Global Veterinaria 5 (5): 248-254, 2010
ISSN 1992-6197

Incidence of Brucella Species in Slaughtered Food Animals and its Edible Offal at Beni-suef, Egypt

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Abstract: This work was done to determine the incidence of brucella species among slaughtered food animals and their edible offal at Beni-Suef abattoir, Egypt .A total of 80 positive brucella slaughtered food animals was used in this study. The collected edible offal of these animals included liver, spleen, kidney, lung, hearts and lymph nodes. Samples were separately collected in sterile polyethelene bags. The collected samples were bacteriologically and histopathologically examined for the presence of brucellae species. Positive samples were subjected for boiling and roasting. Results showed that *brucella melitensis* and *brucella abortus* could be detected with various percentages in edible offal whereas the incidence of *brucella melitensis* was greater than *brucella abortus*. The histopathological reactions for *brucella melitensis* showed different granulamatous reaction in spleen, liver and lymph nodes. Boiling could efficiently eliminate *brucella melitensis* from edible offal, but it could be detected after roasting. In conclusion, edible offal of brucella infected animals could constitute public health hazard. .Moreover, the incidence of brucella during the period 2004 to 2007 in cattle, buffaloes, sheep and goats was monitored.

Key words:*Brucella abortus* · *Brucella melitensis* · Edible offal · Slaughtered animals · Liver · Spleen · Lung · Kidney · Heart

INTRODUCTION

Edible offal is a valuable commodity and popular source of protein for Egyptian consumers. It could be a source of some dangerous zoonotic diseases such as burcellosis as it is often consumed under cooked.

Brucellosis is an important re-emerging zoonosis with a world wide distribution. It stills an uncontrolled serious public health problem in many developing countries including Egypt [1,2]. It is affecting all species of live stock and causing sever economic loss[3]. Infection may be transmitted to man, with the clinical manifestations include chills, fever, malaise and headache, requiring prolonged treatment [4]. Currently, *Brucella melitensis* accounts for globally most recorded cases with cattle emerging as an important reservoir with the few cases of *Brucella suis*. Bacterial load in animal muscle tissues is low but consumption of under cooked traditional delicacies such as liver has been implicated in human infection [5].Other means of human infection include skin abrasions or inhalation of airborne animal manure particles. Contamination of skin wounds may be a problem for persons working in slaughterhouses or meat packing plants or for veterinarians [6]. Although brucellosis and its means of transmission were discovered over 100 years ago, the disease is a world wide problem, predominantly so in the developing countries. The transmission of brucella infection and its prevalence in a region depends upon several factors like food habits, methods of processing [7]. Brucellosis is accounting for the annual occurrence of more than 500000 cases [8]. *Brucella melitensis* is the most invasive species and produces the most serious infection in human and animals [9]. Also Sadler [10] investigated the role of carcasses and its edible offal in transmission of brucellosis. He reported that about 1.25% of the cattle carcasses and 1.15-3.5% of the swine carcasses processed for meat are contaminated with one or more of the three species of brucella organisms in varying numbers; and as a result the processors, butchers, delivery men and others are open to repeated and often massive exposure with a resultant high incidence of recognized infection and an unknown

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incidence of unrecognized infections. A substantial proportion of the retail supply of meat and meat products is contaminated to varying degrees with one or more of the three species of brucella organisms. As a result housewvives, chefs, retailers and persons eating rare meat are subjected to potential exposure of undetermined frequency and magnitude resulting in an unknown incidence of recognized and unrecognized infection. El-Neser *et al*. [11] revealed the presence of individual variations with respect to affected organs , extension and severity of lesions of brucella. Also granulomatous reactions were detected in lymphoreticular tissues, in the liver, kidneys, lungs and udder. Therefore this work was done to estimate the incidence of brucella species among slaughtered food animals and their edible offal through bacteriological and histopathological examination, as well as the effect of different cooking methods on its viability whereas there is little data about brucellosis and its control in Egypt.

MATERIALS AND METHODS

Collection of Data: Data concerning brucella infection among slaughtered food animals at Beni-Suef Aabattoir, Egypt during the period from 2004 -2007 were analyzed for the incidence.

Collection of Samples: A total of 80 serologically brucella positive slaughtered food animals, 20 each of cattle, buffaloes, sheep and goats during the survey of the year 2007 was investigated . Samples included liver, spleen, lung, kidney, heart and lymph nodes. Samples were separately collected in sterile polyethylene bags and then rapidly transferred to Laboratory of Food Hygiene Department in ice box with minimum of delay.

Experimental Techniques

Isolation and Identification of Brucellae: Samples were examined for isolation and identification for brucellae according to the technique recommended by Alton *et al*. [12] as follows:

Direct plating from the examined tissue after surface sterilization on two media 1- Oxoid blood agar base with 10% equine serum and 1% glucose added aseptically after autoclaving (Farrell's medium). Oxoid brucella selective supplement (BBS) is added to this base [13]. 2-Trypticase soy agar with 5% sheep blood. The plates were incubated at 37°C for 4 days in an aerobic atmosphere containing 10% CO2. The isolated organisms were then identified by Gram's method; slide agglutination tests with (anti-S brucella serum, anti-R brucella serum); Lactose fermentation on MacConkey agar; Haemolysis on blood agar; Motility at 37°C; Motility at 22°C; Oxidase positive; Urease positive; Nitrate reaction; sensitivity to dyes and Citrate utilization.

Histopathological Examination: Specimens were collected from serologically positive brucella slaughtered food animals just after slaughtering and postmortem examination. Specimens included liver, spleen, kidney , lung , heart and (prescapular and supramammary lymph nodes). Samples were fixed in 10% formalin solution and processed by conventional method. Paraffin sections 5-7u were prepared and stained by Hematoxylin and Eosin according to Bancroft and Stevens [14].

Effect of Cooking on the Viability of *Brucella Melitensis* in Infected Edible Offal

Effect of Boiling: A piece of each of liver (100 g), spleen, lung, kidney and heart of serologically and bacteriologically positive *brucella melitensis* slaughtered food animals was boiled at 100°C for half hour then left to cool. The cooked samples were bacteriologically examined for brucella as previously mentioned.

Effect of Roasting: A piece (100 g) of each of liver, spleen; lung; kidney and heart of serologically and bacteriologically positive animal for brucella melitensis was cut into small pieces (at size of 2 x 4cm) then roasted in a cooking pan for about 10 minutes (the most common cooking method used in Egypt) with an internal temperature of roasted samples of 70 °C then examined for presence of brucellae.

RESULTS AND DISCUSSION

The data obtained in table 1 and figure 1 showed that the incidence of brucella species were higher in examined slaughtered goats during the year 2004, 2005 and 2007 (8.8, 11.4 and 10.6, respectively) as compared with slaughtered buffaloes(7.6, 9.0 and 10.2), sheep(5.0,5.9 and 6.4, respectively) and cattle(4.0, 3.7 and 4.45, respectively). Comparatively the data concerning 2006 showed high incidence in buffaloes followed by goat, sheep and cattle constituting 12.5, 10.0, 5.1 and 4.6, respectively. However, lower figures were obtained by Mitrov *et al*. [18]. The high incidence of brucellae indicated that the brucella infection was wide spread in different species of animals marketed in Beni-Suef governorate. The method of control is negligible due to

Table 1: Incidence of brucella species among slaughtered food animals at Beni-Suef abattoir in a period (2004 - 2007)

	2004			2005			2006			2007			
	Positive cases			Positive cases			Positive cases			Positive cases			
Species of animal	Total No.*	No.**	%	TotalNo.*	No.**	%	TotalNo.*	No.**	%	Total No.*	No.**	%	Positive cases ***
Cattle	1800	72	4.0	1600	59	3.7	2000	92	4.6	2000	89	4.45	13
buffaloes	300	23	7.6	350	32	9.0	360	45	12.5	350	36	10.2	11
Sheep	400	20	5.0	420	25	5.9	450	23	5.1	450	29	6.4	8.0
Goats	250	22	8.8	280	32	11.4	300	30	10	330	35	10.6	8.0

*= Total number of slaughtered animal/year

**= number of positive cases/year

***=Positive cases for bacteriological examination (n = 20 for each species of

Table 2: Incidence of brucella melitensis in edible offal of slaughtered food animals at Beni-Suef abattoir (n==20).

	Liver		Spleen		Lung		Kidney		Heart		Lymph node	
Species of animal	No	%	No	%	No	%	No	%	No	%	No	%
Cattle	8	40	10	50	3	15	4	20	3	15	10	50
Buffaloe	6	30	8	40	3	15	3	15	4	20	8	40
Sheep	7	35	8	40	2	10	3	15	2	10	8	40
Goats	7	35	8	40	2	10	3	15	3	15	9	45

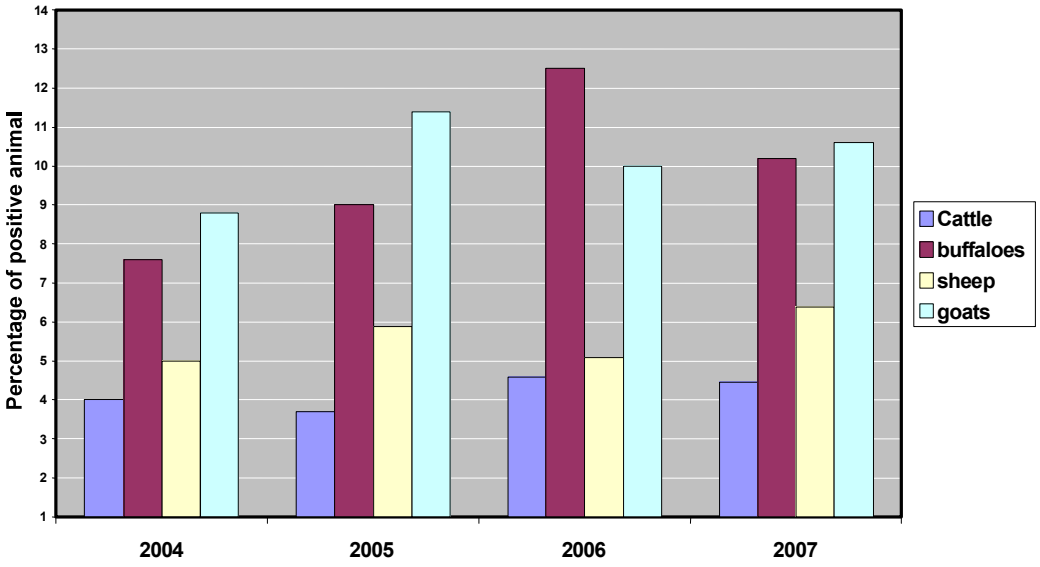


Fig. 1: Incidence of Brucella species among slaughtered food animals at Beni-Suef abattoir during 2004- 2007

the presence of food animals in small holder farms at different localities in the governorate. This held the view reported by Awad [6]. Data in table 2 nd figure 2 showed that *brucella melitensis* was isolated from edible offal of serologically positive slaughtered food animals at Beni-Suef Abattoir constituting 40, 50, 15, 20, 15 and 50% for liver, spleen, lung, kidney, heart and lymph nodes of cattle respectively. It could be isolated with incidence 35,40,10 and 15 for liver, spleen ,lung and kidney of slaughtered sheep and goats, while from the heart and lymph nodes it constituted 10 and 15 sheep and 40 and 45 in goats, respectively. From the present data, it could be concluded that the incidence of *brucella melitensis* was the highest in lymph nodes, spleen and liver of cattle and the lowest in kidney, heart and lung of sheep. In this respect, Ali [1] recorded brucella infection in cattle,buffalos, sheep, goats and camel. Nearly similar results were reported by El-Nesser *et al.* [11] .Variation in incidence of isolation from different organs as well as in different species may be attributed to the changes in stage of infection as well as

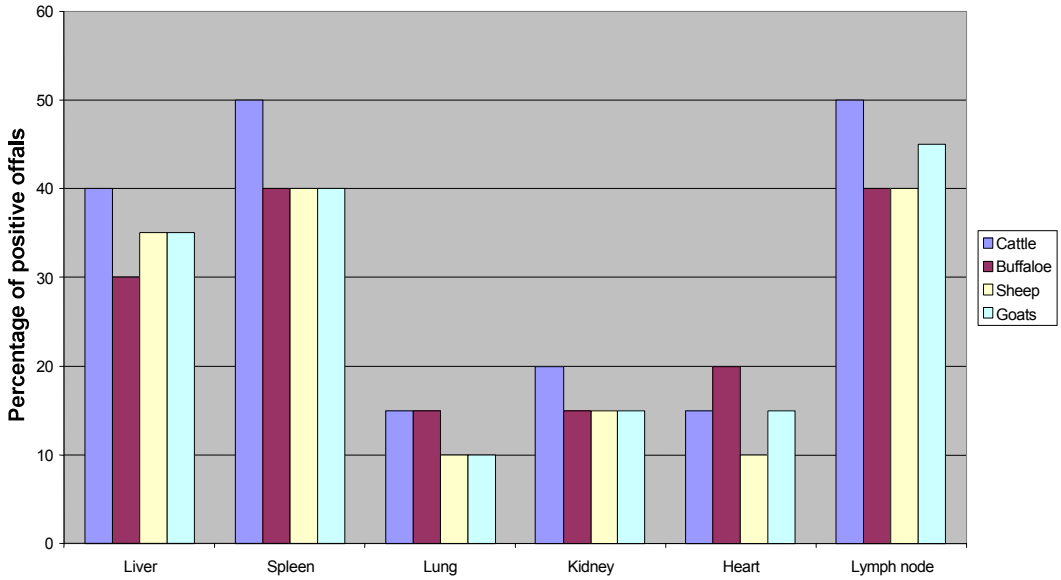


Fig. 2: Incidence of Brucella melitensis in edible offal of slaughtered food animals at Beni-Suef abattoir n=20

Table 3: Incidence of brucella abortus in edible offal of slaughtered food animals at Beni-Suef abattoir (n==20).

	Liver		Spleen		Lung		Kidney		Heart		Lymph node	
Species of anima	No	%	No	%	No	%	No	%	No	%	No	%
Cattle	2	10	3	15	ND	0.0	1.0	5	1.0	5	3	15
Buffaloe	3	15	3	15	1.0	5.0	2.0	10	0.0	0.0	3	15
Sheep	ND	0.0	ND	0.0	ND	0.0	ND	0.0	ND	0.0	ND	0.0
Goats	ND	0.0	ND	0.0	ND	0.0	ND	0.0	ND	0.0	ND	0.0

ND= not detected

the sensitivity of microbiological techniques used for isolation of the organism from serologically positive slaughtered animals. For cattle, infection is usually caused by *B. abortus*. However, *B. melitensis* and rarely *B. suis* can also establish themselves in cattle and the mode of transmission is then similar to that for *B. abortus*. These infections are particularly dangerous to humans because of the high virulence of most *Brucella melitensis* and *Brucella suis* strains and of the large numbers of bacteria that are excreted by these animals. However, the parenchymatous organs are considered the predilected site for the organism; this agrees with that reported by Alton *et al*. [12] and Mitrov *et al*. [18].

Also the high incidence of *Brucella melitensis* may be attributed to that the brucella is usually transmit from animal to animal by contact following an abortion. Pasture or animal barn may be contaminated and the organisms are probably most frequently acquired by ingestion but inhalation, conjunctival inoculation, skin contamination and udder inoculation from infected milking cups are other possibilities. The groups in which the occupational risk of infection is greatest include those whose work brings them in direct contact with infected animals or their products. These include farmers, stockmen, shepherds, goatherds, abattoir workers, butchers, dairymen, artificial inseminators, veterinarians and those involved in the processing of viscera, hides, wool and skins. Persons involved in the maintenance of buildings or equipment used for these purposes may also be at risk. This is in accordance with that reported by Corbel *et al*. [15]. On the other hand, Mitrov *et al*.[18] reported that brucellosis in cattle caused by biovars of *Brucella abortus*. In some countries, particularly in southern Europe and western Asia whereas cattle are kept in close association with sheep and goat; infection can also be caused by *Brucella melitensis*. Data obtained in table 3and figure 3 revealed that *Brucella abortus* could be detected with various percentages in edible offal. In cattle, it could be detected in spleen, lymph nodes and liver with percentages 15,15 and 10% , respectively and could be detected in lower

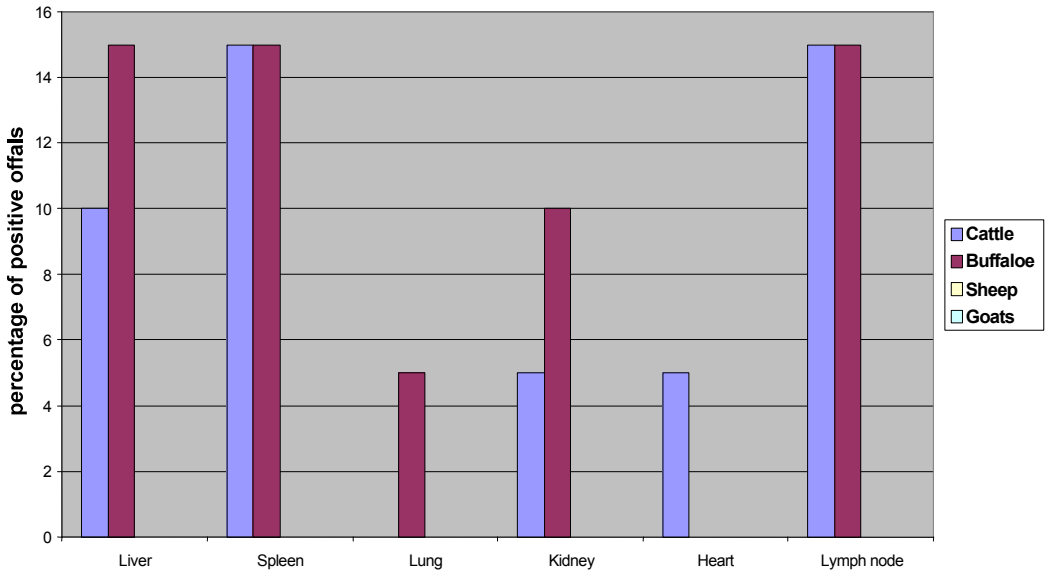


Fig. 3: Incidence of *Brucella abortus* in edible offal of slaughtered food animals at Beni-Suef abattoir n= 20

percentages in kidney ,heart and lungs which were 5, 5 and 0.0%, respectively. In buffaloes, the percentages were 15,15, 15 ,5 and 0.0 in spleen ,lymph nodes, liver, kidney and lungs, respectively. *Brucella abortus* could not be detected in sheep and goats in any examined organs. This may be attributed to host specificity. This agrees with that reported by *Alton et al.* [12] and Corbel *et al*. [15].

The histopathological changes in the liver revealed degenerative changes associated with focal leucocytic infiltration (Figure 4-a). In this respect, Young *et al*. [16] reported that poorly formed hepatic granuloma, composed of leucocytic infilteration with or without necrosis, was demonstrated in mice infected with *brucella melitensis*. In contrast to *brucella abortus* infected mice, well formed hepatic granuloma could be detected. The examined lymph nodes showed granulomatous reactions of different patterns and epithelioid cell granulomas were characteristically seen in most of the lymph nodes (Figure 4-b). In lungs, Epithelioid and giant cell reaction was observed (Figure 4-c). Concerning heart muscle, no pathological alterations related to brucellosis could be detected (Figure 4-d). The kidney revealed the presence of degenerative changes, focal leucocytic infilteration, (Figure 4-e). The spleen showed mild hyperplastic activation of the white pulp with the presence of abundant histiocytes and plasma cells around the medullary cords of the red pulp. Active proliferation of reticulum cells was the characteristic picture in most cases. Epithelioid and giant cell granuloma was also detected (Figure 4-f). Similar results were recorded by

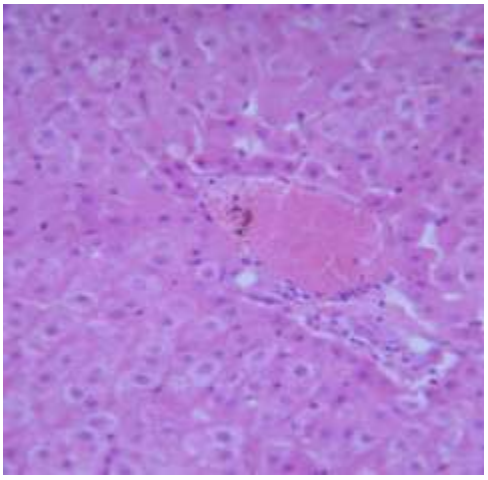
Table 4: Effect of different cooking methods on survival of brucella melitensis in edible offal (n = 10 samples) in cattle

	Cooking methods	
Organ	Boiling	Roasting (positive samples)
Liver	ND*	3
Spleen	ND*	3
Lung	ND	ND
Kidney	ND*	2
Heart **	ND*	2

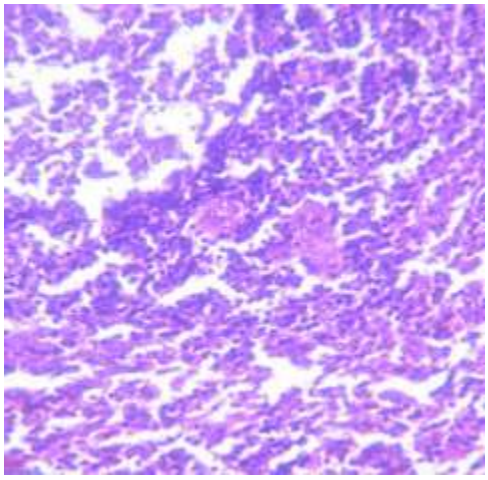
*mean significantly differ at p< 0.05

El-Nesser et al. [11] who also reported that failure to detect lesions in the heart and in one or other organs by other investigators may be related to that the lesions were overlooked due to their microscopical size and missing of getting samples grossly

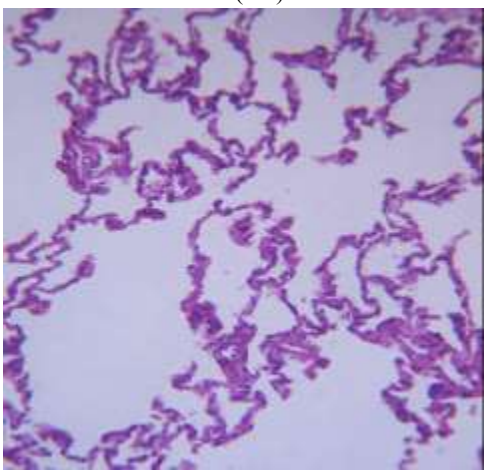
Data obtained in table (4 showed that boiling of liver, spleen, lung, kidney and heart significantly eradicate *brucella melitensis* (p< 0.05) than roasting. Muscle tissue is unlikely to contain more than low concentrations of brucella organisms and their numbers are further reduced if the meat is correctly stored before consumption. Kidney, liver spleen, udder and testes may contain much larger numbers. None of them present a serious hazard from brucellosis if thoroughly cooked. However, in some cultures, raw or undercooked meat may be eaten through choice. This practice and the consumption of fresh blood, either alone or mixed with milk, should be discouraged.



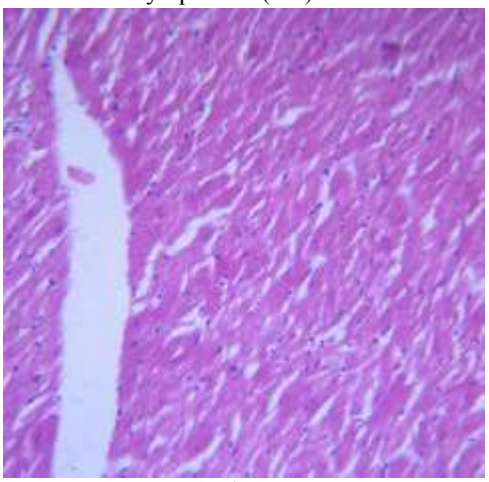
Liver (4-a)



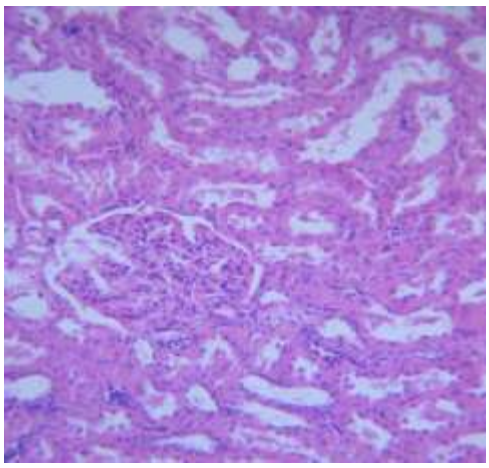
Lymph node(4-b)



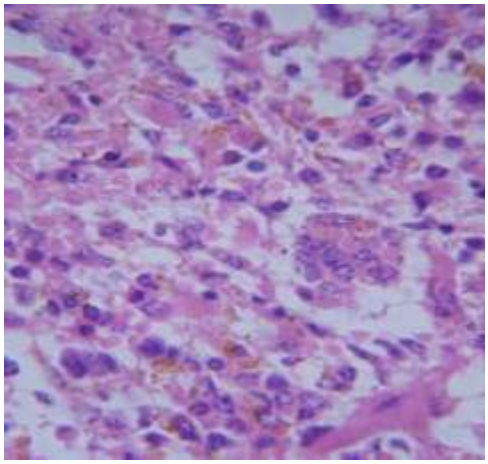
Lung (4-c)



Heart (4-d)



Kidney (4-e)



Spleen (4-f)

Fig. 4-a-f: The histopathological changes in samples of liver (4-a); Lymph node (4-b); Lung (4-c); heart (4-d); kidney (4-e) and spleen (4-f) stained by Hematoxylin &Eosin (x 1000).

The handling and preparation of infected meat and offal without proper hygienic precautions may be also lead to the contamination of other foods.

Corbel *et al.* [15] reported that drying, salting and smoking are not reliable methods for killing brucella. Similarly, the organisms survive well under refrigeration or deep freeze conditions. It is strongly recommended that all meat products are thoroughly cooked before consumption as *brucella melitensis* is highly pathogenic and constitutes a serious risk to public health [9,17] .

In conclusion, *Brucella melitensis* was wide spread in slaughtered food animal in Beni-suef abattoir as well as the lymph node showed the high incidence as compared with edible offal (spleen, liver, kidney, heart and lung). However, the boiling was effective for destruction of *B. melitensis* in edible offal , while roasting cause incomplete destruction. These offal constitute public health hazard for handlers and consumers. Strict authorized regulations for slaughterhouses with condemnation of edible offal of Brucella positive slaughtered animals .

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