Assessment of Critical Control Point in Dairy Farms in Khartoum State, Sudan

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ABSTRACT

An attempt was made to apply Hazard Analysis Critical Control Points (HACCP) system in dairy farms in Khartoum State and to suggest control limits based on the international standard. World Health Organization and Food and Agriculture Organization recommended the HACCP system as a modern tool for disease prevention. Critical points were investigated in 150 farms using California Mastitis Test and Rose Bengal Plate Test for detection of bovine mastitis and brucellosis, respectively. The results revealed that the overall prevalence rates of bovine mastitis and brucellosis were 69.3% and 36%, respectively. Other critical points were obtained from the owners of the dairy farms by a questionnaire, using non-probability sampling method or willingness of the owners for interview. Tick infestation was found in 94% of the farms which could be a risk factor and the application of the odds ratio (OR) was 1.694. The use of antibiotics for treatment of animals was, also, practiced in 54.7% of the farms. The critical points associated with environment in dairy farms and distribution of milk in Khartoum State was investigated, using a questionnaire survey and non probability sampling method. The condition of beddings was poor in 70% of the dairy farms. Absence of cleaning and disinfection of the teats were recorded in 108 and 104 farms, respectively. Furthermore, distribution of the milk depended on either vehicle without chilling (50.7%) or donkey carts (49.3%). Quantification of the risk indicated that hand washing of the milkers could be a risk factor (OR = 2.574), while the condition of the housing as well as the distribution of the milk could be a protective factor (OR=0.383 and 0.278, respectively). In conclusion, many critical points associated with animals or environment were observed in dairy farms in Khartoum State. Diseases of animals included infectious and zoonotic disease, flies and tick infestation, use of antibiotics for treatment and absence of veterinary care. The critical points associated with environment were poor hygiene during handling, storage and distribution of the milk. Moreover, the control limits for all mentioned critical points were as follow: Access to veterinary service, vectors control, improvement of general hygiene in the dairy farms during different practices taking in consideration the low temperature during storage and distribution of the milk.

Key words: Critical control points; dairy farms; Khartoum State; Sudan

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INTRODUCTION

The objectives of food safety were to reduce food illnesses and fatalities. The health and hygiene of the cow, the environment in which the cow is housed and milked, hygiene during milking and storage equipment influence microbial contamination of milk. Milk is considered a good medium for bacteria growth including pathogenic organisms which have a great impact on public health (Ibtisam and Mohboba, 2007). Hazard Analysis Critical Control Points (HACCP) concept was introduced in the United States in 1971 at the Conference of Food Protection where it was recommended for widespread use (Bauman, 1974). The call for change was galvanized in the early 1990s with a tragic outbreak of *Escherichia coli* O157:H7 food borne illness in the North West of the United States. Zoonotic diseases such as brucellosis and tuberculosis can be transmitted by using unhygienic milk or milk products. These diseases have been reported from different parts of Sudan. Abdalla (2006) reported that the prevalence rate of brucellosis in Khartoum State was 23.2% using Rose Bengal Plate Test (RBPT). Abbas (2007) confirmed the presence of tuberculosis in both cattle and man in Khartoum State using Single Intradermal Comparative Tuberculin Test (SICTT), bacteriological procedures and Nested Polymerase Chain Reaction (NPCR).

Mastitis in dairy cows is a multifactorial disease with a long history among the economic losses due to loss of milk production, treatment costs, extra labour and premature culling of chronically infected cows. Hygiene at all levels should be addressed: housing, feeding, cows in the barn and milkers. When an udder health control (UHC) programme is designed and implemented, it warrants a persistent and protocol-based approach by both the farmer (and his co-workers) and a coaching veterinarian in all areas of udder health (Hancock and Dargatz, 1995). Raw milk is approximately 19.4-21.1°C as it comes from the cow, and needs to be chilled to 8°C as fast as possible. For transporting fresh raw milk, a cooler or ice chest is needed in order to keep the milk cold (4.4^{o}C) or lower at all the times. Pre-milking sanitation (udder washing) is a critical point for controlling the bacteria in milk (Mossel *et al*., 1995). Hazard-aware dairy workers, work with dairy animals or work in and around dairy operations, know what personal protective equipment (PPE) wear for certain jobs and what safety precautions to follow when handling chemicals in areas of hazardous atmospheres. HACCP addresses product quality through the control of the production process. It was originally developed for the NASA space programme to safeguard astronauts from chemical, physical and micro-biological hazards through food (Hulebak and Schlusser, 2002).

Objectives of the study:

1\ To apply HACCP in dairy farms in Khartoum State in order to clarify that this approach may yield better results than conventional methods.

2\ To determine the critical points associated with animals, environment in farms and distribution of milk which can affect the quality of the milk for human consumption.

3\ To suggest the control limits for the different critical points based on the recommended international standards.

MATERIALS AND METHODS

Study area

Khartoum State is located in central Sudan (altitude 382 m (1253 ft); the average temperature is 29.8°C (the average monthly temperatures is 42°C in May and June and 16°C in January). The average monthly rainfall is 164 mm. The driest weather is in January, February, March, November and December. The wettest weather is in August with an average of 72 mm Mean relative humidity is 21.8% and on a monthly basis it ranges from 13% in March and April to 42% in August. Hours of sunshine range between 8.4 hours per day in July and 11.1 hours in February.

Determination of the HACCP associated with animals

Collection of milk and blood samples

Milk and serum samples were collected from dairy farms from Khartoum, Omdurman and Khartoum North in order to determine the presence of bovine mastitis and brucellosis. Selection of the farms was based on the willingness of the owners. That meant not all the dairy farms had the same chance for being selected and this is called non-probability sampling as described by Thrusfield (2007). A total of 150 milk samples (50 in Khartoum, 50 in Omdurman and 50 in Khartoum North) were collected at the animal level for determination of the presence of bovine mastitis. Before the collection of milk samples, the teats were disinfected with cotton wool moistened with 70% ethyl alcohol. California Mastitis Test (CMT) was immediately employed after collection of the milk samples in the dairy farms using the method described by Schalm *et al*. (1971) and Quinn *et al*. (1994). Equal volumes of commercial CMT reagent and milk were mixed and formation of gel was observed. The interpretation of the result was done as described by Quinn *et al.* (1994) (Table 1). For analysis, negative (0) and trace (±) gel formation were considered as negative results and different intensities of positive gel formation (1, 2 and 3) were considered as positive results. A total of 150 serum samples (50 in Khartoum, 50 in Omdurman and 50 in Khartoum North) were collected for determination of the presence of *Brucella* antibodies. Blood samples were collected by venopuncture of the jugular veins using syringes. Sera were separated from the clots, placed in the plastic vials and preserved at -20^{o}C

Other Critical Points Associated With Animals

Other critical points associated with animals were determined by the means of questionnaire of owners of the dairy farms. A total of 150 owners responded to the questionnaire (50 in Khartoum, 50 in Omdurman and 50 in Khartoum North). Selection of the owners was based on their willingness for interview (Non-probability sampling method) as described by Thrusfield (2007). Information on tick infestation, presence of clinical cases, history of abortion, access to veterinary services, presence of zoonotic diseases and additions of antibiotics to the diet or drinking water were recorded.

Critical Points Associated With Environment and Distribution of Milk

Information on general conditions of environment in the dairy farms and transportation of the milk were obtained from the owners by the means of a questionnaire using Non-probability sampling methods as described by Thrusfield (2007). Information such as type of housing, condition of housing, condition of bedding, use of detergent, cleaning or disinfection of the teats and milkers hands and storage and distribution of the milk were recorded.

Data analysis

IBM SPSS version 19 was used for data analysis. The results were presented as descriptive statistics in tables using frequency and percentage. Analytical statistics using Chi-square (x^2) and Odds Ratio (OR) were employed for the purpose of getting significance level and estimation of the risk associated with critical points. For Chi-square (x^2), the interpretation depended on *p*-value. Odds Ratio (OR) was used only for significant association and when the OR was greater than one. The factor could be a risk factor, but when it was less than one the factor could be a protective factor.

RESULTS

The study was carried out in dairy farms in Khartoum state to determine the critical points that can influence the quality of the milk as well as to suggest the control limits. The results revealed that the overall prevalence rate of bovine mastitis based on CMT at animal level was very high 69.3% (104/150) 27.3% (n=41), 26.7% (n=40), and 15.3% (n=23), for Khartoum, Omdurman and Khartoum North, respectively. While, the overall prevalence rate of *Brucella* antibodies based on RBPT was 36% (54/150), 10% (n=15), 13.3% (n=20) and 12.7% (n=19) for Khartoum, Omdurman and Khartoum North, respectively (Table 2).

Other critical control points associated with animals were investigated using questionnaire survey. The main results showed that tick infestation was present in the most of the dairy farms in Khartoum state 94% (n=141 out of 150). Moreover, the presence of zoonotic diseases and the use of antibiotics for treatment of infected animals

were confirmed in this study: 54.7% (82/150) and 75.3% (113/150), respectively. It was observed that access to veterinary services was absent in 70% (105/150) (Table 3). Estimation of some risk factors associated with animals based on access to veterinary services revealed, that tick infestation could be a risk factors (OR = 1.694), while the use of antibiotics for the treatment of animals could be a protective factor (OR = 0.453) (Table 4).

On the other hand, the critical points associated with environment in dairy farms in Khartoum state were investigated using questionnaire survey. The main results showed that the condition of beddings was poor in 105/150 (70%). Absence of the cleaning and disinfection of the teat were recorded in 108/150 (72%) and 104/150 (69.3%). Moreover, hand washing was practiced by milkers in only 32% (48/150) of the dairy farms. Storage of the raw milk at the room temperature was in all farms (100%). Distribution of milk depended on either vehicle without chilling (50%) or donkey cart (49.3%) (76 and 74 out of 150, respectively) (Table 5). Estimation of some risk factors associated with environment on the basis of regular examination of the milk revealed that hand washing of the milkers could be a risk factor (OR = 2.574). While, the condition of housing as well as the distribution of the milk could be protective factors (OR = 0.383 and 0.278, respectively) (Table 6).

DISCUSSION

This study was conducted in dairy farms in Khartoum state in order to apply Hazard analysis critical control points (HACCP). Both the critical control points associated with animals or environment were determined by using screening tests and questionnaire survey. As seen from the results, the morbidity rate of bovine mastitis and prevalence of *Brucella* antibodies were was very high in dairy farms in Khartoum state. These diseases are known as ones of critical points associated with animals. A number of researchers in Sudan confirmed the presence of infectious diseases particularly zoonotic diseases in intensive and semi-intensive production system. For instance, Abdalla (2006) using RBT, reported a prevalence rate of bovine brucellosis in Khartoum state as 23.2%. Similarly, Mahmoud (2010) showed that the MRT prevalence rate of the disease in West Kordofan State was 24.6%, 13.6% and 5% by RBPT, Serum Agglutination Test (SAT), ELISA and Milk Ring Test (MRT), respectively. Both clinical and sub-clinical mastitis were, also, observed in dairy farms in Khartoum state (Elsayed, 2000; Mahady, 2010). Moreover, some pathogens such as *Staphylococcus aureus*, *Streptococcus pyogenes* and *E. coli* are considered major pathogens that cause bovine mastitis as well as of major public health concern. Many authors have isolated the above mentioned pathogens from different parts of the country (Mohamed-Ahmed, 2005; Omer, 2007, Nahid and Ibtisam, 2007). Abbas (2007) confirmed the presence of tuberculosis in both cattle and man in

Khartoum state using Single Intradermal Comparative Tuberculin Test (SICTT), bacteriological procedures and Nested Polymerase Chain Reaction (NPCR).

Regarding, detection of antibiotics in milk, most of the owners used antibiotics for treatment and some of them were added to feed or drinking water. Similarly, Abdel Rahman (2001) stated that minimum detectable concentration for oxyteracycline was 2 mg/ml milk, 48 mg/ml milk for benzyl penicillin and 25 mg/ml milk for Tylosin. He found that all the milk samples collected from bulk milk of the farms and shops were free of antibacterial residues, whereas as 76.6% of the samples collected from treated cows with intramammary infusion were positive for antibiotic residues. Furthermore, Abdalla (2005) found that 25% of milk samples which were collected from the central market in Khartoum state in summer were positive for antibiotic residues using *Bacillus subtilis* ATCC – bb33 as the test organism. Her results, also, revealed high positive samples (37.9%) for antibiotic residues in winter. Control of brucellosis and other zoonotic diseases could be achieved by vaccination, culling or treatment of infected animals. Withdrawal period should be considered in case of addition of antibiotics to feed and drinking water or use of antibacterial agents for the treatment of infected animals. Good ventilation, avoidance of overcrowding and regular removable of waste are known as the best methods for controlling bad conditions of housing and bedding. While, temperature and good hygienic practice are necessary for protection of raw milk from growth of microorganisms during different practices in the dairy farms, handling, storage and distribution of the milk.

The results of the questionnaire survey confirmed presence of flies, tick infestation, abortion and zoonotic diseases as well as there was no access to veterinary services in the most of the dairy farms. All the above-mentioned factors have a great impact on animal health affecting both quantity and quality of the milk for human consumption.

Many critical points associated with the general conditions of the environment in dairy farms were observed. For instance, bad conditions of housing and beddings were recorded for most of the dairy farms. Use of detergents, cleaning or disinfection of the teats and hand washing by milkers were at low scale in the current study. Moreover, most of the owners did not consider the importance of low temperature for storage and distribution of the raw milk. The current findings are in agreement with Nahid and Ibtisam (2007) who stated that hygienic quality of milk that were collected from shops in Khartoum State was very low due to high bacterial counts, and detection of *Brucella* antibodies by MRT. Cleaning and removal of soil, beddings and manure from udders and flanks of cow before milking is necessary to prevent contamination of milk. Milk production in Sudan is faced with several problems such as poor husbandry practices, bad handling of raw milk during storage at the farm and during transportation, bad information infrastructure, poor cooling facilities, high ambient temperature and the long distance between places of production and consumption centers.

An attempt was made in Sudan to determine HACCP in dairy farms in Khartoum State. Abdel Gadir (2009) reported that in dairy farms in Khartoum State reported that poor building construction, poor water supply poor hygiene of farms as well as milkers and accumulation of dung and animal waste were critical. She stated that there were two critical points assigned for milk distribution chain; the first critical point was hygiene of raw milk production and distribution chain, while the second critical point was to control milk temperature.

Conclusion

Many critical points associated with animals or environment were observed in dairy farms in Khartoum state: 1\ The study revealed infectious and zoonotic diseases, presence of flies and tick infestation, use of antibiotics for treatment or in feed and drinking water and absence of veterinary care in the most dairy farms. 2\ Critical points associated with environment were poor hygienic conditions, handling, storage and distribution of the milk. The control limits for the mentioned critical points were as follow: access to veterinary service, use of disinfections and detergents, vectors control, improvement of general hygienic conditions in the environment in the dairy farms as well as the consideration the issue of the temperature during storage and distribution of the milk.

Recommendations

1\ Dairy farms should implement a documented food safety management system based on HACCP principles.

2\ Using bacterial counts or Somatic Cell Count (SCC) as well as regular examination of the milk using screening tests such as CMT and MRT are required for evaluation of the quality of the milk.

3\ An attention should be made for increasing awareness of the owners, technical and financial resources, an effective institutional frame work, trained man power and sufficient information about hazards and risks involved.

4\ It is important that milk distribution chain should be monitored by health and veterinary authorities to ensure safe milk to consumers.

Table 1: Interpretation of CMT results and relationship with Somatic Cell Counts (SCC)

CMT	Interpretation	Visible Reaction	Total Cell Count

score			
0	Negative	Milk fluid and normal	0-200,000 0-25% Neutrophil
±	Trace	Slight precipitation	150,000-500,000 30%-40% Neutrophil
1	Weak positive	Distinct precipitation but no gel formation	400,000-1,500,000 40%-60% Neutrophil
2	Distinct positive	Mixture thickness with a gel formation	300,000-5,000,000 60%-70% Neutrophil
3	Strong positive	Viscosity greatly increased strong gel that is cohesive with a convex surface	>5,000,000 70%-80% Neutrophil

Source: Quinn *et al.* (1994)

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Table 2: The presence of bovine mastitis and *Brucella* antibodies in dairy farms in Khartoum State

Study sites	**Mastitis**	*Brucella* antibodies
	No. examined/No. positive (%)	**No. examined/No. positive (%),**

1-Khartoum **2-Omdurman** **3-Khartoum North**	50/41(27.3) 50/40(26.7) 50/23(15.3)	50/15(10) 50/20(13.3) 50/19(12.7)
Overall	150/104(69.3)	150/ 54(36)

- Bovine mastitis based on CMT & scores of 1, 2 and 3 were considered positive
- *Brucella* antibodies based on RBPT.

Table 3: Summary of questionnaire survey responses with regard to critical points that associated with animals

Unit	Site Khartoum	Omdurman	Khartoum North	Total	Chi-square *p*-value	Interpretation
1- tick infestation **1- Yes** **II- NO**	50(/100%) 0(0%)	50(/100%) 0(0%)	41(82%) 9(18%)	141(94%) 9(6%)	$x^2_{=19.1}$ *P*=0.00 (p<0.05)	Significant
2-presence of flies **1- Yes** **II- NO**	35(70%) 15(30%)	38(76%) 12(24%)	28(56%) 22(44%)	101(67.3%) 49(32.7%)	$x^2_{=4.8}$ *P*=0.09 (p>0.05)	Not significant
3-Access to Vet. Service **1- Yes** **II- NO**	11(22%) 39(78%)	16(32%) 34(68%)	18(36%) 32(64%)	45(30%) 105(70%)	$x^2_{=2.5}$ *P*= 0.3(p>0.05)	Not significant
4- presence of abortion **1- Yes** **II- NO**	25(52%) 24(48%)	16(/32%) 34(68%)	31(62%) 19(38%)	73(48.7%) 77(51.3%)	$x^2_{=9.3}$ *P*=0.009 (p<0.05)	Significant
5-Presence of zoonotic diseases **1- Yes** **II- NO**	18(36%) 32(64%)	21(41%) 29(58%)	86%) 7(14%)	82(54.7%) 68(45.3%)	$x^2_{=30.1}$ *P* =0.005 (p<0.05)	Significant
6- Use of antibiotics for treatment **1- Yes** **II-NO**	39(78%) 11(22%)	37(74%) 13(26%)	37(74%) 13(26%)	113(75.3%) 37(25.7%)	$x^2_{=0.3}$ *P*= 0.9 (p>0.05)	Not. Significant
7- Addition of antibiotics to feed **1- Yes** **II- NO**	18(36%) 32(64%)	11(22%) 39(78%)	4(8%) 46(92%)	33(22%) 117(78%)	$x^2_{=11.4}$ *P*= 0.003 (P<0.05)	Significant
8- Addition of antibiotics to drinking water					$x^2_{=0.2}$	

1- Yes	12(24%)	14(28%)	13(28%)	39(26%)	*P* =	Not.
II- NO	38(76%)	36(72%)	37(74%)	111(74%)	0.9(P>0.05)	significant

Table 4: Estimation of some risks associated with animals based on access to veterinary services

Factor	Chi – square *P* – value	Odds Ratio (OR) 95% CI	Interpretation
1- Tike infestation	$\chi^2 = 4.103$ $P = 0.043^*$	OR = 1.094 95% CI (1.03 – 1.16)	Risk factor
2- Presence of flies	$\chi^2 = 1.976$ $P = 0.797$		Risk can not be estimated
3-Presence of abortion	$\chi^2 = 1.221,$ $P = 0.269$	-	Risk can not be estimated
4-Presence of zoonosis	$\chi^2 = 0.866, p = 0.352$		Risk can not be estimated
5- Using antibiotics for treatment	$\chi^2 = 4.102, P = 0.643^*$	OR = 0.453 95%CI(0.21– 0.98)	Protective factor
6-Addition of antibiotics to feed	$\chi^2 = 1.778$ $P = 0.182$	-	Risk can not be estimated
7- Addition of antibiotic to water	$\chi^2 = 0.873$ $P = 0.350$	-	Risk can not be estimated

- *P*-value was significant ($p < 0.05$), - 95% CI = 95% Confidence Interval

Table 5: Summary of questionnaire responses with regard to critical points associated with environment in dairy farms and distribution of the milk.

Unit	Site Khartoum Omdurman Khartoum North	Total	Chi-square p-value	Interpretation
1- Conditions of beddings **Excellent** **Good** **Bad**	12(/24%) 12(/24%) 21(42%) 38(76%) 38(76%) 29(58%)	45(30%) 105(70%)	$\chi^2_{=5.14}$ *P* = 0.076 (*p* > 0.05)	Not. significant
2- Conditions of housing **Excellent** **Good** **Bad**	26(52%) 26(52%) 42(84%) 24(48%) 24(48%) 8(16%)	94(62.7%) 56(37.3%)	χ^2=14.6 *P*=0.001(p< 0.05)	Significant

3- Using of detergents **Yes** **NO**	9(18%) 9(18%) 13(26%) 41(82%) 41(82%) 32(64%)	31(20.7%) 119(79.3%	$\chi^2_{=1.30}$ *P*=0.522 (p > 0.05)	Not significant
4- Cleaning teats **Yes** **NO**	14(28%) 14(28%) 14(28%) 36(72%) 36(72%) 36(72%)	42(28%) 108(72%)	$\chi^2_{=0.00}$ *P*=1.000 (p > 0.05)	Not significant
5- Disinfection of the teats **Yes** **NO**	18(36%) 18(36%) 10(20%) 32(64%) 32(64%) 40(80%)	46(30.7%) 104(69.2%	$\chi^2_{= 4.01}$ *P*=0.134(p > 0.05)	Not significant
6- Regular examination of the milk **Yes** **N0**	25(50%) 37(74%) 18(36%) 25(50%) 13(26%) 32(64%)	80(53.3%) 70(46.7%)	$\chi^2_{=14.84}$ *P*=0.001(p< 0.05)	Significant
7- Hand wash by milkers **Yes** **NO**	10(20%) 20(40%) 18(36%) 40(80%) 30(60%) 32(64%)	48(32%) 102(68%)	$\chi^2_{=5.147}$ *P*=0.076(p > 0.05)	Not. significant
8\ Storage of raw milk in the farms **I-Room temperature** **II-Refrigerator**	50(100%) 50(100%) 50(100%) 0(0%) 0(0%) 0(0%)	150(100%) 0(0%)	$\chi^2_{=7.061}$ *P*=0.029(p< 0.05)	Significant
9\ Distribution of milk **Chilling vehicle** **Open vehicle without chilling** **III- Donkey cart**	- - 32(64%) 21(42%) 23(46%) 18(36%) 29(58%) 27(54%)	76(50.7%) 74(49.3%)	$\chi^2_{= 5.5}$ *P* = 0.064(*p*> 0.05)	Not. significant

Table 6: Estimation of some risks associated with environment in the dairy farms and distribution of the milk.

Factor	Chi – square P – value	Odds Ratio (OR) 95% CI	Interpretation
1- Conditions of beddings	$\chi^2_{= 7.574}$ $P = 0.006$*	OR = 0.385 95% CI (0.191 –0.765)	Protective factor
2- Condition Of housing	$\chi^2_{= 0.510}$ $P = 0.475$		Risk can not be estimated
3- Using of detergents	$\chi^2_{= 1.648}$ $P = 0.306$	-	Risk can not be estimated
4- Cleaning of the teats	$\chi^2_{= 0.021,}$ $P = 0.884$		Risk can not be estimated
5- Disinfection of the teats	$\chi^2_{= 0.027,} P = 0.868$		Risk can not be estimated

6- Hand washing by the milkers	$\chi^2_{= 6.741,}$ $P = 0.009^{**}$	OR = 2.574 95%CI(1.248– 5.310)	Risk factor
7- Store of raw the milk	$\chi^2_{= 0.602,}$ $P = 0.43$		Risk can not be estimated
8- Distribution of the milk	$\chi^2_{= 14.254}$, $P = 0.000^{**}$	OR = 0.278 95%CI(0.142 – 0.547)	Protective factor

- 95% CI: 95% confidence interval
- Risks was estimated based on regular examination of the milk