except Friday. Assuming that a minimum of 40 buffaloes were slaughtered per day, the numbers of buffaloes slaughtered in December 2013, January

2014, April 2014 and May 2014 were 1080, 1040, 1040 and 1040, respectively. So the total study population was 4200 (2120 for winter and 2080 for summer). Based on clinical examination, abomasal ulcers have been observed in bovines of Punjab with a prevalence of 3.7% (SHAH, 2010). But this prevalence is not a true representation of the general population of cows and buffaloes, owing to the inclusion of only those cows and buffaloes that were confirmed to have gastrointestinal dysfunction. As the disease has not been reported in apparently healthy buffaloes from Punjab, on the basis of expert advice and clinical experience, we expected the prevalence of abomasal ulcers to be 10%. Assuming that 10% of the animals in the population had abomasal ulcers, and a population size of 4200, this study required a sample size of 134 for estimating the expected proportion with 5% absolute precision and 95% confidence (DHAND and KHATKAR, 2014).

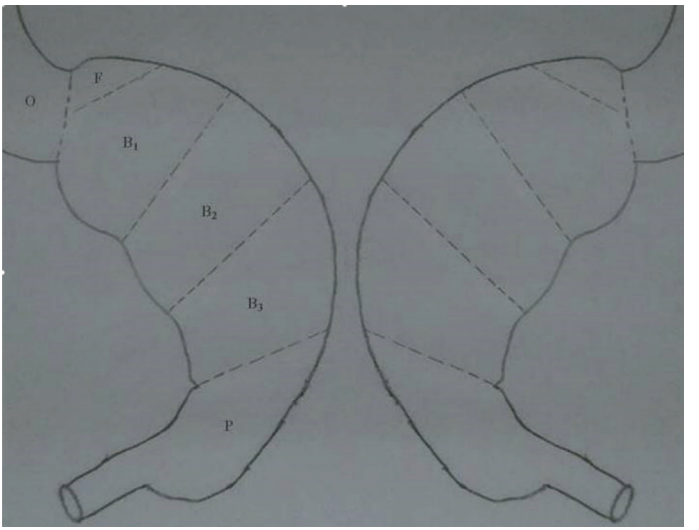


Fig. 1. Schematic representation of the omasum and abomasum. Left: Interior of the visceral surface of the abomasum. Right: Interior view of the parietal surface of the abomasum. O: omasum F: Fundus of the abomasum, B1: One third of the body of the abomasum towards the fundus, B2: Middle third of the abomasal body, B3: One third of the body of the abomasum towards the pylorus, P: Pylorus of the abomasum

*Examination of the abomasum and collection of tissue samples for histopathological examination.* Immediately after slaughter, the abomasum was separated from its surrounding viscera, incised along the lesser curvature and the content rinsed away with water. The abomasum was thoroughly examined for abomasal ulcers. The abomasal ulcers, and their location, number, and subtype were recorded photographically and on a predesigned sketch diagram (Fig. 1). The diagram represented both the parietal and

visceral surfaces of the abomasum and all anatomical parts of the abomasum (fundus, body and pylorus). Further, the body part of abomasum was roughly divided into three subparts *i.e.* one third towards the fundus, the middle third and last third towards the pylorus. Representative samples from all the gross lesions were dissected from grossly abnormal regions using forceps and a scalpel blade. All layers of the abomasal wall, from the epithelial to the serosal surface, were included. These collected tissues were placed in 10% neutral buffered formalin and then underwent routine processing and wax embedding. Sections (4-5 μm thick) were stained with haematoxylin and eosin for microscopic examination. Pathological classification of type 1 ulcers into four subtypes, 1a, 1, 1c and 1d, was performed using their gross characteristics, as per the guidelines of BRAUN et al. (1991) with some modifications, as shown in Table 1. The macroscopic findings were confirmed by histopathological examination.

Table 1. Classification criteria for type 1 abomasal ulcers in water buffalo

| Ulcer subtype | Macroscopic appearance | Microscopic appearance |
|---|---|---|
| Type 1a | Erosions with minimal mucosal defects. Reddish or brown in colour (Fig. 2a) | Superficial erosion of mucosal layer with no or negligible inflammatory response (Fig. 2b) |
| Type 1b | Well-defined dark red to black spots with punched out appearance. The centre of the lesion was always clearly depressed (Fig. 3a) | Mostly trough-like erosions of the mucosal layer with mononuclear cell or neutrophil infiltration. The necrosis of epithelium sometimes extended as far as the sub mucosa (Fig. 3b) |
| Type 1c | Craters with a superficial coating of dead organic matter, faecal matter. The margins usually formed a bulge (Fig. 4a). | Deeper sloughing of the mucosal layer with chronic inflammatory response in the surrounding area, extending up to the sub mucosa (Fig. 4b). |
| Type 1d | Ulcers with radial wrinkles and sometimes with ulcers on the mucosal folds (Fig. 5a). | Superficial or deep erosions of the mucosal layer with chronic inflammatory response in the surrounding area (Fig. 5b) |

*Statistical analysis.* The categorical data were presented as frequency and/or percentage. The Chi square test was used for comparisons between binary outcome and binary explanatory variable. The prevalence was compared between the two seasons, three age groups and between three anatomical parts of the abomasum. For all statistical procedures a value of $P < 0.05$ was considered significant.

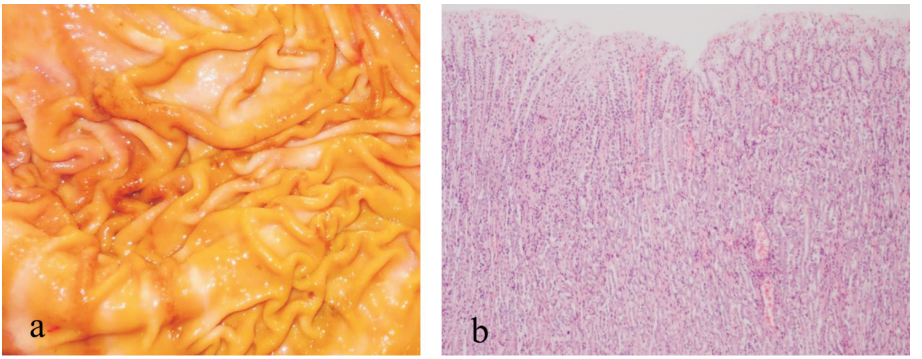


Fig. 2. Gross (a) and microscopic (b, ×100) appearance of a Type 1a abomasal ulcer

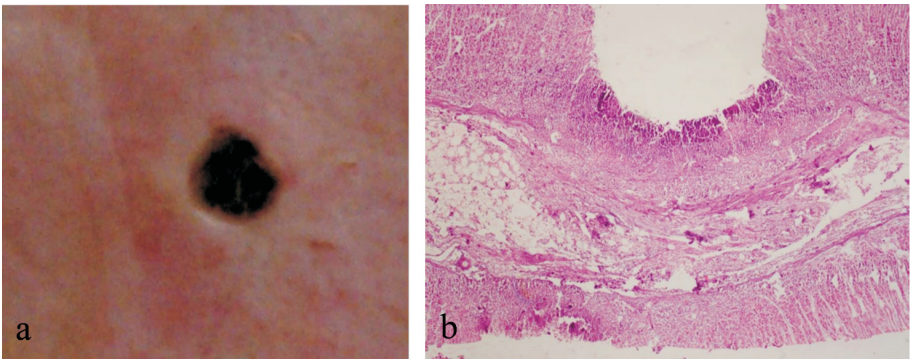


Fig. 3. Gross (a) appearance of a Type 1b abomasal ulcer; section of the abomasum (b) showing a trough-like Type 1b ulcer (×100)

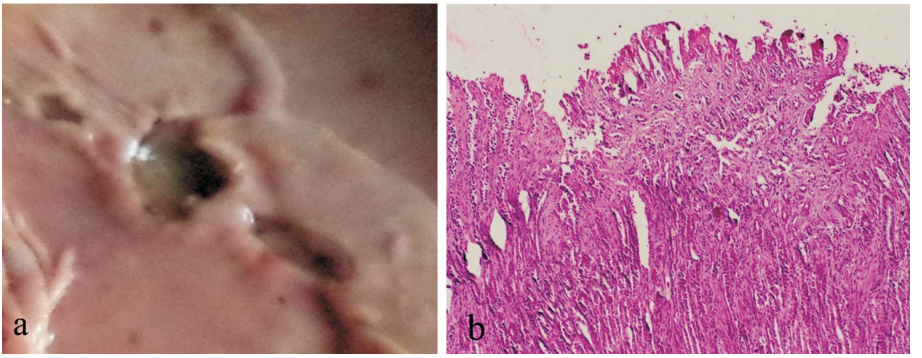


Fig. 4. Gross (a) appearance of a Type 1c abomasal ulcer; Section of the abomasum (b) showing an ulcer with chronic inflammatory response (×100)

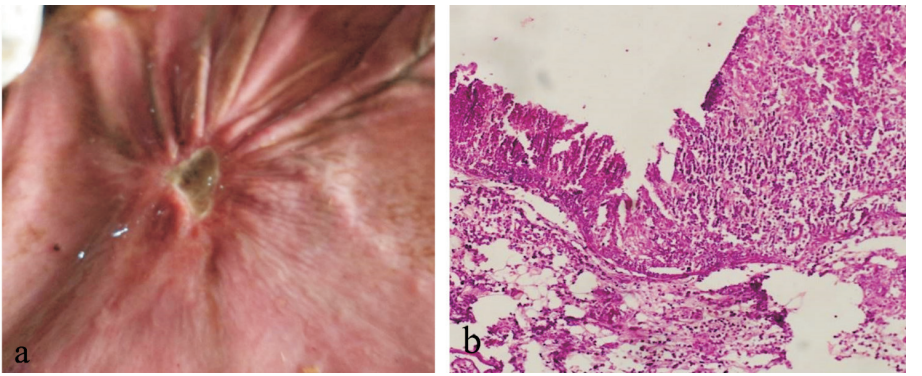


Fig. 5. Gross (a) appearance of a Type 1d abomasal ulcer, section of the abomasum (b) showing an ulcer with inflammatory response and fibrosis (×100)

**Results**

A total of 134 abomasa were examined and graded for type 1 abomasal ulcers. The overall prevalence of type 1 abomasal ulcer was 66.42%, being significantly higher during the summer (78.87%) than the winter season (52.38%) (Table 2). Buffaloes studied during the summer had 3.39 times greater odds of having a higher prevalence of type 1 abomasal ulcers than buffaloes studied during the winter. In addition to Type 1ulcers, 15 abomasa had healed ulcers/scars, while sand was observed in six abomasa.

Table 2. Prevalence of type 1 abomasal ulcers in water buffalo during summer and winter

| | Ulcer Positive | Ulcer Negative | Total | Odds | |
|---|---|---|---|---|---|
| Summer | 56 (78.87) | 15(21.12) | 71(100) | 56/15 = 3.733 | P = 0.01 |
| Winter | 33(52.38) | 30(47.62) | 63(100) | 33/30 = 1.1 | |
| Total | 89(66.42) | 45(33.58) | 134(100) | | |

The numbers in parenthesis represent row percentages

The prevalence was much higher in age group III, but did not differ significantly between the different age groups (P = 0.07, Table 3). In the present study, the odds of abomasal ulcers for the three age groups are shown in Table 3. Taking age group III as standard, the odds ratios for group III with respect to groups I and II were 2.14 and 2.56 *i.e*. the group III animals had 2.14 times and 2.56 times greater odds of having abomasal ulcers than group I and group II, respectively.

Table 3. Age wise prevalence of type 1 abomasal ulcers in water buffalo

| Age group | Ulcer positive | Ulcer negative | Total | Odds | |
|---|---|---|---|---|---|
| Group I | 18 (58.1) | 13 (41.9) | 31 (100) | 18/13 = 1.38 | P = 0.07 |
| Group II | 15 (53.6) | 13 (46.4) | 28 (100) | 15/13 = 1.15 | |
| Group III | 56 (74.7) | 19 (25.3) | 75 (100) | 56/19 = 2.95 | |
| Total | 89 (66.42) | 45 (33.58) | 134 (100) | | |

The numbers in parenthesis represent row percentages

The prevalence of type 1 ulcers was significantly higher in the body region of the abomasum compared to the fundus and pylorus of the abomasum, but did not differ significantly between the fundus and pylorus (Table 4). The odds of abomasal ulcers for the three anatomical parts of the abomasum are shown in Table 4. Taking the abomasal body as standard, the odds ratios for the abomasal body with respect to the fundus and pylorus were 12.32 and 6.12, *i.e.* the abomasal body had 12.32 times and 6.12 times greater odds of having abomasal ulcers than the fundus and pylorus, respectively.

Table 4. Location of type 1 abomasal ulcers in different age groups of water buffalo

| Location of ulcer | Ulcer Positive | Ulcer negative | Total | Odds |
|---|---|---|---|---|
| Body | 63 (47.01)[a] | 71 (52.98) | 134 (100) | 63/71 = 0.887 |
| Fundus | 9 (6.7)[b] | 125 (93.3) | 134 (100) | 9/125 = 0.072 |
| Pylorus | 17 (12.69)[b] | 117 (87.31) | 134 (100) | 17/117 = 0.145 |

The numbers in parenthesis represent row percentages; The values with different superscripts differ significantly ($P<0.05$)

Table 5. Location of type 1 abomasal ulcers in different age groups of water buffalo

| | Ulcer location | | | | |
|---|---|---|---|---|---|
| Age group | Body | Fundus | Pylorus | Total | |
| Group I | 11 (61.1) | 3 (16.7) | 4 (22.2) | 18 (100) | P = 0.282 |
| Group II | 9 (60) | 2 (13.3) | 4 (26.7) | 15 (100) | |
| Group III | 43 (76.8) | 4 (7.1) | 9 (16.1) | 56 (100) | |
| Total | 63 (70.8) | 9 (10.1) | 17 (19.1) | 89 (100) | |

The numbers in parenthesis represent row percentages

The location of ulcers did not differ significantly between the three age groups (Table 5). Out of 89 abomasa with type 1 ulcers, 67 abomasa had ulcers over both the parietal and visceral surfaces, 20 had them over the parietal surface only, and two abomasa had ulcers over the visceral surface only. Although ulcers were commonly observed over both surfaces of the abomasum, they were more concentrated in the caudal third (towards the pylorus) of the parietal surface of the abomasal body, along the greater curvature,

followed by the middle third of the parietal surface of the abomasal body, along the greater curvature.

Table 6. Percentages of different type 1 ulcers in water buffalo

| Ulcer type | N | Percentage of examined abomasa (134) | Percentage of affected abomasa (89) |
|---|---|---|---|
| 1a | 18 | 13.43% | 20.22% |
| 1b | 35 | 26.12% | 39.33% |
| 1c | 29 | 21.64% | 32.58% |
| 1d | 7 | 5.22% | 7.86% |
| Total | 89 | 66.42% | 100% |

The percentages of various subtypes of type 1 ulcers are shown in Table 6. Type 1b (39.33%) and type 1c (32.58%) were the most frequently observed ulcers. On histological examination, types 1a and 1b were mostly acute or sub-acute while 1c and 1d were always chronic. Types 1b and 1d were mostly observed in the body region of the abomasum (68.57% and 85.71%, respectively) while type 1a was mainly observed in the pyloric region (44.44%, Table 7).

Table 7. Topographic distribution of various type 1 abomasal ulcers in water buffalo

| Ulcer type | N | Fundus | Body | Pylorus | Body and pylorus |
|---|---|---|---|---|---|
| 1a | 18 (100) | 2 (11.11) | 5 (27.78) | 8 (44.44) | 3 (16.67) |
| 1b | 35 (100) | 5 (10.9) | 24 (68.57) | 2 (5.71) | 4 (11.43) |
| 1c | 29 (100) | 1 (3.45) | 15 (51.72) | 7 (24.14) | 6 (20.69) |
| 1d | 7 (100) | 1 (14.28) | 6 (85.71) | 0 (0.0) | 0 (0.0) |
| Total | 89 (100) | 9 (10.11) | 50 (56.18) | 17 (19.1) | 13 (14.61) |

Numbers in parenthesis indicate percentage

Table 8. Frequency of each subtype of type 1 ulcer in water buffalo

| Ulcer type | N | Number of ulcers per abomasum | | | |
|---|---|---|---|---|---|
| | | 1-5 ulcers | 6-10 ulcers | 11-20 ulcers | >20 ulcers |
| 1a | 18 (100) | 6 (33.3) | 5 (27.78) | 5 (27.78) | 2 (11.1) |
| 1b | 35 (100) | 6 (17.1) | 8 (22.9) | 11 (31.4) | 10 (28.6) |
| 1c | 29 (100) | 17 (58.6) | 8 (27.6) | 3 (10.3) | 1 (3.4) |
| 1d | 7 (100) | 4 (57.1) | 2 (28.6) | 1 (14.3) | 0 (0.0) |
| Total | 89 (100) | 33 (37.1) | 23 (25.8) | 20 (22.5) | 13 (14.6) |

Numbers in parenthesis indicate percentages

The number of type 1 ulcers per abomasum varied among the animals (Table 8). Thirty-three abomasa (33/89) had 1 to 5 ulcers, 23 abomasa had 6 to 10 ulcers, 20 had 11

to 20 ulcers and 13 abomasa had more than 20 ulcers. Usually 1a and 1b were observed in large numbers, while 1c and 1d were much less common.

**Discussion**

To our best knowledge, this is the first study to describe the prevalence of abomasal ulcers in Indian water buffalo, so the required sample size was calculated using a standard epidemiological method. The sample size of this study meant that we selected a random sample of 134 from a finite population of 4200 buffaloes, and determined that 10% of subjects had the factor of interest. We were 95% confident that between 5% and 15% of the subjects in the population had the factor of interest. The prevalence of type 1 abomasal ulcers in this study was greater than expected, and we suggest that type 1 ulcers in buffaloes of this region are a frequently occurring event, without noticeable clinical symptoms. The earlier literature indicates a high prevalence of type 1 abomasal ulcers in buffaloes in Iran (GHADRDAN-MASHHADI et al., 2010; TAJIK et al., 2012). However, in adult cattle the reported prevalence is highly variable (20.5% to 57%) among different studies (JOHANNSEN et al., 1989; BRAUN et al., 1991). The prevalence of abomasal ulcers varies in different studies and is hard to compare due to differences in the examined sample size and study design. At slaughter or necropsy, all types of ulcers can be diagnosed, but during clinical evaluation type 1 ulcers are difficult to diagnose. We believe that the findings of this study are a true representation of buffalo in this region because the sample size was calculated by a standard method and all lesions were established by histopathological examination. Even though some abomasa contained sand, those buffaloes did not seem more prone to abomasal ulcers compared to buffaloes with normal abomasal content. The higher prevalence of abomasal ulcers in summer was in agreement with earlier literature on dairy cattle (AUKEMA and BREUKINK, 1974; RADOSTITS et al., 2007). However, in contrast to the present study, TAJIK et al. (2012) reported that the prevalence of type 1 ulcers in Iranian water buffalo was higher. This may be attributed to the different agro climatic conditions in our area, sampling technique and sample size.

Abomasal ulceration is mostly associated with stress, feeding with a high concentrate diet, and metabolic disturbances (FUBINI and DIVERS, 2008; RADOSTITS et al. 2007). The types of stress reported to be associated with abomasal ulcers in ruminants are environmental, nutritional, physical, genetic, hyperacidity, lactic acidosis, and coarse rations (CEBRA et al., 2003; BLOWEY, 2004; FARSHID et al., 2006; MOSTAGHNI et al., 2008). However, in this preliminary study these risk factors were not taken into consideration. The higher incidence in older animals may be attributed to longer exposure to stress of any kind. The present findings with respect to age difference are in agreement with the literature that suggested no significant difference between either sex and between

the different age groups of buffaloes in the prevalence of type 1 abomasal ulcers (TAJIK et al., 2012). However, the incidence of bleeding abomasal ulcers is reported to vary with the age of cattle, and is low in cattle of two years of age (AUKEMA and BREUKINK, 1974). In the present study the higher percentage of type 1b ulcers was somewhat unexpected compared to the literature, where the percentages of cattle abomasa with type 1a, type 1b and type 1c ulcers were almost equal (BRAUN et al., 1991).

Currently, we assume that the pathogenesis of abomasal ulcers in the pyloric region is different from ulcers in the body region of the abomasum. The present findings with respect to the location of different subtypes of type 1 ulcers are in agreement with the literature that suggests that each type of ulcer has an affinity for a particular location in the abomasum (BRAUN et al., 1991). However, for unknown reasons, BRAUN et al. (1991) only compared the location of ulcers between the pyloric and fundic regions of the abomasum and observed type 1a and 1c ulcers mainly in the pyloric region, and types 1 b and 1d mainly in the fundic region. Although the abomasal body had greater odds of having type 1 ulcers than the fundus and pylorus, the ulcer location did not change with increasing age. The occurrence of multiple abomasal ulcers in this study was much more frequent than the occurrence of solitary ulcers, which is in agreement with previous reports (TAJIK et al.. 2012). TAJIK et al. (2012) observed different localizations of ulcers between the different age groups in Iranian buffaloes. However, our results did not show the same pattern in Indian water buffaloes.

*Study strengths and limitations.* This study had many strengths. Firstly, in the present investigation, sampling was performed on many days over two seasons, and the time of inclusion might have influenced the results. The same protocol was used for examination of the abomasa and histopathology examination throughout the study, to avoid this bias. Secondly, histopathological examination was used for confirmation of ulcers in order to avoid bias in sub classification of type 1 ulcers. It is therefore believed that all the examined abomasa were affected. Thirdly, from our experience from necropsy examination of clinical cases, irrespective of primary aetiology, we anticipated that type 1 ulcers were commonly located along the greater curvature of the abomasum. So, the abomasa were cut open along the lesser curvature in order to avoid damage to the greater curvature.

In addition to its strengths, the present investigation also had some limitations. The investigated animals are not a representative sample of all affected farms in Punjab, India, so the findings of this study should be extrapolated to farms in this region with caution. The aetiology of the disease could not be established in this preliminary study. Several management and environment variables related to practices or farm characteristics are likely to have changed over the study period. However, the potential risk factors were not evaluated in this study, given abattoir records were found to be minimal and of poor

quality. In future studies, the role of possible management factors contributing to the formation of abomasal ulcers in buffaloes in this region should be evaluated. This will help in establishing the pathogenesis, probable causes, and risk factors of abomasal ulcers in water buffaloes in the area. The clinical signs and laboratory alterations of type 1 abomasal ulcers in cattle are reported to be non-specific and faecal occult blood seems to be the only reliable method for diagnosis of such ulcers in healthy animals. However, the diagnostic capability of faecal occult blood tests was not evaluated in this study because our main aim was to establish this disease in this area. Given the high prevalence of type 1 abomasal ulcers in buffaloes, we suggest that the sensitivity and specificity of faecal occult blood tests should be evaluated for early diagnosis of this disease.

*Conclusion and potential relevance.* This preliminary study used gross and histological examination to highlight the prevalence of type 1 abomasal ulcers in a mixed population of Indian water buffaloes. The results of this study suggest a high prevalence of type 1 abomasal ulcers in buffaloes of this region. Type 1b and type 1c were the most frequently observed ulcers. The type 1 ulcers were commonly observed over both the parietal and visceral surfaces of the abomasum, they were more concentrated along the greater curvature in the caudal third of the parietal surface of the abomasal body. Irrespective of the age of the animals, the predominant location for type 1 ulcers in the present study was the abomasal body. Although type 1 abomasal ulcers do not cause obvious clinical signs, we believe that these may be an important cause of decreased production in buffaloes in India. This anticipated decreased production may lead to economic losses of varied degrees, depending upon the number and type of ulcers in the abomasum. The economic effect of abomasal erosions in ruminants has not been investigated so far, and may be taken into consideration in future studies. Helicobacter organism is an important cause of gastric ulceration in humans and dogs, but in calves abomasal ulcers have been attributed to *Clostridium* spp. Therefore, in future studies ulcer positive abomasa/animals should be screened for infectious aetiologies such as Helicobacter and Clostridium organisms. Further research regarding the range of pathology in a larger and more diverse group of buffaloes is required.

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**SAŽETAK**

Iako su čirevi sirišta kod goveda poznati dugo vrijeme, još uvijek nedostaju epidemiološka istraživanja s podacima o njihovoj učestalosti i topografskom položaju kod vodenih bivola. U ovom istraživanju parova procijenjena je prevalencija čireva tipa 1 u sirištima zaklanih bivola u Indiji. Veličina uzorka izračunata je standardnom epidemiološkom metodom. Položaj, broj i vrsta čireva zabilježeni su fotografski i na unaprijed skiciranom dijagramu, koji je predstavljao sve anatomske dijelove i obje površine sirišta. Na temelju makroskopskog i mikroskopskog izgleda, čirevi tipa 1 klasificirani su u četiri podtipa, 1a, lb, lc i 1d. Od 134 pretražena sirišta, 66,42 % imalo je čireve tipa 1. Prevalencija je bila znakovito viša tijekom ljeta nego zimi. Bivoli čija su sirišta pretražena tijekom ljeta imali su 3,39 puta veću šansu za višu prevalenciju čireva tipa 1 nego što su to imali bivoli čija su sirišta pretražena tijekom zime. Iako se prevalencija nije znakovito razlikovala između različitih dobnih skupina bivola, starije životinje imale su veće izglede za pojavu čireva u sirištu. Tip 1b i 1c bili su najčešće opaženi podtipovi čireva. Šansa za pojavu čira u tijelu sirišta bila je 12,32 puta veću u odnosu na fundus sirišta i 6,12 puta veću u odnosu na pilorus. Navedeni odnosi nisu se mijenjali s porastom dobi bivola. Svi čirevi, osim tipa 1a, bili su više koncentrirani u kaudalnoj trećini parijetalne površine tijela sirišta, duž velike krivine i obično su bili višestruki. Smatra se da čirevi sirišta, zbog visoke prevalencije i višestruke etiologije, mogu biti važan uzrok smanjene proizvodnosti vodenih bivola u Indiji.

**Ključne riječi:** bivol; čir sirišta; prevalencija; izgledi