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Epidemiological and Diagnostic Studies on Subacute Ruminal Acidosis in Dairy Cows

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Abstract

Subacute ruminal acidosis (SARA) has a serious effect on both animal health and final herd profitability. The present study was conducted to investigate the prevalence of SARA in Egyptian dairy herds and the associated risk factors. Moreover, ultrasonographic and biochemical investigation on such disease were also attempted. A total of 80 dairy cows were selected randomly from eight herds raised in El-Behara province. For each cow, clinical examination, rumenocentesis and ultrasonographic examination of liver were carried out. Questionnaire was constructed to evaluate the risk factors of SARA. The prevalence of SARA-positive cow and SARA-marginal cows were 26.25% and 13.75%, respectively. The pH of ruminal fluid in SARA-positive and SARA-marginal cows was 5.29±0.027 and 5.68±0.018 respectively The risk factors include feeding on finely ground concentrates, neglecting application of exogenous buffer as sodium bicarbonate, neglecting yeast mixing with ration, mixing ground bread and beet tops with feed as well as absence of roughages incorporation. A significant hypo-albuminemia ($p<0.05$) and a significant increase in the activity of AST, ALT and GGT were recorded in SARA-positive cows ($p< 0.05$). The results of the present study indicate that SARA is a common problem in dairy herds in El-Behera. Risk factors associated with such disease should be avoided to construct the ideal methods for prevention. Liver function in suspected cases should be monitored to avoid deterioration of the condition.

1. INTRODUCTION

Subacute ruminal acidosis (SARA) represents one of the most important metabolic subclinical disorders of high producing dairy cows. Feeding on diets rich in readily fermentable carbohydrates maximizes milk production, but simultaneously decreases ruminal pH, leading to a widespread prevalence of SARA (Brzozowska et al., 2013). During SARA, rumen pH is depressed for several hours per day due to accumulation of volatile fatty acids and insufficient rumen buffering (Plaizier et al., 2009). Ruminal acidosis represents a significant economic problem due to direct effects caused by alterations in the ruminal metabolism that could lead to death and indirect effects, which could lead to rumenitis, liver abscesses and laminitis (Nocek, 1997 and Miranda et al., 2005). Clinical signs of SARA include decreased dry matter intake (DMI), laminitis, rumenitis, liver abscesses, and pulmonary bacterial emboli (Nordlund and Garret, 1994; Nordlund et al. 1995; Kleen et al., 2003). Abomasal displacement (Sarashina et al., 1990), mastitis, metritis (Enemark et al., 2002) and low fertility (Britt, 1995) were also recorded. In clinical practice, definite diagnosis of SARA is only established by determining the pH of rumen fluid either at a specific time-point after feeding (collected by stomach tubing or more credibly by rumenocentesis) or continuously (using electronic rumen boluses) (Duffield et al., 2004). Ultrasonography has markedly enhanced the diagnosis of hepatic diseases

in cattle as hepatic abscess, hepatic lipidosis, fascioliasis, caudal vena cava (CVC) thrombosis and ultrasound-guided liver biopsy (Lechtenberg, and Nagaraja, 1991).

To the best of the author's knowledge, Studies on SARA at dairy herds in Egypt are scare. Consequently, the aim of the present study was to investigate the prevalence of SARA in Egyptian dairy herds and the associated risk factors. Moreover, ultrasonographic and biochemical investigation on such disease were also attempted.

1. MATERIAL AND METHODS

2.1. Animals

A total numbers of 80 high lactating recently parturient (5 -90 days) dairy cows belonging to El-Behera governorate were used in this study . From these cows, 10 cow were selected randomly to perform rumenocentesis. Ruminal fluid was collected from each cow under investigation at 5-8 hrs after feeding on total mixed ration (TMR) distribution and 2-4 hours after individual distribution of concentrates .

2.2. Management system

In five farms, the feeding system was TMR, while in remaining three farms the system was manual distribution of concentrates. Yeast (2-5gm/day/cow) and sodium bicarbonate (0.8 % of dry matter) mixing with ration were applied in three farms. 5 farms incorporated roughages as well as ground bread was mixed with ration while 3 farms mixed beet tops with the ration. Concentrates were consisted of barley, wheat bran, maize grains, cotton seed meal and were mixed with soya bean. Furthermore, a purpose-built questionnaire was created in order to evaluate some possible risk factors for SARA development in the dairy herds .

2.3. Clinical examination

All animals were subjected to thorough clinical examination; rectal temperature, ruminal contractions, respiratory and heart rates were evaluated (Kelly, 1984 .(

2.4. Ultrasonographic examination

Abdominal ultrasonography was the principal examination protocol of examined cows; an ultrasound scanner (EXAGYNE) with a micro convex probe 3.5 MHz. Ultrasonographic examination was carried out while the animals were standing using 3.5 MHz microconvex transducer. In preparation for ultrasonography, the 3rd to 12th intercostal spaces and the entire abdomen were clipped, shaved and swabbed with alcohol to remove excess oil, and coupling gel was finally applied. Abdominal ultrasonography was carried out according to Mohamed and Oikawa, (2007.(

2.5. Sampling

Twenty mL of ruminal fluid was obtained by rumenocentesis (Nordlund and Garrett, 1994) without sedation, using a 16- Gauge and 13 cm long stainless steel needle (fig.1). Ruminal fluid pH was measured on-site, at room temperature, right after collection of all samples, by using a portable pH-meter (AD1030, pH/mV and temperature Meter, Romania, Europe). The rumen fluid was immediately examined for color, odor, consistency, and methylene blue reduction time (MBRT) (Dirksen, 1969) (fig.2). Protozoa motility (Misra and Singh, 1974) and sedimentation activity time (SAT) (Nicholus and Penn, 1958) were also evaluated.

Figure 1: Aspiration of ruminal fluid.

Figure 2: Methylene blue reduction test.

Blood sample was collected from jugular vein of each animal without anticoagulant for colometric determination of serum albumin, gama glutamyle transferase (GGT), asparate aminotransferase (AST) and alanine amino transferase (ALT) using commercial test kits (EliTech diagnostics Co.)

according to Gendler,(1984); Heersink et al. (1980); Murray, (1984a) respectively.

Fresh 100 ml raw milk samples were obtained from cows. Samples of milk were cooled down until 6°C was reached. Samples were kept at the same temperature during the determination of milk fat % by milkoscan analyzer.

Urine samples were collected by catheterization in clean non-leak container for determination of urine pH. The pH of urine samples were determined directly by pH meter at room temperature.

About 20-30 gm of fecal samples was collected from each examined animal for physical examination. The samples were obtained from the rectum or at the time of defecation in clean non-leak container. Examination was carried out as soon as possible according to the method described by Ewing, (1974).

2.6. Statistical analysis

Data analysis was performed using SPSS) software package, version 13. One-way analysis of variance (ANOVA), followed by Duncans's multiple range test was used to compare differences among SARA positive, suspected and SARA negative cows. All results were expressed as means ± SD (standard deviation), and differences were considered significant at $P<0.05$.

3. RESULTS

3.1. Clinical findings

The examined dairy cows were suffering from diarrhea, lameness, decrease in feed intake and milk production as well as loss of body condition. The rectal temperature was 38.6 OC (38.3-38.9 OC), the heart rate was 74 bpm (61-87), and the respiratory rate was 24 cpm (12-36). The mucous membrane was bright red in most cows. Congested and pale mucous membrane were recorded in 3 and 2 cows respectively. Tachycardia (12.5%), polypnea (15%), ruminal hypomotiliy (6.3%), lameness (18.8%), and diarrhea (20%), inappetance (21.3%) were also observed in diseased cows.

3.2. Physical characters of the ruminal fluid:

The color of ruminal fluid was related to the feed type and degree of pH. It was greenish, greenish brown to yellowish brown in color in SARA-negative cows and yellowish brown in SARA-marginal cows while in SARA-positive cows was slightly milky brown. The consistency of ruminal fluid was slightly viscous in SARA-negative and SARA marginal cows, while in SARA-positive cows was watery. The odor of ruminal fluid was aromatic in SARA-negative and SARA-marginal cows, but in SARA-positive cows was sour (tab.2& fig.3).

3.3. Prevalence of SARA

The results of ruminal pH of all examined Egyptian dairy cows were evaluated according to Garrett et al., (1997). The prevalence of SARA-positive cows, SARA- marginal cows, and SARA-negative cows were 26.25%, 13.75% and 60% respectively.

Table 1: Mean values (±SD, range) of ruminal pH, SAT, MBRT and protozoa motility

Parameter	SARA- positive cows	SARA-marginal cows	SARA-negative cows
pH	5.09 -5.49 5.29 ± 0.027^{a}	5.6 -5.77 5.68 ± 0.018^{b}	5.83 -6.74 6.14 ± 0.034^{ab}
SAT	12.00 -18.00 $14.9\pm.33^{a}$	3.50-8.00 $6.4\pm.47^{b}$	3 - 8.45 $5.4\pm.20^{ab}$
MBRT	8.00 – 16.00 12.4 ± 0.55^{a}	2.20 -6.00 4.3 ± 0.36^{b}	1.30 – 5.5 2.9 ± 0.13^{ab}
otozoa motility	1.00 ± 0.00^{a} (+)	2 - 3 2.2 ± 0.12^{b} (++)	2 - 3 2.9 ± 0.04^{ab} (+++)

Means with different subscripts in the same raw are significantly different ($P<0.05$)

Table 2: Physical characters of the ruminal fluid in SARA-positive, SARA-marginal and SARA-negative cows.

Parameter	SARA positive cows (n=21)	SARA marginal cows (n=11)	SARA negative cows (n= 48)
Color	Slightly milky brown	Yellowish brown	-Greenish -Greenish brown -Yellowish brown
Odor	Sour	Aromatic	Aromatic
Consistency	Watery	Slightly viscous	Slightly viscous

Table 3: Mean values (±SD) of milk fat%, milk amount and urine pH in SARA-positive, SARA-marginal and SARA-negative cows.

Parameter	SARA-positive cows (n: 21)	SARA-marginal cows	SARA-negative cows (n: 59)
Milk fat %	2.84 ± 0.05^{a}	3.32 ± 0.05^{b}	3.53 ± 0.06^{ab}
Milk amount Kg/cow/day	9.56 ± 0.56^{a}	21.03 ± 0.22^{b}	22.13 ± 0.12^{ab}
Urine pH	7.87 ± 0.12^{a}	8.02 ± 0.11^{b}	8.21 ± 0.13^{ab}

Means with different subscripts in the same raw are significantly different ($P<0.05$)

Table 4: Mean values (±SD, range) of serum albumin, ALT, AST and GGT activities in in SARA-positive, SARA-marginal and SARA-negative cows.

Parameter	SARA- positive cows (n: 10)	SARA-marginal cows (n: 10)	SARA-negative cows (n: 10)
Albumin g/L	(13-22) 17.2 ± 0.9^{a}	(23-28) 25.6 ± 0.6^{b}	(27-33) 29.7 ± 0.6^{ab}
ALT Units/L	(33-70) 55.4 ± 3.8^{a}	(34-47) 40.7 ± 1.4^{b}	(18-42) 30.7 ± 2.3^{ab}
AST Units/L	(72-115) 95.5 ± 0.4^{a}	(64-84) 73.3 ± 2.0^{b}	(62-80) 71.5 ± 1.9^{ab}
GGT Units/L	(16-27) 22.6 ± 1.1^{a}	(14-22) 16.9 ± 0.9^{b}	(11-17) 13.6 ± 0.6^{ab}

Means with different subscripts in the same raw are significantly different ($P<0.05$)

3.4. Physical characters of feces:

Physical characters of feces changed in relation to the depression in the ruminal pH, as consistency, fiber particle size and presence of undigested food. The consistency was soft and the fiber particle size was greater than 2.5cm in length in addition to presence of undigested grains in feces in SARA-positive cows (fig.4).

Figure 3: Slight milk brown color of ruminal fluids in SARA-positive cows.

Figure. 4: Undigested grains in feces.

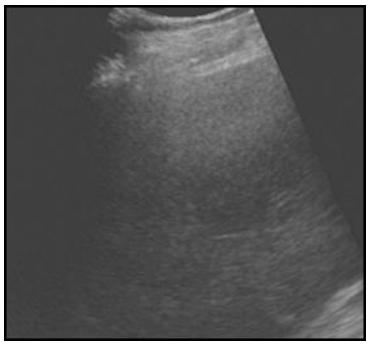
3.5. Risk factors related to SARA

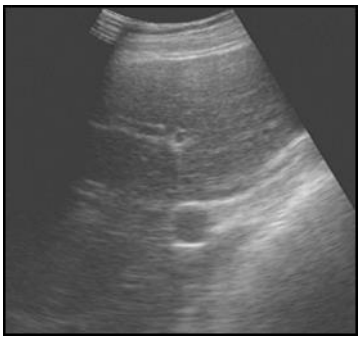
The risk factors associated with SARA in dairy farms were food particle size (coarse or finely ground), sodium bicarbonate inclusion(yes or no), yeast mixing with ration(yes or no), bread feeding and beet tops feeding(yes or no) in addition to roughages incorporation (yes or no.(

3.6. Ultrasonographic findings

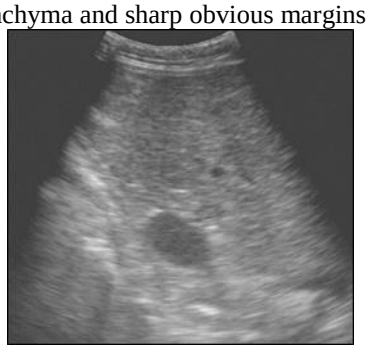
The ultrasonographic assay revealed presence of fat deposits in the liver tissue. Deposition of fat in the liver tissue resulted in increased hepatic size, rounding of liver margins, increased of coarseness

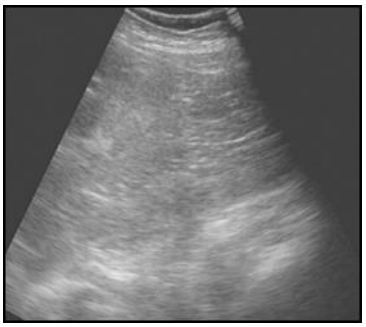
of echoes, increased echogenisity of the liver parenchyma near the abdominal wall (fig. 4-7).





Figures 4, 5: Ultrasonogram of normal liver; homogenous granular echo texture of parenchyma and sharp obvious margins of blood vessels.





Figures 6, 7: Ultrasonogram of fatty liver; bright pattern, vessel blurring and deep attenuation are present.

4. DISCUSSION

The examined dairy cows were suffering from diarrhea, lameness, decrease feed intake, loss of body condition and decrease in milk production. The mucous membrane was bright red in all animals. Congested and pale in 3and 2 cows respectively. These findings may be due to rumenitis, rumen parakeratosis, liver abscesses and pulmonary bacterial emboli as was reported by Duffield et al. (2004); Grove-White,(2004).

Sub-acute or chronic laminitis was observed in SARA affected cows with an evidence of discoloration of the hoof, sole hemorrhages, sole ulceration and misshapen hooves. These findings were in agreement with that obtained by Nordlund et al. (1995). It is suggested that accumulation of fecal substances and some infectious agents may be predispose factors to occurrence of lameness This explanation is supported by the findings reported by Cook et al. (2004); shaver, (2005). In addition, histamine release may have a role in occurrence of lameness as was recorded by Dirksen, (1985).

Ruminal hypomotility was recorded in SARA affected cows. Bacterial endotoxins and increased ruminal osmolarity may lead to decrease of rumen contraction. This finding is confirmed by previous results (Eades, 1997). On the contrary, ruminal contraction has not been changed in cows with SARA (Tajik et al. (2009)

Decrease of dry matter intake was also recorded in SARA affected cows. The reasons for the decrease feed intake include reduced fiber digestibility and volatile fatty acids accumulation, and increase the osmolarity in the rumen. Similar finding was obtained by Bipin et al. (2016).

It was found that the prevalence of SARA-positive cows in Egyptian dairy herds was 26.25 % while the prevalence of SARA-marginal cows was 13.75 % (tab.1). Prevalence of SARA differed from country to another where the prevalence of SARA in Wisconsin was 20.1% (Oetzel et al., 2000), but in Australia, about 10% of cows sampled were at a high risk of acidosis (Bramley et al., 2005). In Ireland, 11% of cows were SARA-positive (pH ≤5.5) and 42% were marginal (pH 5.6–5.8) (O'Grady et al., 2008) and in the Dutch province of Friesland was 13.8% (Kleen at al., 2009).

The prevalence of SARA in Egyptian dairy herds was higher than most other countries, and this may

be due to presence of some risk factors which were recorded in this study as bread and beat tops mixing with ration as well as neglecting application of exogenous buffers as sodium bicarbonate and magnesium hydroxide. Decrease roughages percent in the feed and absence of yeast mixing with ration also may play role.

The ruminal pH was significantly lower in SARA positive cows than in SARA negative and SARA marginal cows (P<0.05). The depression of ruminal pH in dairy cattle with SARA is mainly due to the total accumulation of VFAs alone and is not due to lactic acid accumulation in the rumen as was recorded by Krause and Oetzel, (2006).The time of sedimentation and the time required for reduction of methylene blue significantly increased in SARA-positive cows than in SARA-negative and SARA-marginal cows (P<0.05) (tab.2).

These results similar to the results reported by Slyter, (1976); Dirksen, (1990); This could be ascribed to the destruction of normal micro flora (cellulolytic bacteria) and a shift in their pattern from predominantly Gram negative to amylolytic Gram positive nature and this agree with Randhawa et al. (1989). There was a significant decrease (P<0.05) in ruminal protozoa motility in SARA positive-cows (tab.2). These results are in agreement with Goad et al. (1998) who found that motility and count of ruminal protozoa decreased at rumen pH value of 5.2.

There were some changes in color, odor and consistency in ruminal fluid of SARA positive cows (tab.3). These changes may be due to decrease in activity of ruminal protozoa, bacterial changes from gram negative to gram positive bacteria and these findings agree those obtained by Dirksen, (1990); Garry, (2000).

There was a significant decrease in milk fat % (P<0.05) in SARA-positive cows. The fat % of the total milk in the bulk tank was within normal range (tab.4). These results agree with those reported by Kleen et al. (2003); Nordlund, (2004); Enjalbert et al. (2008) and Bipin et al. (2016) but not agree with Oetzel, (2005); Tajik et al. (2009) who reported that SARA had no effect on milk fat % in farm condition.

The milk amount decreased significantly in SARA-positive cows than SARA-marginal and SARA-negative cows (P<0.05) (tab.4). These findings were in agreement with Dong et al., (2013); also these results were confirmed by Bipin et al. (2016).

Urine pH in all examined cows was alkaline. There was a significant difference between SARA-positive, SARA-marginal and SARA-negative cows (P<0.05) (tab.4). These results agree with those reported by Kleen, (2004); Tajik et al. (2009); Ceroni and Shabani, (2011) but not agree with Enemark et al. (2002) who believe that urine acidity is efficient parameter in SARA diagnosis.

Some physical characters of feces have been changed in relation to the decrease in the ruminal pH, as consistency, fiber particle size and presence of undigested food in the feces. It was recorded that feces of 10-20 % of cows in SARA-positive herds were soft and contain fiber particle size greater than 2.5cm in length in addition to undigested grains. These changes in fecal characters similar to that enumerated by Bolton and Pass, (1988).

There was a significant difference (P<0.05) in the albumin level between SARA-positive, SARA-marginal and SARA-negative cows (tab.5). In this study there was a mild hypoalbuminemia in SARA-positive cows and this decrease in the level of albumin may be due to infiltration of liver with fat and effect of endotoxines produced from the rumen on liver performance. These results agree with what reported by Sevinc et al. (2001).

There was a significant difference in the ALT, AST and GGT activities between SARA-positive, SARA-marginal and SARA-negative cows (P<0.05) (tab.5). These results agree with Lechowski, (1997) who recorded a significant increase in serum ALT, AST and GGT activities in cows subjected to sub acute ruminal acidosis for two months before parturition in comparison with the control group. The increase in levels of these enzymes may also due to deposition of fats in the liver and these findings were approved by Sevinc et al. (2001).

In this study we recorded some important risk factors, which may lead to occurrence of SARA in the dairy farms such as food particle size (finely ground or coarse), sodium bicarbonate and yeast (mixed with ration or no), roughages incorporation in feeding system (yes or no) as well as bread and beet tops feeding.

The ultrasonographic assay revealed presence of fat deposits in the liver tissue. Deposition of fat in the liver tissue resulted in increased hepatic size, rounding of liver margins, increased of coarseness of echoes, increased echogenisity of the liver parenchyma near the abdominal wall (figs. 1, 2, 3 and 4). This finding of fatty liver agrees with Kimura, (1989); Braun, (1996) who reported that B-

mode ultrasonograms of fatty infiltration of the liver in human and cows are characterized by increased parenchymal echo (bright pattern) and coarseness of echoes, decreased echo penetration at deeper areas of the hepatic tissue (deep attenuation) and vessel blurring.

There was an association between SARA and infiltration of liver with fat . These findings may be due to pervious deposition of fats in liver before parturition. Based on our knowledge, there is no previous literature about relation between SARA and fatty liver. Accumulation of fat in the liver of dairy cattle starts from 2-3 weeks before parturition and continues to 2 weeks postpartum because of the negative energy balance, and then the fat begins to leave the liver (Andrews, 1998).

The limitation o the study should be acknowledged

5. CONCLUSION

The results of the present study indicate that imperfect feed practice may lead to occurrence of SARA in dairy farms with subsequent poor performance and profitability.

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