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Critical Review

Literature Review about Libyan Brucellosis in Ruminants and Humans

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ABSTRACT

First report of brucellosis in Libya since 1931 in goat where researcher **(**Medulla,1931**)** was able to document after that , the reports were repeated but cow in the 1970s public milk station (Aboudaya. 1992). Brucellosis in camels in a small flock of camels imported from Sudan registered by Hosni and Aboudaya in 1992. In 1980-1982 the first survey and eradication program in and around Tripoli and in public cow station for animal production in Benghazi city. Than in 1997 to end of 2000 the Libyan authorities conducted second survey and eradication program. From1931 to 1984 almost of the examination was by culture in laboratories outside Libya isolations *Brucella melitensis* (serotype I and serotype II) than in 1997 serological methods TAT, CFT, RBPT and BAPT. SAT and ELIZA biotype III and MRT in milk. This review therefore is to determine the prevalence of the Brucellosis in Libyan animal farmers (sheep, goats, cow and camel), human and the eradication programs that were conducted.

KEYWORDS: Brucellosis, Libya, Sheep, Goats, Cow, Camel, Human.

INTRODUCTION

Sheep and goats are very important livestock in Libya than cow and camel their population represents about 7o% of the total size of the Libyan animal wealth, they produced more than 40% of the total consumption of the red meat and represent the traditional job for the most of the Libyan farmers of the whole Libyan regions.

Brucellosis is endemic in Mediterranean and Middle East regions as it considered a one of the main bacterial diseases causes abortion in animals and the important zoonotic diseases which has a public health hazard. Many Brucella species cause infection to cattle, small ruminants, horses, dogs, and several other animals including camelids (OIE, 2016).

Brucellosis is worldwide distributes disease and is an important disease of Libyan sheep and goats, which are the most frequently infected animals with *Brucella melitenses* and *Brucella abortus*. Infection with Brucellosis spreads by aborted fetuses, milk and vaginal discharges. Several routes can transmit the infection to animal including the vagina, digestive system, conjunctiva and skin also in human It can attack the genital organs, mammary glands and Lymph nodes and causing abortion of the pregnant of the last stage of the gestation period in sheep and goats.

The disease causes economic losses is not confined only in the incidence of abortion and death of the fetus, but in the incidence of infertility for the animal, the low rate of milk and losses resulting from the wrong treatment ,who consequent consumption of money and time, which is compounded with the condition of the animal in addition to future problems from improper use of medications, and so the early diagnosis of the disease reduces the time and effort to treat both for humans or animals also determine the incidence of this disease is very important in terms of public policy upon which to combat and control the disease.

In some countries of the near East region, brucellosis was reported in almost all domestic animals particularly cattle, sheep and goats**.** *Brucella melitensis* biotype 1 has reported in Libya (Refai, M. 2002). Although many of the brucella diseases are well known, there is still little available literature about their prevalence among the Libyan animal farmers. This review therefore is to determine the prevalence of the Brucellosis in Libyan animal farmers (sheep, goats, cow and camel) and the eradication programs that were conducted.

DISCUSSION

History of Brucellosis in Libya

1-Brucellosis in Sheep and Goats

The first study of animal diseases survey in Libya was initiated after the Italian occupation of Libya 1911-1943. So the beginning of recorded history of animals brucellosis in Libya was 1931 where researcher **(**Medulla, 1931**)** was able to document. Either in the year 1935, **(**Viglietta1935**)** referred to a storm of epidemiological disease in animals in 1934 in Derna, goats and sheep, which examined during that epidemic revealed the disease by 19% in goats and 17% in sheep and therefore suggested the importance for community health and explained the secret of an epidemic in the city of Derna due to attract herds of animals infected from weshishilia Italy and Tripoli as well as Egypt. After that in 1943 when Libya became under the authority control of British console administration established a first diagnostic laboratory for animal diseases in Tripoli. In 1953 Kanter record an epidemic outbreak of the disease in Libya with importing sheep from Malta as well as researcher he shows that there are two types of *Brucella* in

Libya are *Brucella meltiensis* and *Brucella abortus*. And recorded for five years 1963-1959 as a result of Bacteriological isolation of 116 cases of the bacterium *Brucella meltiensis* and these cases were distributed in all of the cities of Tripoli, Benghazi and Sebha in the 1960s by **(**Kanter, 1967**)**.

(Mustafa, A.A. 1984**)** introduced a report subjected to the economic losses of sheep and goats due to infection with Brucellosis, which causes abortion, sterility, Lamb mortalities and decreases of milk production. It confirmed the occurrence of Brucellosis in sheep and goats of Al-jabel Al-Gharby at the western Region of Libya by discovering of two strains of *Brucella melitenses* (serotype I and serotype II) and indicated that about 36.4% of the examined flock be infected with Brucellosis.

A local serological survey at the Al Jabal al Gharbi University in the western mountains region in 1997 found that 8.5% of sheep, 28.4% of goats, and 3.5% of camels were positive for brucellosis. (Elarbi A, 1997).

Also in the same date there is research described that a total of 320 blood serum samples of goats were serologically examined for brucellosis using TAT, CFT, RBPT and BAPT. Milk samples from 65 lactating goats were subjected for MRT and bacteriological examinations for *Brucella* isolation. The result revealed that CFT and BAPT gave the highest correlation with *Brucella* isolations.

Brucella melitensis biotype III could be isolated from 9 milk samples.(0.2%)(Azwai, S.M. 1997). A Moreover, (Sassi, 1998) reported a prevalence of (4.5%) in 420 ewes using Rose Bengal Plate Test and Tube agglutination Test in Tripoli.

In 2006 to 2008 from (340 goat, 188sheep) when tested to detected of *brucella* using Rose Bengal test, tube agglutination test and ELISA assay were seropositive animals, 104 (31 %) of goat.24% of sheep, 45 were positive for brucellosis indicate a substantial increase over the past ten years in this area (Ahmad.et al 2010). The surveillance of sheep and goats in 2008-2009 showed prevalence rates of 8.3% in sheep and 14.8% in goats (AL Ghanay 2009). A prevalence of (4.86%) was recorded by Fawzia Abo El-Khirat (2010) in El Ogilat which in the western area using Rose Bengal Plate Test from 720 sheep serum samples. When serum sample collected from 10562 animal (sheep 6840, goats 2665,) were tested by RBPT for Brucella antibodies the percentage of infection recorded 17.4% in sheep (6840), 41.7% in goats (2665), The highest percentage of infection in males recorded in sheep (18.9) but in females recoded in goats (47.8) and highest percentage of abortion recorded in sheep (19.4) (Elkhouly and Alhireereeq, 2021).

In eastern part of Libya, there is no study about brucellosis in small ruminants until 2008 (AL Talhy 2008) and after that from 866 flocks were found to be subjected to different conditions of repeated abortion. Out of them 180 flocks were subjected to cases of abortion but no samples were taken from them because not known the time of abortion.

3000 serum sample collected from 686 flock of small ruminant with abortion history were examined by RBPT as screen test and ELIZA for brucellosis only 252 sample given positive by

Table (1) Comparison Results between AL Talhy and Essa at AL Jabal AL khaddar Area

Location	Total sample	Positive	%Incidences	%	
Al-Goba	1296	111	8.6%	%44	AL Talhy
AL-Beyeda	1229	120	9.8%	48%	
Derena	475	21	4.4%	8%	
Total	3000	252	8.4%	100%	
Al-Goba	240	79	33%	%52	**ESSA**
Al-Beyeda	107	53	49%	%35	
Derena	53	20	38%	%13	
Total	400	152	38%	100%	

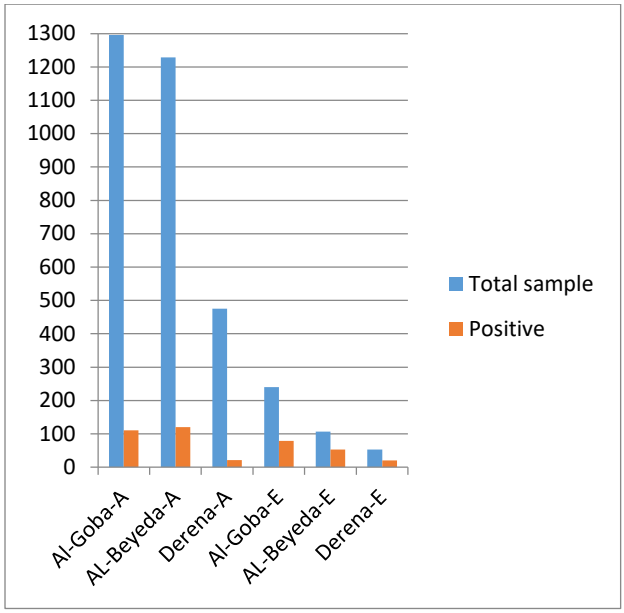


Fig (1) Comparison Results between AL Talhy and Essa at AL Jabal AL khaddar Area

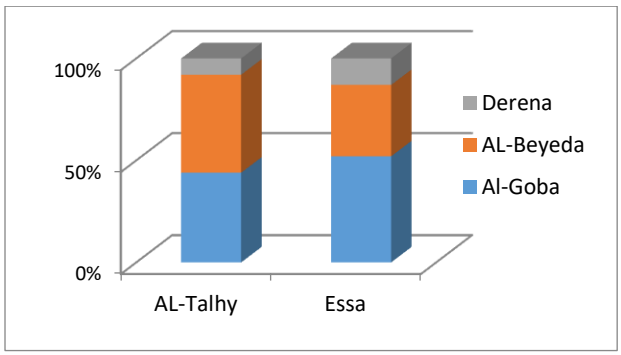


Fig (2) Comparison Rsults between AL Talhy and Essa at AL Jabal AL khaddar Area

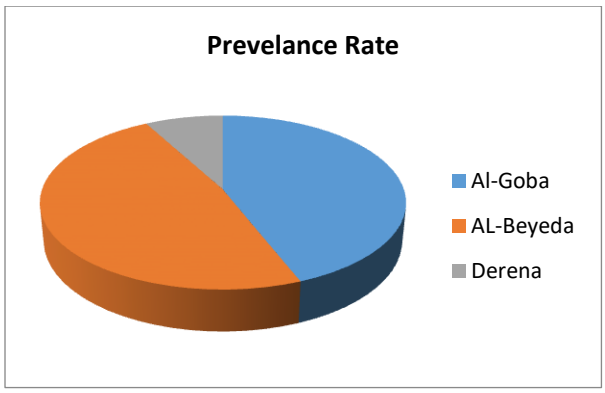


Fig. (3) Prevalence rate of brucellosis by AL Talhy at AL Jabal AL khaddar Area

2-Brucellosis in Cows

Although reference was made to the existence of *Brucella abortus* in the 1960 by (Kanter, 1967) but there is no documentation of brucellosis in cows in that period and earlier as well as in the 1970s and the problem of RBPT and 284 by ELIZA (0.104%) divided in to (1.0% mal, 98.3% female).(ALTalhy 2012).

In a similar study during the period from January 2015 to mid of 2016. a total of 400 blood samples were randomly collected from the same regions All the serum samples were tested for detection of *Brucella* antibodies by using the serological test Rose Bengal Plate Test (RBPT). When the Positive samples were 152 with prevalence of brucellosis was 38%.

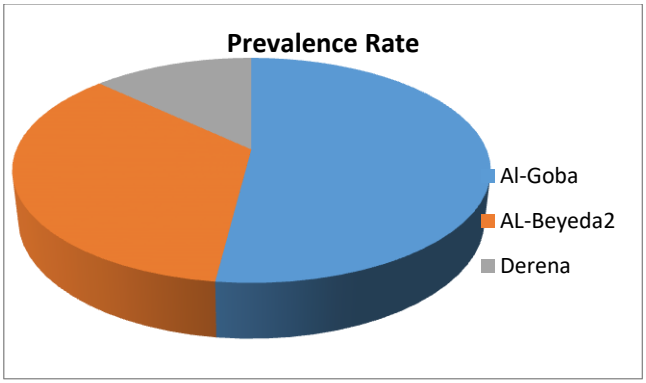


Fig. (4) Prevalence rate of Brucellosis Essa at AL Jabal AL Khaddar Area

Table 2 Incidence Rate of Brucellosis in Sheep and Goat

Years	Sample	References	infection %
1934	Goats& Sheep	Viglietta 1935	19%, 17%
1960	Sheep	Kanter 1967	116
1984	Sheep &Goats	Mustafa, A.A.	36.4%
1997	Sheep& Goats	Elarbi	8.5%, 28.4%
1997	Goats	Azwai, S.M	0.2%
1998	Sheep	Sassi	4.5%
2008	Sheep &Goats	ALTalhy	9.5%
2009	Sheep& Goats	AL Ghanay	45.4%,47.7%
2010	Goats	Ahmad *et al*	31 %,24%
2010	Goats& human	El-Khirat, F	4.86%, 37.5%
2011	Sheep& Goats	NCAH report	8.3% . 14.8%
2012	Sheep& Goats	NCAH report	47.5%
2012	Sheep& Goats	ALTalhy	0.104%
2013	Sheep	Abo Rokia *et al*	4%
2017	Sheep& Goats	Al-Griw *et al*	9.2% , 33.4%
2017	Sheep& Goats	Eissa *et al*	18.6%,69.3%
2018	Sheep& Goats	ALTalhy	8.16%
2021	sheep & goats	Elkhouly, Alhireereeq	17.4% ,42%
2021	Sheep& Goats	Al Garmi *et al*	13, 15.5%

contagious abortion in cows began to appear in draft cattle in 1972 established a number 3225 head of cattle spread over five breeding stations and cattle were imported from West Germany and the Netherlands, Britain, Denmark and the United States and Canada since that date Herd began increasing the number of stations then to fifteen stations distributed in different parts of Tripoli.

Contagious abortion cases in 1972 began to appear at the three stations, in Al Zawia , Al geria, and Godaim. Blood samples were sent to the central veterinary laboratory and Diagnosis proved positive for brucellosis. In the years of 1977, 1978, 1979 the infectious abortions had been increased and blood samples sent from these stations to the Veterinary laboratory in Tripoli were they diagnosed and confirmed as brucellosis.

In the end of 1979, the number of abortions, retention placenta and premature birth increased in Al zawia station making public administration for animal production and veterinary services contemplate a comprehensive survey of brucellosis on those stations. Indeed, blood samples collected from cows and sent to the laboratory were confirmed the existence of the disease in cows in Al Zawia station by 16.1% and 32.7% Algeria station whereas in station of Godaim was 17.1% . (Aboudaya. 1992).

El sanousi and oumer in serology survey of Brucella in Benghazi cow station by SAT and CFT they recorded that in cows breeding project in Benghazi was less than Tripoli which the general morbidity rate was (0.3) (ELSanousi& oumer 1985) . Seroprevalence of brucella Study was conducted in north – western Libya (western mountain region). Blood samples collected over 13 months in the period December 2006 to January 2008 from 528 animal (goat, sheep) and tested for *brucella* using R. B.P T, tube agglutination test and ELISA assay .

Amongst livestock, 31 % of goat and cattie 42% . Human samples showed a high seropositive 40%, with 95 (43%) of the 221 positive sample for IgM, indicating active or recent infection (Ahmad et al. 2010).

Prevalence of the disease in 2011did not exceeds 0.2% in cattle, 0.1% in camels, 8.3% in sheep, and 14.8% in goats. After that in 2014 and april 2016. Cattle from 255 positive 12 with 4.7%, Camels from 35 positive 2 with 5.7%, Goats from 854 positive 285 with 33.4%, Sheep from 468 positive 43with 9.2% (ALGriw, 2017) .

From 33 the positive was 8 with % 47.06 (Al Garmi *et al*, 2021) The husbandry methods may be causes of increased prevalence in different animal species. Some research has reported that controlling this disease in goats and sheep can be effective in reducing infection in other livestock.

The role of goats in perpetuating brucellosis and in disseminating the disease among humans has also been highlighted.

Table 3 Brucellosis Rate in Cattle between 1997 until 2009 (AL Ghanay 2009)

Year	Samples	Positive	positive rate
1997	29814	60	0.2%
1998	97957	98	0.1%
1999	50364	51	0.1%
2000	74040	75	0.1%
2001	58350	176	0.3%
2002	3475	3	0.1%
2003	3230	1	0.0%
2004	658	2	0.3%
2005	1292	5	0.4%
2006	37	1	2.7%
2007	353	3	0.8%
2008	929	0	0.0%
2009	1295	2	0.2%

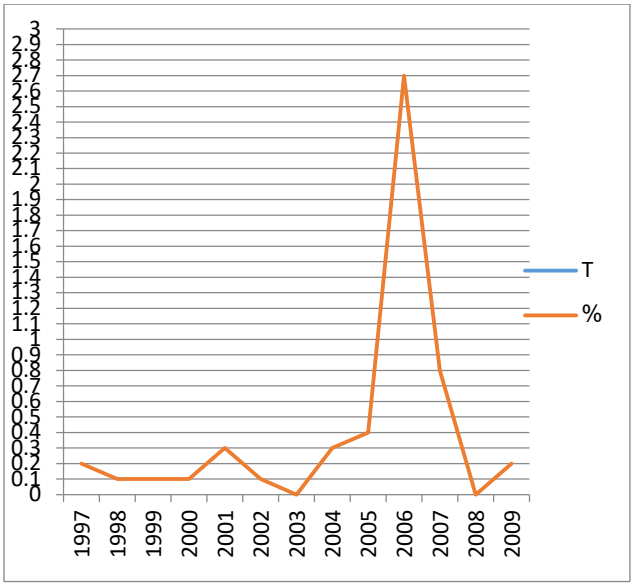


Fig (5) Rate of Brucellosis in Cattle between 1997 until 2009

3-Brucellosis in Camels

Camel brucellosis was reported in Libya since 1990 (Ben Faraj, 1990, Gameel et al, 1993, Hosni and Aboudaya, 1992, Azwai and carter., 1995, Azwai et al, 2001, El-Boshy et al, 2009). Camelid brucellosis caused by *B. melitensis* and *B. abortus* has been reported in all camel-rearing countries except Australia and the incidence appears to be closely related to breeding and husbandry practices (Richard D.1980) Camels brucellosis in Libya, the first case recorded was in 1990 (Ben Faraj) and has been documented through the work of Hosni and Abudayia in 1992 and they recorded the infection (0.09%) in a small flock of camels imported from Sudan for breeding using rose Bengal test (RBPT) and stacking tubular test (SAT). In 1993 Gameel and others conduct biodiversity surveys and microbiology for 967 male and female camels and they recorded prevalence 4.1%. Samples collected for cultural technique revealed 9 isolates. Five isolates were from milk samples, 3 from aborted fetuses and one from a vaginal swab. All isolates were identified and biotyped as *Brucella melitensis* biovar 1. (Gameel SE *et al* 1993). Report prepared by Azwai and carter in 1995 said that only been diagnosed by conventional serological tests (RBPT, SAT and ELIZA)which were shown to be inadequate in camels. Direct ELIZA together with western blotting for detection of IgG and IgM to B. abortus and *B meltiensis* in camel In 2001 introduced unpublished report subjected by ALTalhi to the economic infection with Brucellosis, which causes abortion in 81 camel. It confirmed the occurrence of Brucellosis in this flock which at Heshet AL Thuban in south of Al Jabel Al Akhdar at the east Region of Libya all the herd were positive when tested by RBPT and ELIZA. According to the random sample testing during the interval between January, 2002 and December, 2003 when about (109244) heads were tested and the morbidity rate was (0.7%). The highest rate was recorded in Al Jabal Al Akhdar area (Ben-Soliman 2005). From 14 of camels only 2 were testing positive with 14% positive rate (Ahmed et al 2010).

Table 4 Percentage of Brucellosis in Camels Cses according to the Year of Research

Year	Tested Samples	Positive cases	Incidences rate	References
1990	666	25	3.75	Ben Faraj *et al.*
1992	n/a:	n/a:	0.09%	Hosni & Abudayia
1993	967	40	4.1%.	Gameel et al
1995	n/a:	n/a:	n/a:	Azwai and carter
1997	n/a:	n/a:	3.5 %	Elarbi A
2001	81	81	100. %	ALTalhy
2001	520	18	3.5%	Azwai
2002	109244	765	0.7%	Ben-Soliman
2003				
2006	14	2	14%	Ahmed et al
2008				
2009	-	-	0.5%	EL Ganay
2017	35	2	5.3%	EL Griw *et al*
2021	940	117	6%	Elkhouly, Alhireereeq
2021	40	25	62.5%	AL garmi *et al*

n/a: Information not available

Table 5 Brucellosis Rate in Camels according to Year of Survey

Year	Tested samples	Positive cases	Incidences rate
2002	60645	313	0.5%
2003	46597	481	1.0%
2004	3163	27	0.9%
2005	1584	6	0.4%
2006	2890	7	0.2%
2007	2019	11	0.5%
2008	2860	10	0.3%
2009	2590	21	0.8%

Later surveillance programmers by National Centre of Animal Health, from 1997 until 2009, reported that, Seroprevalence rate of bovine brucellosis below 0.2%, Survey about Camel brucellosis survey between 2002 until 2009 by National Centre of Animal Health revealed Seroprevalence rates 0.2% (AL Ghanay, 2009) .

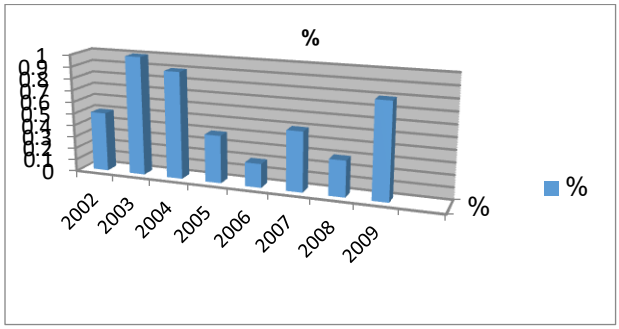


Fig. (7) Rate of Brucellosis in Camel by Survey from 2002 - 2009

4-Brucellosis in Human

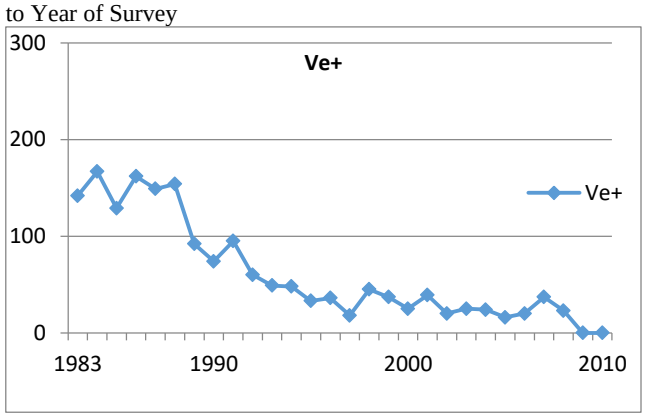
Brucellosis in Libya has been reported in man since 1983 on the AL-Jabal AL-Gharbi region (Azwai, 1987). The success measure of any brucellosis eradication program is the extent of the disease in humans. When we look at the table (7), we see that the rate of infection continues to decrease until 1991 than which it increase in 1992, despite the continuation of the eradication program.

This indicates the inaccuracies of the result due to the use of the RBPT and also its don to cows, and then camel and exclusion of goats and sheep which may be the essential reason for return the disease to cows and then to humans, or infecting human directly from goats, especially the study was carried out on the AL-Jabal AL-Gharbi region as it is an area where there is an faceted But when compared the result to Table (6) the rate of infection remains high. Unfortunately, AL Toumei-2012 this study is the only one that has studied the incidence of the disease in humans for several years,

Table (6) Brucellosis Rate in Human according to Year of Research

Years	Sample	References	%
1990	110	Ben faraj etal	29.1%
1993	967	Gameel	4.1%
2006-2008	546(332 m , 214 f)	Ahmed et al	40%
2010	16	El-Khirat, F	37.5%
2012	3423	EL-Tumi	50.2
2021	3	Elshagmani et al	33%

Fig. (8) Brucellosis Rate in Human from 1983-2010 according to Year of Survey



(AL Toumei -2012).

Although it was limited to one region not all Libya and when comparing the results with a study conducted on the same area among humans the prevalence seropositivity in Yafran municipality was 40%, with Jado (47%) and Yifrin (46%) having the highest proportion of brucellosis brucellosis-positive people (Table 8). Human blood samples were also collected from 546 selected individuals (332 male and 214 female) data indicate a substantial increase over the past ten years. The prevalence of IgG and IgM antibodies of *Brucella* in seropositive individuals was 57% (126/221) and 43% (95/221), respectively, suggesting that a substantial proportion of the population in this region were actively or recently infected (Ahmad *et al*, 2010). Serological examination By RBPT then by blood culture for three blood samples were collected in the admission day, and based on the presentation of fever and examination one sample was positive without history of travelling abroad or animal contact only in two months ago she was drinking goat's raw milk. (Elshagmani *et al*, 2021)

Table (7) Rate of Brucellosis in Human according to the Year of Survey (ALToumei-2012)

Year	Total Sample	Positive sample	Incidences rate	Infectious rate
1983	227	142	62.6	8.3%
1984	216	167	77.3	9.7
1985	213	129	60.6	7.5
1986	239	162	68.6	9.4
1987	196	149	76.0	8.7
1988	232	154	66.4	9
1989	154	92	59.7	5.4
1990	165	74	44.8	4.3
1991	176	95	54.0	5.5
1992	119	60	50.4	3.5
1993	124	49	40.0	2.9
1994	132	48	36.4	2.8
1995	124	33	26.6	2
1996	121	36	29.8	2.1
1997	100	18	18.0	1.1
1998	128	45	35.2	2.6
1999	120	37	30.8	2.2
2000	99	25	52.2	1.5
2001	108	39	36.1	2.3
2002	75	20	26.7	1.2
2003	60	25	41.7	1.5
2004	76	24	31.6	1.4
2005	49	16	32.7	0.02
2006	48	20	32.7	1.2
2007	73	37	41.7	2.2
2008	45	23	51.1	1.3
Prevalence %	3423	1719	50.2	100%

5-Diagnostic Brucellosis Tests Used in Libya

From1931 to 1984 almost of the examination was by culture don in laboratories outside Libya *Brucella* isolations. *Brucella melitensis* (biovar I and biovar II). Than in 1997 the serological methods TAT, CFT, RBPT, BAPT, SAT, ELIZA, Rivanol test and MRT in milk see table (8). But the first time to used Molecular methods Real- time PCR assay to diagnosis of brucella in sheep and goats was by (Younis, Abd-Elstar, and Altalhi. 2018) . EL-Sanousi 1985 and Aboudaya 1986 First evaluation of Serological method to diagnosis of brucella in cattle Moreover Sassi, (1998) see table (8) . Rose Bangle test it is the most method used to detection brucellosis in Libya, although it is not accurate, and that some research was only through RBPT (Essa, 2017). Serological test gave different results when diagnosing brucellosis in Libya. see table (9) An incorrect diagnosis of brucellosis may occur when based on serology alone , where they study was collected overall 720 serum sample from goats where examined by RBPT only 35 were positive , 35 (4.86%)by Buffered acidified plate test (BAPT), 34(4.72%) by Rivanol test and TAT 38(5.28%) and 86 milk sample (20.93%) by ring test and 16 human serum sample were tested by RBPT, Rivanol test, TAT positive was 6 (37.5) (Fozia Algurat, 2010). A similar study when collected 320 blood serum samples from goats were serologically examined to detected Brucella by TAT, CFT, RBPT and BAPT. Milk samples from 65 lactating goats were subjected for MRT and could be isolated from 9 milk samples (0.2%) (Azwai, S.M. 1997).Also in study by the same author Azwai, S.M. in (2001) from 520 Camel were tested by RBPT, ELISA IgM, ELISA IgG and SAT was 1.4%, 3%, 3.5%, 1.2 respectively (Ahmed *et al* 2010) from 546 samples was collected from human and tested by Rose Bengal test Rose, tube agglutination test and ELISA assays , was higher in males than females, with 66% of samples among males and 34% of samples from females positive for *Brucella* infection in this study. In addition to cross-reactivity with other bacteria that make the serological diagnosis of brucellosis more difficult this requires a procedure to the correct diagnosis like molecular methods. New techniques- such as Real- time PCR assay - allowing identification and sometimes quick typing also was more sensitive to *Brucella* agent than other methods of diagnosis and are in use in certain diagnostic laboratories (Khamesipour et al., 2015). Most serological methods which used to diagnoses of Brucella infection were primarily standardized and immediately designed for tested of sera. There are national, regional and international demands for sensitivity and specificity of serological methods when used for diagnoses Brucella infection in various animal species. real-time PCR using for detecting Brucella DNA and to determine the species level of Brucella diseases in animal sera with short time (OIE, 2016). bacteriological examinations for *Brucella* isolation which resulted that CFT and BAPT more sensitive than RBPT, TAT and MRT correlation with *Brucella* isolations.

***Brucella melitensis* biotype III**

From the results it is clear that the serologically results are mixed in goats but same results in human. Nevertheless so far the serological tests are still being used detection of brucella in animal as initial test or confirmation test in survey or research which may be this big reason to not disappearing

the disease. Some *Brucella* proteins are responsible for serological cross-reactions between *Brucella* spp. And other bacterial species cross-reactivity exists to: *Yersinia enterocolitica, Escherichia hermannii, E. coli, Francisella tularensis, Stenotrophomonas maltophili, Vibrio cholera, and Salmonella*. Real-time PCR has sensitivity and specificity of 100% when detection of the Brucella DNA in animal sera as detected by real-time PCR was considered as a golden mark referring to the exposure of these animals to Brucella organisms**.** Competitive ELISA detected of Brucella animal blood samples with sensitivity of 91.8% and specificity of 83.6% (Hamdy *et al*, 2017). The same results obtained the relative sensitivity and the specificity were (89.5%) and (83.3%) respectively (Sayour *et al*, 2015).

Table (8) The Dfferent Brucella Diagnostic Method in Libya

Reference	Date	Cu	RBPT	CFT	TAT	Ri	SAT	BAPT	MRT	ELIZA	PCR
Medulla	1931	+									
Viglietta	1935	+									
Kanter	1953	+									
Kanter	1967	+									
Mustafa, A.A.	1972			+			+				
ELSanousi et al	1985			+			+				
Aboudaya	1986										
Sassi, M. F	1990		+		+						
Ben Faraj *et al.*	1990										
Hosni & Abudayia	1992		+				+				
Gamee *et al*	1993	+	+	+			+				
Azwai and carter	1995		+				+			+	
AL Zwai	1997	+	+	+	+			+	+		
Al ghanay	1997		+				+			+	
Sassi	1998		+		+						
Azwai et al	2001	+					+			+	
Altalhy(unp research)	2001		+							+	
B in soliman	2005		+							+	
Altalhy(unp research)	2008		+						+	+	
Al ghanay	2009		+				+			+	
Elkhirat, F	2010		+	+		+	+		+		
Ahmed.*et al*	2010		+	+						+	
Altoumei	2012		+				+			+	
Altalhy("MVSC)	2012		+							+	
Abo Rokia	2013		+			+					
Ahmed.*et al*	2015		+								
Al-Griw	2017		+				+			+	
Essa	2017		+								
Younis.*et al*	2018		+							+	+
Elshagmani et al	2021	+	+								
Elkhouly, Alhireereeq	2021		+								
Algarmi	2021		+								
Total		8	23	6	3	2	11	1	3	11	1

unp research =unpublished research

Table (9) Seropositive Samples by Rose Bengal Tst, then Confirmed by ELIS

author	RBPT	ELISA IgM	ELISA IgG	TAT	R.T	SAT	BAPT	RMT
Azwai 2001	1.4%	3%	3.5%			1.2%		
Altalhy 2008	0.11		0.95					
Ahmed *et al* 2010	40%	17.4%	23.1%					
Fozia Algurat 2010	4.86%			5.3%	4.7%		4.86%	20.9%
ALTalhy 2012	8.4%		0.104%					
Aloumi 2012		43%	57%					
Younis,Abd-Elstar&Altalhy 2018	7.5%	0.5%	7 %		8.2%			

Real-time PCR has sensitivity and specificity of 100% when detection of the Brucella DNA in animal sera as detected by real-time PCR was considered as a golden mark referring to the exposure of these animals to Brucella organisms**.** Competitive ELISA detected of Brucella animal blood samples with sensitivity of 91.8% and specificity of 83.6%. (Hamdy *et al*, 2017) the same result was obtained relative sensitivity and specificity was (89.5%) and (83.3%) respectively (Sayour *et al*, 2015)

The evaluation of Real time PCR versus serological tests for Brucellosis detection in Libya they done by 600 blood samples were tested for detection the anti-body or DNA of *Brucella* by Real time PCR, 45, 42 and 49 were positive by RBPT, ELISA and Real time. PCR, respectively. Some of the serologically negative samples showed positive from Real Time. PCR, however few positive samples were tested negative by Real time PCR. Sensitivity and specificity of RBPT compared with Real time.PCR were 81.25% and 98.9%, while with ELSTA (IgG, IgM) these were 88% and 100%, respectively. The study suggests that the advantages of Real Time. PCR over serological tests it particularly detects the active phase of infection.(Younis, Abd-Elstar & Altalhy, 2018)

6- Antibiotic Susceptibility Test

A sixteen years old Libyan girl life in a rural area, she has presented of intermittent fever since seven days without history of travelling abroad or animal contact. In the past two months, she was occasionally drinking goat's raw milk. She was referred to National Cancer Institute, Misurata-Libya on 15 June, 2020. When diagnostic by serologic tool, bacteriology culture and then doing antibiotic sensitivity test using disc diffusion method (Elshagmani *et al*, 2021). After incubation for three days, diameters of inhibition zone measured and they were as table (10).

Table (10) Inhibition Zone Diameter by Disc Diffusion Method according to(CLSI).

Inhibition zone diameter >30 mm	Inhibition zone diameter 15-20 mm	No inhibition zone
Meropenem (MEM 10µ g)	Cefixime (5 µg)	Vancomycin(VA 30 g)
Imipenem (10µ g)	Cefuroxime (30 µg)	Clindamycin(2µ g)
Ciprofloxacin (5µ g)	Ceftazidime (30µ g)	
Azithromycin (15µ g)	Erythromycin (15µ g)	
Cefotaxime (30µ g	Bactrim(trimethoprim-sulfamethoxazole,25µ g)	
Ceftriaxone (30µ g),		
Chloramphenicol(30µ g)		
Augmentin(30µ g)		
Doxycycline (30µ g)		

7- Eradication Brucellosis Program in Libya

In Libya 1980-1982 the first survey and eradication program in and around Tripoli and in public cow station for animal production in Benghazi city. In Tripoli from 8.607 head in public station only 125 are positive and from 35942 head in private farm only 59 are positive. While only from public station of Benghazi 12 are positive from 3753 sample (0.3%) (without privet farms) after that the Government used a brucella disease experts like

prohska in 1987 who advised immunization the calf under 6 month by C19 vaccine and calf greater than 6 month with the other animal species by 45/20 vaccine but Dr Bruce suggested slaughtered all the positive and negative animals in the cows stations (aboudaya, 1997) . While Margaret from USA mentioned in her report 1982 three choices the first one was vaccination and the second was slaughtered all animal from public cow station and last choice was slaughtered the positive cases and keep the negative animals, as for private farm used tested and slaughter the positive (Meyer, 1982). As a result, the government chose to use tested and slaughter the positive in the public cow station and retests after a year and then evaluation the program. In Libya at 1980-1982 the first survey and eradication program in and around Tripoli and in public cow station for animal production in Benghazi city. In Tripoli from 8.607 head in public station only 125 are positive and from 35942 head in private farm only 59 are positive. While only from public station of Benghazi 12 are positive from 3753 sample (0.3%) (without privet farms), although, historically of surveillance has been Start between 1997 and mid-1999, a National Bovine Brucellosis Eradication Programme found the prevalence rate of bovine brucellosis was 0.129% (215 positive cases out of 166388 animals tested) (Table 5) at the end of 1999 (FAO1999).

Later surveillance programmes, from 1997 until 2000, suggested a prevalence rate of bovine brucellosis below 0.2% from 252197 was tested. The first case recorded In Camel was since 1990 (Ben Faraj) camel milk has benefits for the digestive tract. In the other hand *B. meltiensis* biovar 1 has been isolated from camel milk has been isolated in Libya (Gameel et al., 1993).

But brucellosis eradication program happened too late between 2002 until 2003 eradication program has been done to some camel herds when about (109244) heads were tested and the morbidity rate was (0.7%). The highest rate was recorded in Al Jabal Al Akhdar area. (Ben-Soliman, 2005). Prevalence rates (Ben-Soliman, 2005). The serological survey of the diseases continued until 2009 revealed prevalence rates between 0.2% and 1%..

In contrast, the first report of small ruminants brucellosis in Libya since 1931 in goat by researcher **(**Medulla, 1931**)** And recorded for five years 1963-1959 as a result of Bacteriological isolation of 116 cases of the bacterium *Brucella meltiensis* and these cases were distributed in all of the cities of Tripoli, Benghazi and Sebha in the 1960s by **(**Kanter, 1967**)**.

Table (11) Survey and Eradication Program1980-1982 around Tripoli & Public Cow Station

Year	Total sample	Positive case	Positive rate
1980	8312	135	0.06%
1981	44549	181	0.4%
1982	3046	80	0.26%

The only study about immunization was conducted between March and July 1989 at the western region in the country by used local Chinese *Brucella suis* S2 vaccine in the goats milk. Goats milk have flavor in people who living in country side especially at the east and western part of Libya usually consumed raw from camel and goats without pasteurization or boiling. Under such conditions public health hazard due to brucellosis definitely exist.

Table (12) Comparison between Public Cow Station Private Cow Farm.

Tripoli 1981	public cow station	private cow farm	Total
Total sample	8607	35942	44.549
Positive case	125	56	181
Positive rate	1.45	0.15	0.40
Suspected case	20	19	39

Table (13) Frst Survey and Eradication Program Benghazi-1982.

Benghazi-1982	public cow station	private cow farm	Total
Total sample	3753	-	3753
Positive case	12	-	12
Positive rate	0.3	-	0.3
Suspected case	55	-	55

Table (14) Surveillance Programmers', from 1997 until 2009.

All Libya -	private cow farm	Total
Total sample	252197	252197
Positive case	318	318
Positive rate	0.126%	0.126%
Suspected case		

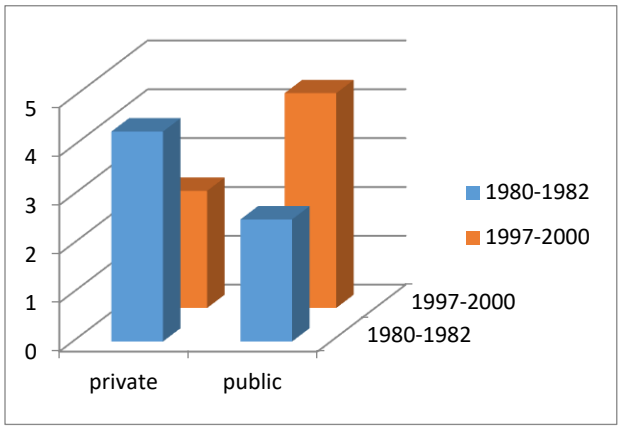


Fig. (10) Comparison between Public Cow Sation Private Cow Farms

About small groups of small ruminate to study the usefulness of using this vaccine locally. Random selection of small ruminants (one in 10 animals) was tested to determine the prevalence of the disease in the region (Table 15). The campaign comprised vaccination of 213933 animals from 932 herds from three regions, Nalut, Kabaw and Zwara. The recorded data showed that the vaccinated number of sheep was higher than the goats throughout the three regions (135501 to 78432 respectively). This may attribute to the desire of most people breeding sheep more than goats, preferring to eat sheep meat and limiting the eating of goat meat to the spring season while utilizing goat milk to produce Ghee. (Mustafa and Abusowa, 1993). Slaughtered the positive animal used as eradication program as well as the vaccine is use in some countries on small scale.

We most used vaccination programs for sheep, goats and camel to carry out of brucellosis because biosecurity measures on most farms are lacking, and most producers do not test animals for brucellosis or other diseases when purchasing replacements also too much mobility in search of pasture makes the application of slaughtered the positive animal used as eradication program very difficult.

Unfortunately, In all Libyan survey and eradication program done only about cow and camel with neglected sheep and goats Although *B. melitensis or B. abortus* which present in the sheep and goats that may cause the disease to return to cows and camels again (OIE, 2016),(Mustafa and Corbel 1988).

Table (15): Compare between Vaccinated Animals in some Areas of Western Libya at 1989.

Region	herds	vaccinated sheep	vaccinated goats	Total
Nalut	246	56564	26964	83528
Kabaw	315	54175	34323	88498
Zwara	371	23660	10707	34367
Total	932	135501	78432	213933

In addition, most of the people in eastern part and southwest region prefer to drink goat's milk than cow.

Despite prohska in 1987 advised about immunization young's calf with the other animal species by 45/20 vaccine. In addition to the experience of immunization that was done by Mustafa and Abusowa, 1993. So far, vaccinations program have not been entered as protection against disease program in sheep and goats.

Very important used eradication program to prevent disease transmission from the small ruminants to different animal species and to reduce the risk of human exposure. Except that slaughtered the positive animal when used as eradication program for brucella disease in the sheep and goats is very expensive and difficult because the number of flock of sheep is large and separated in big area.

As well as the small ruminant flocks owners managements due to their income are classical and almost the same. They cannot implement close management system which, however is more benefit but it is expensive the flocks feeding is usually depends on the open pasture system and on the residual of the cereal crops which remain at pastures after the crops harvesting and mostly drinking from the scattered rain water wells. The small ruminants indeed are raised on the nature for the most months of the year and depend on the seasonal rain full, therefore many of the flocks owners transmit their flocks to another region when the rains is not enough for grass growing at their localities. However, the uncontrolled movement of the flocks between the regions and un-cleaned used trucks for flock transportation were the main causes of the disease distribution and eradication slaughter program is very expensive and difficult. Also from the problems of breeding is not to perform routine test for the herd or conducting tests before bring or

buy any new heard In a year of 2012, the laboratory of the National Centre of Animal Health reported that Brucella infection in 217 head mixed of sheep and goats in the Alkhums region. Seroprevalence rate of brucellosis was 47.5%. The owner of flock stated that these animals brought from Bani Walid region.

CONCLUSION

For Brucella vaccines, however the vaccine programs have not authorized to be implemented in Libya yet. It seems from the previous reports of the National Animal Health and Breeds improving Center that *Brucella melitenses* is the major dangerous cause of the abortion disease in Libyan sheep and goats of all ages.

Therefore we suggest to use vaccine programs and the vaccines produced locally from the local strains also reconsider about the analysis development program by adoption of r.time PCR as a confirmatory test to approximate the number of positive cases rounded to the real number due to cases that have been lost due to use of serological tests and to help establish the role of Real time in the expansion of the diagnostic rate lead detect to positive case when the infected animal in the early stage that unavailable by serological method in eradication program of Brucellosis.

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الملخص

أول تقرير عن داء البروسيلات في ليبيا منذ عام 1931 في الماعز، حيث تمكن الباحث (ميدولا، 1931) من توثيقه. بعد ذلك، تكررت التقارير في محطة ألبان عامة في سبعينيات القرن الماضي (أبودايا، 1992). كما سُجلت حالة إصابة بداء البروسيلات في قطيع صغير من الإبل المستوردة من السودان، وذلك بواسطة حسني وأبودايا عام 1992. وفي الفترة من 1980 إلى 1982، نُفذ أول مسح وبرنامج استئصال في طرابلس وما حولها، وفي محطة أبقار عامة لإنتاج الثروة الحيوانية في مدينة بنغازي. ثم في الفترة من 1997 إلى نهاية عام 2000، أجرت السلطات الليبية مسحًا ثانيًا وبرنامج استئصال. من عام 1931 إلى عام 1984، تم إجراء معظم الفحوصات عن طريق زراعة العينات في مختبرات خارج ليبيا، حيث تم عزل بكتيريا البروسيلا الملتية (النمط المصلي الأول والنمط المصلي الثاني). وفي عام 1997، تم استخدام الطرق المصلية TAT وCFT وRBPT وBAPT. تم فحص النمط الحيوي الثالث من بكتيريا البروسيلا (SAT وELISA) وبكتيريا البروسيلا المقاومة للميثيسيلين (MRT) في الحليب. تهدف هذه الدراسة إلى تحديد مدى انتشار داء البروسيلات بين مربي الماشية الليبيين (الأغنام والماعز والأبقار والإبل)، وكذلك بين البشر، بالإضافة إلى برامج الاستئصال التي نُفذت.

الكلمات المفتاحية: داء البروسيلات، ليبيا، الأغنام، الماعز، الأبقار، الإبل، البشر.