EAS Journal of Nutrition and Food Sciences
Abbreviated Key Title:EAS J Nutr Food Sci
ISSN: 2663-1873 (Print) & ISSN: 2663-7308 (Online)
Published By East African Scholars Publisher, Kenya

Volume-2 | Issue-3 | May-June; 2020 | DOI: 10.36349/easjnfs.2020.v02i03.006

OPEN ACCESS

Research Article

Risk Assessment of Brucellosis in Dairy Cows in Bahri North Locality, Sudan

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Article History
Received: 04.06.2020
Accepted: 19.06.2020
Published: 27.06.2020

Journal homepage:
https://www.easpublisher.com/easjnfs

Quick Response Code

Abstract: Milk Ring Test (MRT) was conducted to determine the seroprevalence of bovine brucellosis in dairy cows in Bahri North locality, Sudan, to evaluate the associated risk factors of brucellosis as well as to estimate the qualitative risk of brucellosis to public health from consuming raw milk contaminated with *Brucella*. The Seroprevalence of brucellosis in dairy cows screened was found to be 33.3% using MRT. Chi-square test showed significant association between brucellosis and risk factors such as breed (P-value.030), rearing system (P-value.008), sharing of bull- for fertilization with other farms (P-value.008), and testing of new animals before introducing them into the farm (P-value.030). All results comparing the association between Chi square and the rearing system were found to be significant in the Logistic Regression (P-value.041).Value chain using the OIE frame work for risk analysis with some adjustments as to fit with an assessment for an endemic disease, together with value chain analysis designed by FAO for disease management were used to qualitatively assess the risk for the spread of brucellosis amid dairy cows and hence milk produced. The release pathway of brucellosis in value chain represents the probability of spread of *Brucella* into the farm and prodsucing contaminated milk , this was found to be high which means the risky event is likely to occur this year or in frequent intervals. In this study the exposure risk represents probability of producing and marketing of milk that contaminated with *Brucella*, which was assessed to be medium, which means the risky event is likely to occur more than once in the next three years as explained by the DEFRA model. The overall risk estimation for brucellosis was found to be high, which means the risky event is likely to occur among farms workers, and consumers this year or at frequent intervals.

Keywords: Brucellosis, risk assessment, Sudan , Bahri

INTRODUCTION

According to the World Health Organization (WHO), yearly, there are millions of people becoming sick due to food sourced zoonosis. In terms of public health one of the most significant zoonosis is brucellosis which is caused by *Brucella spp* (Pappas *et al.*, 2006).

Brucellosis is considered to be a serious problem in at least 86 countries (Hamdy and Amin, 2002), it is also considered as one of the major zoonotic diseases transmitted by direct contact within animals and/or their secretions, or by consuming milk and dairy products (Díaz, 2013). Except cats which are resistant to *Brucella* infection, almost all domestic species can be affected with brucellosis (Radostits *et al.,* 2000). Economically brucellosis leads to losses due to abortion, premature off springs, and delayed oestrus, weak or dead calves. It also leads to infertility, sterility and decrease in milk yield due to mastitis and interruption of lactation. Despite the fact that brucellosis is well controlled in most developed countries but it remains an uncontrolled diseases in regions with poor animal and public health standards leading to high endemic in Africa, Middle East, the Mediterranean, parts of Asia and Latin America (Refai, 2002). According to the Centre for Disease Control and Prevention, *Brucella* considered as a possible bio-terrorist agent (CDC, 2002). The Centre also classifies *Brucella abortus*, *Brucella melitensis* and *Brucella.suis* as "agents of mass destruction (Elzer, 2002).

Identifying and analyzing of the risk factors for *Brucella* contamination of raw whole milk has great significance for the prevention and control of human brucellosis (Ning *et al.,* 2012). One of the most important factors leading to the spread of *Brucella* infections among humans is milk. This is due to the habits of milk consumption which varies according to cultural habits, unhygienic, and unhealthy factors in the preparation and distribution processes (Sam *et al.,* 2012). Many pathogenic organisms, such as *Brucella*,

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may remain viable if milk is raw or the boiling temperature is insufficient (Chye, *et al.*, 2004). In situations where good hygiene practices are not practiced the development of the disease in humans cannot be prevented.

Several reports indicated that, brucellosis is a common disease in Sudanese animal, although the control measures were adopted, but annually there are new cases reported and the disease is widely spread (Anon, 2001). The first *Brucella spp.* isolated from a dairy farm in Khartoum was *Brucella abortus* (Bennett, 1943), but the disease in human was reported in 1904 in River Nile State, Berber area (Haseeb, 1950), and the first isolation of *Brucella abortus* from an infected person was reported by (Erwa, 1966). *Brucella abortus*, biovars 1, 3, 6, 7 and *Brucella melitensis*, biovars 2 and 3 were found associated with the disease in Sudan (Musa *et al.*, 2008). The disease has great importance for health authorities and policy makers in Sudan as it affects human health and livestock, therefore the disease was studied by many researchers, but for the first time this study tried to estimate the overall risk of brucellosis through the raw milk production value chain qualitatively.

The objectives of the study were to evaluate the associated risk factors which may lead to brucellosis as well as to estimate the qualitative risk of brucellosis for public health when consuming raw milk contaminated with *brucella.*

MATERIALS AND METHODS

Study Population:

The target population for this study was the dairy cows in Bahri North locality during the year 2018.

Data Collection:

A questionnaire was used for collecting data on risk factors. The questionnaire contained questions about some potential risk factors that might have an association with the brucellosis sero-prevalence. It also included general questions that covered husbandry system, housing of animals, vaccination against brucellosis, farm practices, previous/currently infection, farm workers attitudes and types of breed for each farms sampled . Another questionnaire was used to collect data about some risk factors that might have an association with the form of consumption of milk, which varies according to cultural habits and unhygienic factors in the preparation, distribution, processes and consumption of milk. Data about brucellosis sero-prevalence and the numbers of the dairy cows located in the study area was collected from the General Directorate of Animal Health and Epizootic Diseases Control of the Ministry of Animal Wealth. Scientific information about epidemiology of brucellosis was collected from scientific publications on open access e- journals and websites.

METHODOLOGY

Sampling:

Seventy two (72) milk samples and twelve (12) questionnaire data were collected from twelve (12) dairy cows' farms. One hundred and twenty (120) questionnaire data were collected from consumers living in Bahri locality.

Collection of Milk Samples:

About 5 ml of raw milk was taken from each milk bulk tank directly into a labeled sterile plastic container. The samples were kept in an ice box and then transported to College of Veterinary Medicine Laboratory, University of Bahri for MRT.

Milk Ring test:

The antigen used in this test was prepared by the Central Veterinary Research laboratory (CVRL) Soba, Khartoum, following the procedure described by Alton *et al.*, (1988).

Data analysis:

For all appropriate statistical analyses the Statistical Package for Social Sciences (SPSS) for Windows version 21.0 was used. Chi-square test was conducted to test the association between the investigated 19 risk factors and the (MRT) results. In the second step, a logistic regression model was used to predict the association between the significant risk factors (*P*- value$\leq$ 0.05) in the univariate analysis.

Risk Assessment

Methodology for Qualitative Risk Assessment: Qualitative risk assessments are commonly used for screening risks to determine whether they merit further investigation, and can be useful in the 'preliminary risk management activities (FAO/WHO, 2002).The four elements of risk assessment were:

i. Hazard identification:

A hazard is something potentially harmful to animals, human, plant and environment (FAO, 2011).

ii Risk question:

What is the risk associated with dairy cows serologically positive for *brucella* to the farms workers and consumers of milk in Bahri North? (FAO,2011)

iii. Risk pathway:

Scenario tree is appropriate and effective way in depicting biological pathways (MacDiarmid and Pharo, 2003). The events that have to occur in order for the unwanted outcome to occur is called risk pathway and the analysis of this pathway is the main tool used in risk assessment (FAO, 2011).

Release assessment:

In this study it estimated the probability or likelihood of introducing the *Brucella* to the dairy cows within the farm (OIE, 2014).

Exposure assessment:

In this study it estimated the probability or likelihood of the exposure of cattle herds to the Brucellosis, as well as exposure of human population to the *brucella* contaminated milk.

Qualitative estimation for the probability (likelihood): It involves two steps:

1. Information (derived from collected data) were put together with the risk pathway in a tabular frame work in order to make a systematic process and evidence-based assessment and encourage transparency.
2. Logical conclusions were extracted by comparing the requirements for each step with the actual situation (OIE, 2014).
3. The overall assessment of risk is made based on the probabilities along the pathway, the degree of exposure, and the impact of the unwanted outcome, using a qualitative risk assessment scheme used by Defra Figure (1a and 1b)

	H	**M**	**M**	**H**	H*
↑	**M**	L	M	M	H
	L	VL	L	M	M
Likelihood	**VL**	VL	VL	L	M
		VL	**L**	**M**	**H**
		Impact	→		

Figure (1a): Risk estimation scheme by Defra. (OIE, 2014)

Likelihood		Description
VL	**Very low**	Rare (the risky event may occur in exceptional circumstances).
L	**Low**	Possible (the risky event may occur in the next three years).
M	**Medium**	Likely (the risky event is likely to occur more than once in the next three years).
H	**High**	Almost certain (the risky event is likely to occur this year or in frequent intervals).

Figure (1b) : Risk estimation scheme by Defra. (OIE, 2014)

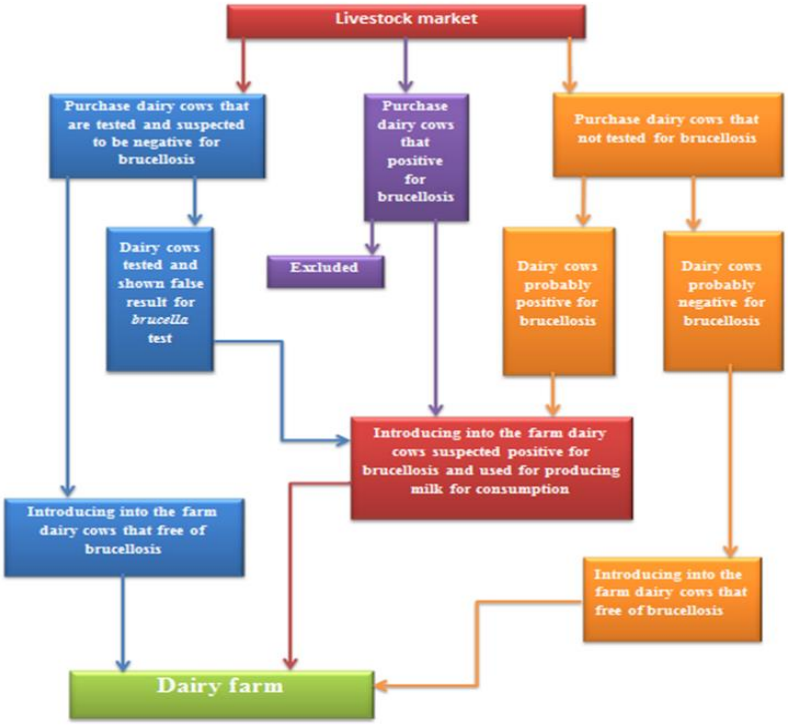

Figure (2): Scenario tree for a release risk assessment pathway

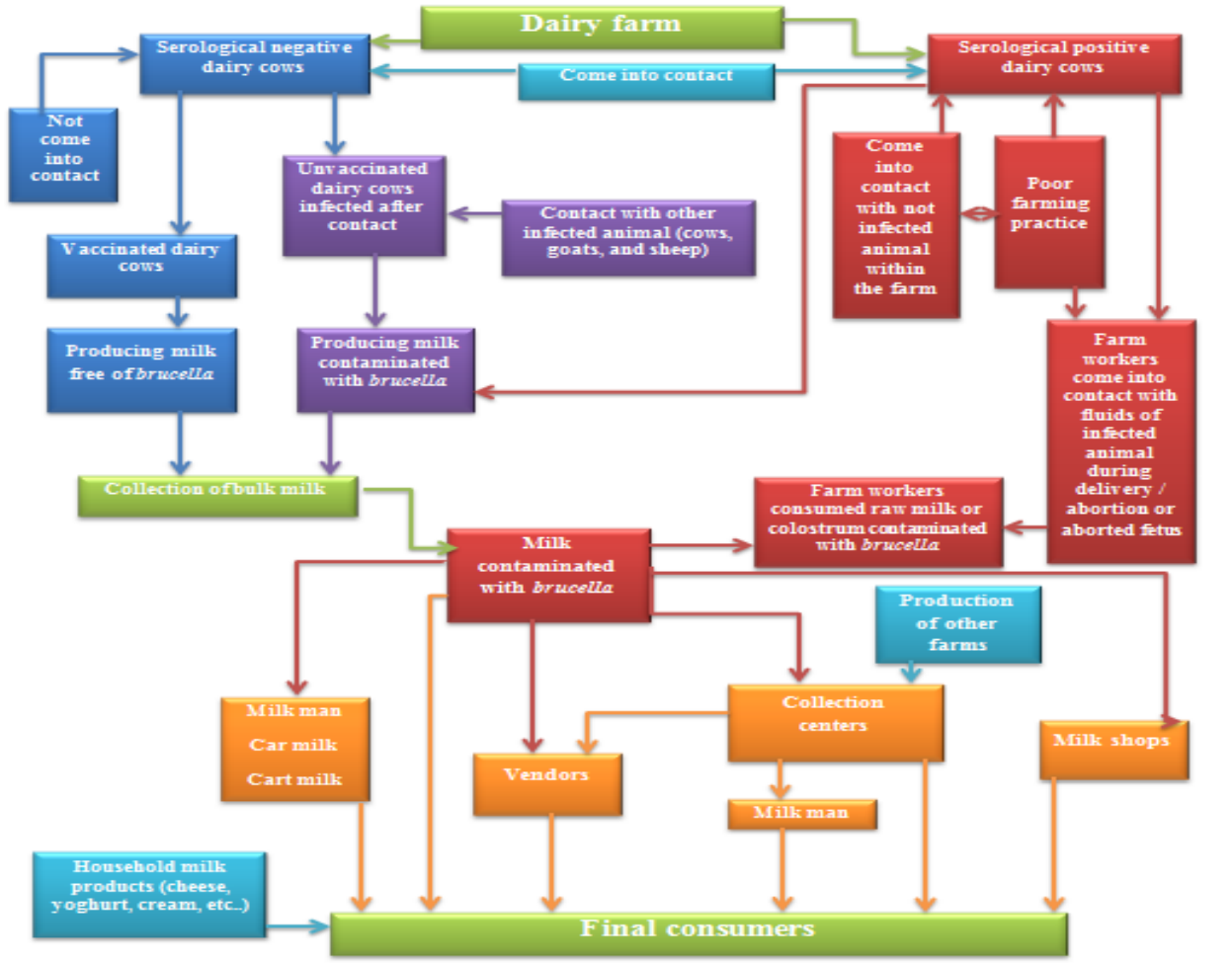

Figure (3): Scenario tree for exposure assessment.

RESULTS

Milk Ring Test (MRT): Form the 72 milk samples, 24 samples (33.3%) were seropositive for *Brucella* using Milk Ring Test (MRT).

Table (1): Frequencies of positive milk samples tested by (MRT) screening test

	Frequency	Percent %
Negative	48	66.6
Positive	24	33.4
Total	72	100.0

Distribution of positive and negative samples according to farms:

The results showed that all animals tested in three farms (2,6,7) were found seropositive while in farm number (4) there were three samples positive from the total of five samples, as well as in farm number (10) there was one sample positive from the total of six samples. Seven farms (1,3,5,8,9,10,11) were serologically negative for MRT.

Table (2): Distribution of positive & negative tested samples of milk for (MRT) between the farms

	Positive		**Negative**		**total**
	N	Percent	N	Percent	
Farm1	-	-	6	100%	6
Farm2	7	100%	-	-	7
Farm3	-	-	6	100%	6
Farm4	3	60%	2	40%	5
Farm5	-	-	6	100%	6
Farm6	7	100%	-	-	7
Farm7	6	100%	-	-	6
Farm8	-	-	5	100%	5
Farm9	-	-	6	100%	6
Farm10	-	-	6	100%	6
Farm11	-	-	6	100%	6
Farm12	1	17%	5	83%	6
	24		48		72

Risk Factors Associated With Brucellosis In Dairy Cows Within The Farm:

About twelve (12) potential risk factors were investigated using structured questionnaire for every sampled farm and other collected data, out of all the risk factors, four factors – breed, rearing system, testing of new animal before introducing into the farm, and using of bull for fertilization in other farms -were found to be associated with seroprevalence of brucellosis (P-value ≤ 0.05) when Chi- square was used. The four factors that were found to be significant in the Chi Square test were subjected to analysis using Logistic Regression model. One factor which was the rearing system was found to have an association with brucellosis seroprevalence (P-value ≤ 0.05).

Table (3): Risk factors associated with brucellosis among dairy cows within the farm using Chi Square test.

	Factor	**frequency**	**Cumulative percent%**	x^2	**Df**	***p*-value**
1	Breed			6.0	1	.030
	Foreign breed	4	33.4 %			
	Local breed	0	0 %			
	Mixed (goats , sheep)	8	66.6 %			
	Total	12	100 %			
2	Rearing system			8.6	1	.008
	Only cows	11	91.7 %			
	Mixed	1	8.3 %			
	Total	12	100 %			
3	Testing of new animal before introducing into the farm			6.0	1	.030
	Yes	4	33.4 %			
	No	8	66.6 %			
	Total	12	100 %			
4	Place where samples are tested			1.34	1	.284
	Veterinary Research Laboratory	6	50 %			
	Veterinary Hospital Laboratory	6	50 %			
	Special Laboratory	0	0 %			
	Total	12	100 %			
5	Type of fertilization			1.1	1	.50
	Natural	12	100 %			
	Artificial	0	0 %			
	Total	12	100 %			
6	Using of bull for fertilization in other farms			857	1	.008
	Yes	5	41.6 %			
	No	7	58.4 %			
	Total	12	100 %			
7	Separation of premises			4.5	1	.50
	Yes	12	100 %			
	No	0	0 %			

	Total	12	100 %			
8	Milking system			2.41	1	.227
	Automatic	2	16.6 %			
	Manual	10	83.4 %			
	Total	12	100 %			
9	Exchanging workers between the farms			4.1	1	.091
	Yes	3	25 %			
	No	9	75 %			
	Sometime	0	0 %			
	Total	12	100 %			
10	Disposing of aborted and dead fetuses			1.34	2	.513
	Burning	3	25 %			
	Burial	1	8.3 %			
	Burning and burial	0	0 %			
	Outside farm	8	66.7 %			
	Total	12	100 %			
11	Exchanging equipment between farms			1.84	2	.4
	Yes	1	8.3 %			
	No	8	66.7 %			
	Sometime	3	25 %			
	Total	12	100 %			
12	Separation of aborted cows			.000	1	.774
	Yes	2	16.6 %			
	No	10	83.4 %			
	Total	12	100 %			

Table (4): Risk factors associated with brucellosis amid dairy cows within the farm using Logistic regression

	Risk factor	***β***	***ρ*-value**
	Constant	**1.618**	**0.19**
1	Breed	**-.088**	**.366**
2	Rearing system	**-.471**	**.041**
3	Testing of new animal before introducing into the farm	**.118**	**.659**
4	Using of bull for fertilization in other farms	**.353**	**.260**

$$Y_i = 1.618 - 0.088x_1 - 0.471x_2 + 0.118x_3 + 0.353x_4$$

Frequencies and distribution of risk factors associated with brucellosis among farm workers:

About seven potential risk factors were found to be associated with farm workers 50 % of farm workers intervened during delivery, and 8.3% of workers were found to drink raw milk without heating. History of previous/present infection with brucellosis was found among 16.7% of the workers.

Table (5): Frequencies and distribution of risk factors associated with brucellosis among farm workers.

	Risk Factor	**frequency**	**Cumulative percent%**
1	Sanitation program		
	Yes	10	83.3%
	No	2	16.7%
	Total	12	100%
2	Intervention during delivery		
	Veterinarian	6	50%
	Veterinary assistant	0	0%
	Worker	6	50%
	Total	12	100%
3	Using of gloves		
	Yes	9	75%
	No	3	25%
	Total	12	100%
4	Exchanging of farm worker		

	Yes	3	25%
	No	9	75%
	Sometime	0	0%
	Total	12	100%
5	Habit of drinking raw milk without heating		
	Yes	1	8.3%
	No	10	83.4%
	Sometime	1	8.3%
	Total	12	100%
6	Habit of drinking colostrum among workers	2	16.7%
	Yes	8	66.7%
	No	2	16.7%
	Sometime	12	100%
	Total		
7	History of previous/present infection of brucellosis among herd	2	16.7%
	Yes	10	83.3%
	No	12	100%
	Total		

Frequencies of risk factors associated with brucellosis amid consumers of milk in Bahri area:

The results revealed that 6.7% of consumers drink raw milk without heating, 8.3 % did not boil to the correct temperature for milk, and 25% of them did not refrigerate milk after it was heated.

Table (6): Frequencies of risk factors associated with brucellosis amid consumers of milk in Bahri locality

Risk factor	Frequency	Percent %
Sex:		
Male	25	20.8 %
Female	95	79.2 %
Total	120	100 %
Age:		
Less than 25	22	18.3 %
From 25-35	60	50 %
From 35-45	25	20.8 %
From 45-55	6	5 %
More than 55	7	5 %
Total	120	100 %
Education level:		
Secondary school	29	24.2 %
Graduate	62	51.7 %
Post graduate	29	24.2 %
Total	120	100 %
Profession:		
Student	20	16.7 %
Business	6	5 %
Medical field	1	0.8 %
Vet. Field	25	20.8 %
House wife	28	23.3 %
Employee	40	33.3 %
Total	120	100 %
Drinking milk daily:		
Yes	79	65.8 %
No	41	34.2 %
Total	120	100 %
Amount of milk per person /day:		
A cup a day	103	85.8 %
more than a cup	14	13.2 %
Total	120	100 %

Place where milk is purchased:		
Farm	16	13.3 %
Distribution center	5	4.2 %
Grocery	29	49.2 %
Car milkman	11	9.2 %
Cart seller	27	22.5 %
Other	2	1.7 %
Total	120	100%

Results of the qualitative risk assessment:

Risk of brucellosis was assessed using the tabular frame for the release risk and exposure risk pathways explained by an influential diagram shown in figure (4)

Release pathway: Medium (R1) x High (R2) = High (R3)
Exposure pathway: Low (P1) x Medium (P2) = Medium (P3)

Risk estimation for Brucellosis among dairy herds, farms workers, and consumers was estimated as explained by an influential diagram shown in figure (4)

Release pathway risk × Exposure pathway risk = High (R3) x Medium (P3) = High (overall risk)

Although overall estimation of risk associated with brucellosis was found to be high, but results of survey among consumers shown that (91.7%) from consumers heated milk after purchased, (93.7%) heated milk to the degree of boiling, (75%) refrigerated milk after it was heated, and (83.3%) from consumers didn't have habit of drinking milk without heating. These treatments could decrease the risk raised from *brucella* to "LOW" at the point of consumption.

Figure (4): Overall Estimation of Risk
*R = probability of release pathway.
*P = probability of exposure pathway.

DISCUSSION

The seroprevalence rate in the present study was lower (33.3%) than those reported by Salman *et al.,.,* (2014) who reported that the prevalence rate in Bahri province was the highest among Khartoum and Omdurman cities. And 100% of the sampled herds were serologically positive and 44.6%, 45.6 %, 42.1% and 47.2% of the tested cows were positive when using the group of tests (MRT, M Elisa, RBPT and the S Elisa, respectively. The seroprevalence rate in the present study was also slightly lower than those reported by Suliaman, (2006) that used some of serological tests included (MRT). His results showed that the prevalence rates of the disease in Khartoum North to be 42.8% based on MRT. In the same previous studies, Khartoum North recorded the highest prevalence rates 45.3% of the disease compared to 37.1% in Khartoum, and 29.8% in Omdurman city based on RBPT, (Suliaman, 2006). According to Suliaman, (2006); Salman *et al.,* (2014) this high prevalence rate of disease in Khartoum North could be due to the mismanagement practices and the great number of animals rose in Khartoum North.

In this study, several potential risk factors were found to be associated with brucellosis among dairy cows within the farm. The type of breed showed significant association with brucellosis (*p*-value .030) which agreed with the results reported by Wegdan *et al.,* (2016); Solafa *et al.,.,* (2014) who reported similar findings with (*P*= 0.020).Whether lending bulls for fertilization from other farms or not, it was found to be significantly associated with spread of brucellosis among dairy farms in this study with (*p*-value .008), but Radostits *et al.,.,* (2007) and Aparicio (2013) reported that bulls may discharge semen that contains *brucella* but unlikely to transmit the infection. The risk of spreading infection by an infected bull is much higher when the semen is used for artificial insemination. Testing of new animal before introducing it into the farm was found to be associated with brucellosis in this study (*p*-value.030). This was similar to the finding reported by Schelling *et al.,.,* (2003) and Aparicio (2013). Rearing system was found to be significantly associated with brucellosis by using multivariate logistic regression model (*p*-value .041). Mixed farming between goats and cattle in the same premises was observed in one farm and most of these goats were imported from foreign countries, and were not tested for brucellosis and they were not recognized as a herd free of brucellosis due to lack of pre-importation certificates. If they were infected by *Brucella melitensis* consequent transmission to cattle is not unlikely although the organism could not be isolated. Reports showed that brucellosis due to *Brucella melitensis* emerged as a new bovine-related public health problem since that this bacteria is capable of colonizing the bovine udder (Banai, 2002; Corbel, 1997). The mixing of different species, especially goats and sheep with cattle is the most important determinant for brucellosis transmission as reported by Al Majali *et al.,.,* (2009) and Omer *et al.,.,* (2000). However, Brucellosis should be investigated in other animal species in close contact with cattle to understand the role of these animals in the epidemiology of brucellosis in cattle. Place where samples were tested, type of fertilization, separation system, milking system, exchanging workers between the farms, disposing of aborted/dead fetuses, exchanging equipment between farms, and separation of aborted cows were suggested in the present study to be potential risk factors influence on the occurrence of brucellosis into the farm but these factors did not show statistical association with occurrence of the disease.

Risk assessment is considered one of three components of risk analysis which provides systematic framework in transparently collecting, analyzing and evaluating relevant scientific and non-scientific information about a chemical, biological or physical hazard possibly associated with food in order to select the best option to manage that risk based on the various alternatives(FAO, 2005).

In the present study the release pathway of brucellosis in value chain was assessed by two events, the first one was the probability that infected cows didn't come into contact with infected animals within the farm, and this was assessed to be "medium", due to seroprevalence (33.3%) detected by using screening MRT test. The second one in release value chain was the probability of infected cows with brucellosis could not be detected and tested, which was assessed to be "high", where 66.6 % from owners did not test animals for brucellosis before introducing them into the farm, in addition to the preventive measures which were not in place, the livestock markets is controlled by a series of brokers and the dairy cow's source, health and vaccination history could not be identified or traced.

About seven potential risk factors associated with farm workers were investigated using structured questionnaire for every workers in sampled farms, 50 % of farms workers intervened during delivery .According to Young (1983) and Schelling *et al.,.,* (2003) this intervention could increase the risk associated with human brucellosis. Also 25% of workers in this study didn't use gloves during intervention. These finding were close to results obtained by Tesfaye *et al.,.,* (2011) who reported that 20.8% of the investigated respondents wear gloves. But the result was very lower than that reported by Anka *et al.,.,* (2014) (71%) who did not use gloves. Also this study revealed that 8.3% of workers drank raw milk without heating. This was akin to that reported by Anka *et al.,* (2014) who stated that 8.5% of the investigated workers used to consume raw milk direct without heating, and lower to that recorded by Tesfaye *et al.,* (2011) who reported that 39.6% consumed raw milk. Reported history of previous/present infection with brucellosis was found to be among 16.7% of farms workers in this study.

Similar finding were reported by Anka *et al.,* (2014). He stated that (19.7%) of workers experienced undulant fever who was later diagnosed as brucellosis.

The results revealed that 6.7% of the respondents consume raw milk without heating, 8.3 % didn't heat milk to degree of boiling, and 25% of them didn't refrigerate milk after it was heated. Although the percentages were low, but it agreed with Nielsen *et al.,* (2005) and OIE (2008) who reported that, the possibility of infection occurring by drinking milk necessitates the pasteurization or boiling of milk. Other studies demonstrated that the brucellosis in human was strongly associated with consumption of raw milk (Godfroid *et al.,* 2011).

The exposure pathway of brucellosis in value chain assessed also by two events, the first event in this pathway is the probability of production of healthy cows mixed with production of infected cows or (production of other animal, goats, sheep) into the farm was assessed to be low, 91.7% from sampled dairy farms rearing cattle only, on the other hand 8.3 % with mixed rearing system (goats and sheep) with cows. Policies of separation different species in different premises and their production adopted by some owners, and arising of awareness of farm owner's about the risk of mixing different production could be measured to reduce the risk arising from mixed milk from different species. The second event in the same pathway was the probability of mixing milk of different dairy farms in collection centers was assessed to be "Medium" .Enforcement of legislations, laws and adoption of standard methods of production to control milk quality are of urgent need. Resulted from the assessment of the last two events that, the exposure pathway of brucellosis in value chain which represents the probability of marketing of milk contaminated with *brucella* was found to be "Medium", that means the risky event is likely to occur more than once in the next three years.

The overall risk estimation for brucellosis among farms workers, and consumers was found to be "High"; since High X Medium = High (figure1a) the risky event is likely to occur this year or in frequent intervals. (Figure1b). Although the overall estimation was "High", but results of survey among consumers showed that 91.7% of consumers heated milk after being purchased, 93.7% heated milk to degree of boiling, 75% refrigerated milk after it was heated, and 83.3%of them didn't have the habit of drinking milk without heating. These treatments could be responsible for decreasing the risk raised from *brucella* to (Low) at the point of consumption. Nevertheless, brucellosis remains public health problem that need more investigation and should be assessed qualitatively and then quantitatively.

CONCLUSION AND RECOMMENDATIONS

It could be concluded that brucellosis in dairy farms in Bahri North, was found to be (Medium), the risk of spread of *brucella* into the dairy farms was assessed to be "High" and the risk of brucellosis among farms workers, and consumers was assessed to be "Medium" and the overall risk estimation for brucellosis among farms workers, and consumers was found to be "High". The study recommended that risk analysis to assess brucellosis shall be conducted qualitatively and then quantitatively over whole country.

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