دراسة مصليه لمرض البروسيلا في الانسان والحيوانات الحقليه في محافظة واسط

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Serological study for Brucellosis in human &domestic animals of Wasit Province

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الخلاصه

مرض البر وسيلا من الأمراض المشتركة الأكثر انتشار في الشرق الأوسط ومنها العراق إذا تم جمع بيانات لمدة أربعة سنوات من 2006-2009 في (الحي ,النعمانيه,الكوت,العزيزيه والصويره) تم اخذ 3962 عينه من الإنسان167 عينة من الأبقار و 148 عينة من الأغنام والماعز ونقلت العينات إلى مختبر البكتريولوجي في مستشفى الكرامة التعليمي وقد قسمت العينات إلى جزئيين الجزء الأول فحص ب الروز بنكال والجزء الثاني تم زرعه على وسط الدم وبعد ظهور النمو تم فحص حساسية البكتريا للمضادات الحياتية وقد أظهرت النتائج إن إعداد كبيره من الإنسان مصابه بالمرض اذا سجلت اعلى نسبة اصابه بالمرض سنة 2006 وكانت 11.5% واقل نسبة اصابه سنة 2008 وكانت 5.2اما الابقار كانت نسبة الاصاب11.1% والاعنام والماعز 23.5% كما اظهرت النتائج حساسية البكتريا للمضادات مثل the aminoglycosides , rifampicin streptomycin , gentamicin ومقاومته ل oxcillin وان العلاج يحتاج لفترة طويلة لان البكتريا توجد داخل الخلايا

Summary

Brucellosis is an endemic anthropozoonotic disease in most Middle East countries including Iraq. It causes considerable loss of economy and energy, particularly in the rural areas. Since 2006-2009 when the prevalence of brucella agglutinin from human , sheep ,cattle and goat was reported in wasit, several time have been conducted including serological studies of brucellosis in the wasit provinces of Iraq (suwayra, Aziziya, Alnomaniya, hay,Kut ,Shake sade). Humans, sheep, goats and cattle have been involved. The studies accure in microbiology lab. In alkarmha hospital teaching& appear the percentage of infection is high inhumin in2006 (11.5%) &lower infection in 2008 (5.2&) but in cattle (11.1%) and in sheep &goat (23.5%). The study included sensitive test and identification of susceptible *Brucella* species, to drug including *chloraphenicol, streptomycin ,nitrofutantion ,nalidixic acid* &garamycin but resistant to oxcillin &evaluate the antibacterial activity of olive oil against the brucella isolated from the animal & humane in kut / Iraq .

Introduction :-

Brucellosis distributed world wide in humans and animals disease . It is problem and one of the most important zoonotic in the Mediterranean region (MR). Although continuous progress is verified in brucellosis control, it still remains a major public health hazard and of great economic importance. This explains the ever-increasing concern in many countries(5)

Brucellosis, a bacterial disease caused by members of the genus *Brucella,* is an important zoonosis and a significant cause of reproductive losses in animals.(4) Brucellosis is usually caused by *Brucellaabortus*in cattle, *B. melitensis*or *B. ovis*in small ruminants, *B. suis*in pigs and *B. canis*in dogs. Abortions, placentitis, epididymitis and orchitis are the most common consequences, although other syndromes are also reported.(7) The main impact is economic; deaths are rare except in the fetus and neonate.(6) Some *Brucella*species are also maintained in wildlife populations.(12) Wildlife reservoirs including feral pigs, bison, elk and European hares complicate eradication efforts for *B. abortus*and *B. suis*. Marine mammal isolates of *Brucella*have recently been recognized in many species of pinnipeds and cetaceans, and there are concerns that these organisms might have a detrimental impact on some species.(13)

The complexity of the epidemiology of brucellosis and the serious difficulties for effective control measures, arise from the involvement in the infection of the main producing domestic animals (cattle , sheep, goats, swine) and humans(2).

The epidemiological surveillance of human and animal brucellosis is among the methods considered as a high priority and of essential strategic importance for endemic and disease-free countries. Experience has proven that this system is one of the pillars upon which any monitoring control programme, irrespective of the country, should be based.(1)

Moreover, essential tools for an organised control of zoonotic diseases in general and of brucellosis in particular are the efficient surveillance system at national level; the effective co-operation and information exchange between public health and veterinary sectors as well as regular co-operation between neighbouring countries and the entire MR. (3)
Most species of *Brucella*can infect animals other than their preferred hosts, when they come in close contact. *B. abortus, B. melitensis, B. suis, B. canis*and marine mammal *Brucella*species are human pathogens.(16) In humans, brucellosis can be a serious, debilitating and sometimes chronic disease that may affect a variety of organs. Most cases are caused by occupational exposure to infected animals or the ingestion of unpasteurized dairy products. In the U.S., *B. suis*has been eliminated from commercial pigs and *B. abortus*has nearly been eradicated from domesticated ruminants. As a result, human brucellosis is rare. However, this disease remains a common and serious problem in some parts of the world. In addition, some species of *Brucella*could be used in a bioterrorist attack (10)
Brucellosis results from infection by various species of *Brucella,* a Gram negative, facultative intracellular coccobacillus or short rod in the family

Brucellaceae. Six named species occur in animals: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*and *B. neotomae*. One or more unnamed species of *Brucella*have been found in marine mammals. Formal names proposed for marine mammal isolates are *B. maris*for all strains, or *B. pinnipediae*for strains from pinnipeds (seals, sea lions and walruses) and *B. cetaceae*for isolates from cetaceans (whales, porpoises and dolphins). Some species of *Brucella*contain biovars. Five biovars have been reported for *B. suis*, three for *B. melitensis*, and up to nine for *B. abortus*.(20)

Each *Brucella*species is associated most often with certain hosts. *B. abortus*usually causes brucellosis in cattle, bison and buffalo. *B. melitensis*is the most important species in sheep and goats, but *B. ovis*can also cause infertility in rams. *B. canis*causes disease almost exclusively in dogs. *B. neotomae*is found in rodents, but has not been linked to disease. *B. suis*contains more diverse isolates than other *Brucella*species, and these isolates have broader host specificity. *B. suis*biovars 1, 2 and 3 are maintained in pigs; European hares are also a reservoir for biovar 2. Biovar 4 mainly affects reindeer and caribou, and is not normally found in pigs. This biovar was formerly known as *B. rangiferi*. Biovar 5 occurs in rodents.(14)

In humans, brucellosis can be caused by *B. abortus*, *B. melitensis*, *B. suis*biovars 1-4 and, rarely, *B. canis*or marine mammal *Brucella*. Live vaccines for *B. abortus*and *B. melitensis*, as well as the *B. canis*M- strain (a less virulent strain used as an antigen for serological testing), are also pathogenic for humans. *B. ovis*, *B. neotomae*and *B. suis*biovar 5 have not been linked to human disease(15)

The antibiotic susceptibility testing of brucella may help the choice of treatment in specific cases & detection of any drug resistance that may be developed (24)

Brucella spp. Were highly susceptible to most antibiotics tested (21)

Olive oil was commonly used as a medicinal herb in Asia ,Africa & south America because of its anti- diabetic, antibacterial , antiviral & chemo preventive functions (8)

Olive oil have recently been shown to inhibit or delay the rate of growth of arrange of bacteria & microfunge but there are no data in the literature concerning the employment of these secoiridoides as antimicrobial agent against pathogenic bacteria in man (3)

Antimicrobial activity 0f olive oil has been demonstrated against several strain of bacteria implication in intestinal & respiratory infection ,due to its hydroxytyrosol ,oleuropein & phenolic compounds which identified as having antibacterial properties (26)

MATERIAL AND METHODS

Since 2006-2009 when the prevalence of brucella agglutinin(3962) sample from human , (167) from cattle,(148)s. from sheep and goat was reported in wasit, this study accure in bacteriology lab. In al karamah hospital teaching .several time have been conducted including susceptible bacteria to antibiotic & serological studies of brucellosis(Rose Bengal test) in the wasit provinces of Iraq (suwayra, Aziziya, Alnomaniya, hay,Kut ,Shake sade). Humans, sheep, goats and cattle have been involved .each sample should be divided into two portions. First portion of sample examination by

Rose Bengal plate test (RBT)

PRINCIPLE OF THE TEST

In the course of human infection with any pathogenic microbiological agent, a variety of antibodies are formed. Among these antibodies are the agglutinins. An agglutinin when combined with homologous antigen (agglutinate) under the properly controlled conditions is capable of causing agglutination. Suspension of Brucella possessing active antigen will agglutinate when exposed to homologous Brucella
antibody. This agglutination forms clumps of bacteria which becomes macroscopically visible.(22)(17)

The Rose Bengal stained Brucella antigen is used forth early detection of Brucella agglutinins (BrucellaAbortus, Melitensis and Suis).

REAGENTS

1- Rose Bengal Brucella Antigen (0.5% phenol)
2- Positive Control (0.01% sodium azide)
3- Negative Control (0.01% sodium azide)

WARNING

Brucella Antigens and control antiserum are to be used for In Vitro Diagnostic Use Only.

STORAGE

Brucella antigen and control antiserum must be stored
at 2-8°C when not in use.

SPECIMEN COLLECTION & PREPARATION

1. Collect 5ml whole blood samples aseptically from the patient.
2. Allow blood to clot and remove serum assoon as possible to prevent excess haemolysis.
3. Store serum at 2-8°C until testing can be performed.

MATERIALS PROVIDED

1. Brucella Antigen, Rose Bengal Stained.
2. Positive Control.
3. Negative Control.
4. White Glass slide.
5. Stirring Sticks.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes for dispensing 40µl serum.
2. Timer.

PROCEDURE

1- Allow reagents and serum samples to reach room temperature for testing.
2- Shake the antigen bottle gently to insure auniform suspension.
3- Place 40ul sample serum onto the selectedring of the slide.

4- Place one drop of the Rose Bengal antigenonto serum sample.
5- Mix serum sample with Rose Bengal antigenusing Stirring stick.
6- Repeat these steps using the positive andnegative controls instead of serum sample.
7- Gently rock the slide for 2 minutes(automatic rotator can also be used).
8- Observe for agglutination after 2 minutesfrom beginning of shaking (this is the
optimum time limit).
RESULTS
Negative: No agglutination
Positive: (Presence of Specific Antibodies):Agglutination

The test is an excellent screening test but may be oversensitive for diagnosis in individual animals, particularly vaccinated ones.(25)(19)
Identification methods was mad on the basis of the requirement of co2 for growth ,production urease &H2s ,gram negative stain & oxidase positive
Ten standard antibiotics which are susceptible and active against brucella including
Rifampicin, streptomycin , chloramphenicol , gentamicin , amikacin , nitrofurantion , naldixic acid , cetaxime , ceftriaxone & ciprofloxacin .
One disc of each antibiotic was placed with sterile forceps of each plate ,incubation , followed by measuring diameter of zone of inhibition on muller-hinton agar
Olive oil (HEMANI -IRP)was purchased from local market & identified at the national Iraq institute for Herbs ,Baghdad ,Iraq .
Well aseptically on each agar plate with diameter 5mm for the olive oil ,0.1 ml of the oil at a concentration of 100% in the well & incubation ,followed by measuring diameter of zone of inhibition on muller –Hinton agar .

Statistical analysis :
Data were expressed as mean ±SD .analysis of data was statistically performed by the use of completely randomized design (CRD) of single factor .
Least significant difference (LSD) has been used to determent the statistically difference between mean values p-value (<o.oo1) was considered significant different (18)

Result &Dissociation

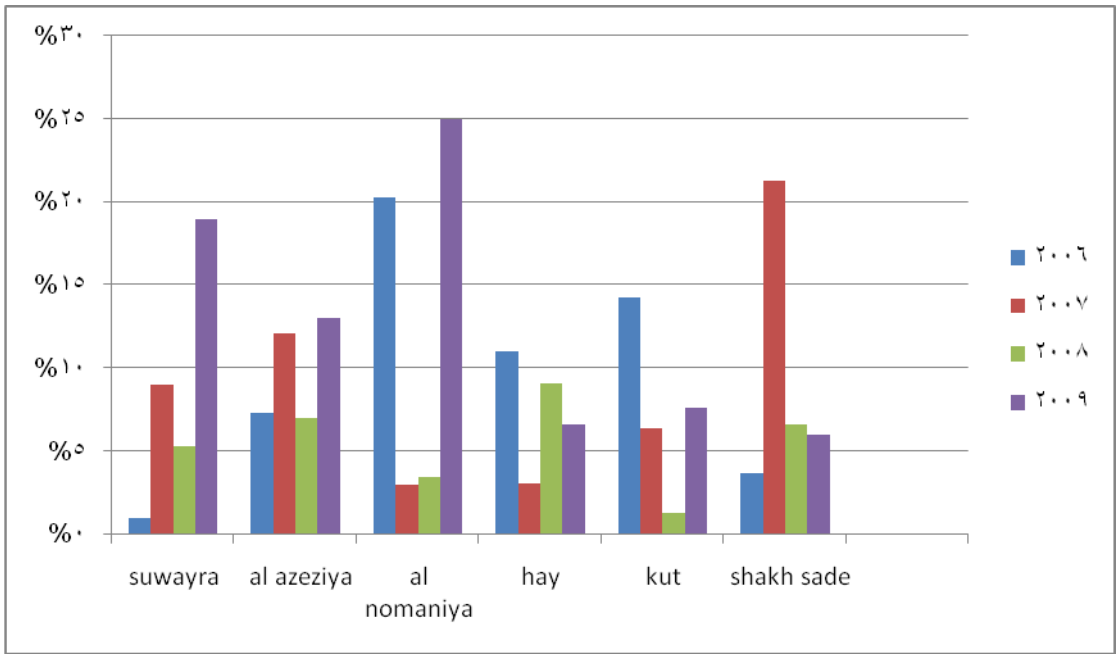

Table (1) show the positive cases in human according to the place &years

Years	2006			2007			2008			2009		
Place	n.	+ve	%	n.	+ ve	%	n.	ve+	%	n.	+ve	%
Suwayra	200	2	1%	300	27	9%	150	8	5.3%	180	35	19.4%
Aziziya	150	11	7.3%	197	24	12.1%	100	7	7%	300	39	13%
Al nomaniya	300	61	20.3%	100	3	3%	140	5	3.5%	100	2	25%
Hay	100	11	11%	95	3	3.1%	120	11	9.1%	150	10	6.6%
Kut	300	43	14.3%	280	18	6.4%	150	2	1.3%	130	10	7.6%
Shakhe sade	80	3	3.7%	150	32	21.3%	90	6	6.6%	100	6	6%
Total	1130	131	11.5%	1122	10 7	9.5%	750	39	5.2%	960	106	11%

Table (1) shows the distribution of positive case in according to the place and years . These result showed the highest infection in 2006 were(20.3%) in numaniyabut in2007 in shakhe sade is (21.3%) &in 2008 in hay was (9.1%) &in 2009 numaniyais (25%) .*Giannacopoulos et al;* (2002) reported the highest incidence of infection was observed in human is(52) . the high incidence ccure due to eating habit &contaminated milk . and the lowest (1%) in suwayra (2006) , and lnommaniya(2007) was (3%), in kut (2008)was (1.3%)&in shakhe sade 2009 was(6%) . Troy et al (2005) reported lowest incidence of infection was observed in human is (28)

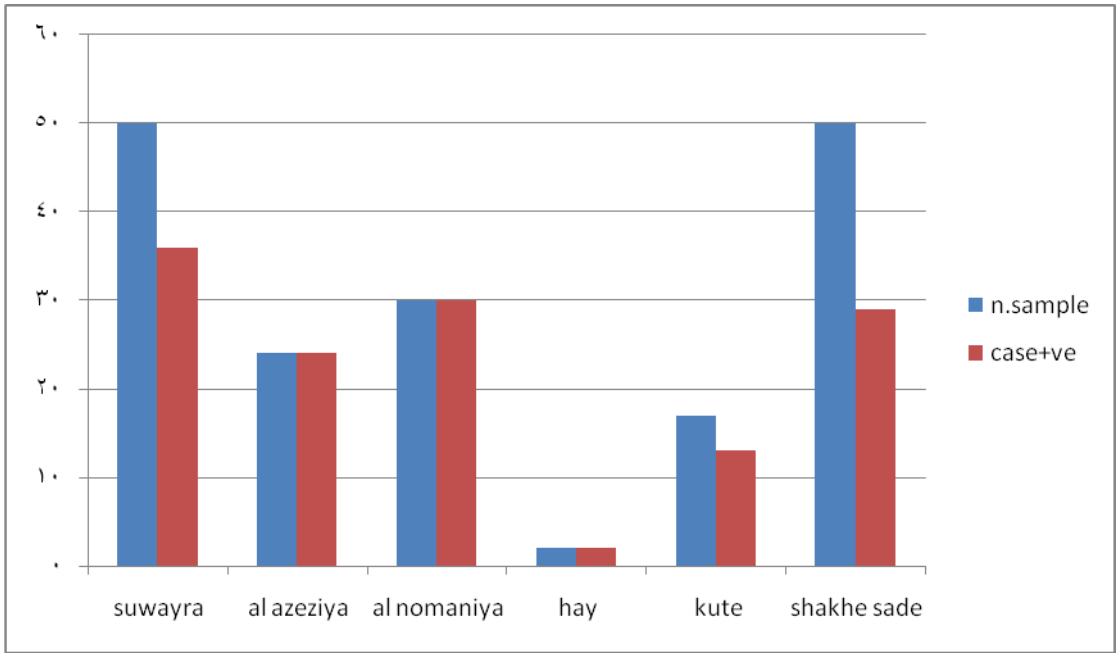

Table (2) show the positive cases in sheep &goat according to the place from 2006 to 2009

Place	Abortion animal	Number of sample	Case +ve	%
suwayra	57	50	36	64.2%
Aziziya	115	24	24	20.8%
Alnomaniya	35	30	30	85.7%
Hay	65	2	2	3%
Kut	225	17	13	5.7%
Shake sade	71	50	29	40.8%
Total	568	148	134	23.5%

Table (2) show distribution of animal abortions (sheep, goat) ,sample take from animal & positive case according to place in four years (2006 to 2009) . These result showed the highest animal abortions in kut (225) but the sample examine (17) &positive case (5.7%)that mean the large number of animal is abortion but not examination &don't found microbiology lab. In veterinary hospital &the

lowest in hay the animal abortions (65) but the sample examination (2) or (3%) World Organisation for Animal Health. (2004)

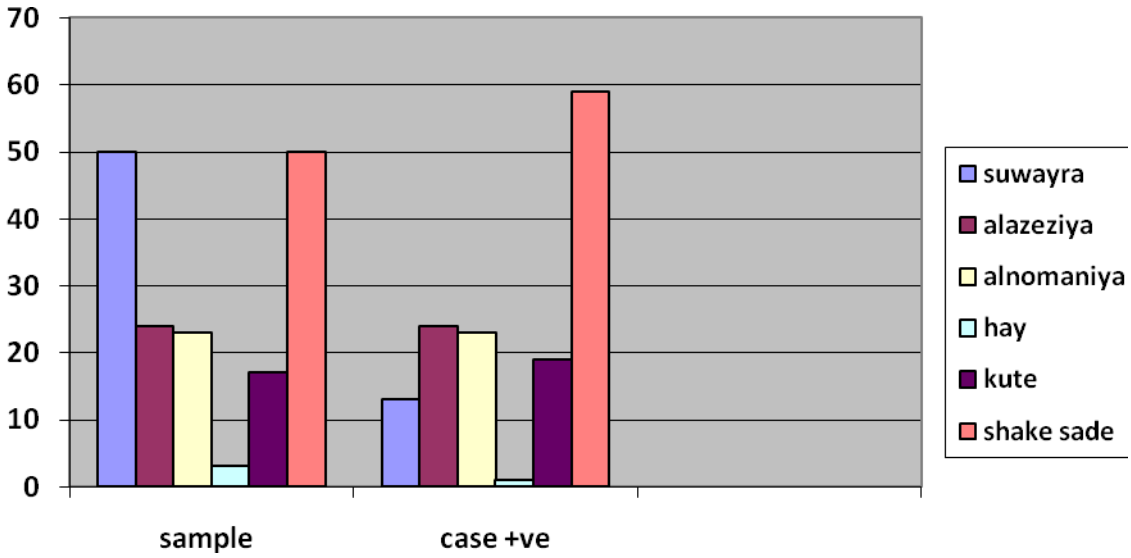

Table (3)show the positive cases in cattle according to the place from 2006 to 2009

Place	Abortion animal	Number of Sample	Case +ve	%
suwayra	107	50	13	12.4%
Al azezia	58	24	2	3.4%
Alnomania	157	23	23	14.6%
Hay	40	3	1	2.5%
Kut	60	17	1	1.6%
Shakhe sade	108	50	19	17.5%
Total	530	167	59	11.1%

Table (3) show distribution of animal abortions(cattle) ,sample take from animal & positive case according to place in four years (2006 to 2009) . These result showed the number of animal abortions in wasit is (530) but the sample examine or sample take from animal (167)that mean the (363) animals not examine & thepositive case of animal examine (59) (11.1 %) & the highest animal examine in shakhe sade &suwayra is (50)but positive case (17.5,12.4) in

hay the animal abortions (40) but the sample examination (3) or (7.5%) the brucella one of large number of agent cause abortion in cattle Choeekaert,A. ; et al (2005)

Table (6) the susceptible of bacteria to the antibiotic

Antibiotic	Susceptible	Resistances	Mean ±SD
Amikacin	+	-	**7.50±0.36e**
Streptomycin	+	-	**12.27± o.86ab**
Chloramphenicol	+	-	**10.05 ± 1.17 bc**
Nitrofurantion	+	-	**7.10±0.51e**
Nalidixic acid	+	-	**6.50±0.33e**
Gentamicin	+	-	**10.56± 1.36 bd**
Ciprofloxacin	-	+	**2.61±0.49f**
Cefaxime	+	-	**7.84±0.70ce**
Ceftriaxone	+	-	**8.48±0.62de**
Rifampicine	+	-	**13.67±2.14a**

Table (6) show antibiotics like rifampicin and the aminoglycosides streptomycin and gentamicin are effective against Brucella bacteria. However, the use of more than one antibiotic is needed for several weeks, because the bacteria incubates within cells.
While the oxacillin ,ampicillin &olive oil were resistant with mean value 0±0

- **The value represent M±SD**
- **The different small letter show significant effect between different antibiotic**

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