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Short Communication

Isolation, identification and biotyping of brucella abortus in cattle and camels in Northern State, Sudan

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Abstract

Brucellosis is a thropo-zoonotic infection which can be considered as a great challenge to development of dairy production in developing countries. It is one of the most economically devastating disease, which causes heavy economical losses resulting both from direct effects on animals such as abortions, irregular breeding, sterility and decreased milk yield. From indirect effects on the animal industry associated with costs of veterinary attendance, replacement of animals and impediment to free animal movement and export. The objective of this study was to isolate and characterize Brucella species and determine the biovars associated with the disease. Seven Brucella a bortus biovar l was isolated and identified from cattel and camels in Northern State, Sudan. Tow isolates were isolated from bovine milk. Four from bovine vaginal swabs and one from a placental specimens from a camel. Placental fragment, vaginal swabs, milk and hygroma fluids were collected. Then cultured into Mueller Hinton agar medium. Isolates were identified as Brucella (spp) on the basis of morphology, staining of the organism, production of catalase, oxidase and urease. The colonies appeared after 4-5 days of incubation and were smooth and transparent. Suspected colonies were stained by Gram stain used Modified Zihl-Neelsen (MZN) methods. Gram- negative coccobacilli were found in smears from the colonies. All biochemical tests of the showed characteristics of brucella. The isolates were agglutinated with Brucella A but not with M Brucella antiserum and lyses by Wb, Tb, Bk and Fi phages. We concluded that the seven isolates were sensitive to the antibodies used for treatment of the disease and one isolate show in vitro resistance to Rifampicin.

Keywords: Brucellos, Diterent animals.

Introduction

Brucellosis is an important public health problem through the world, especially in the Mediterranean region, the Arabian pensiula, Africa, the Indian sub- continent, Mexico and parts of Central and South America[1]. Because of the grave economic consequence of the epizootic of the disease, intensive control measures have been adopted, starting from the farm level[2]. Bannatyne et al.[3] suggested that there must be a full cooperation between all countries and international agencies in disease surveillance and notification of outbreaks. Despite being controlled in many developed countries, the disease remains endemic in many parts of the world and the major source of infection to man and farm animals. The presence of brucellosis in Sudan was first established early in the past century and it was reported from almost all the States[4], but the situation varies widely between States. The disease in animals was extensively surveyed in different parts of the country, while in humans, data on this disease are frequently incomplete.

Materials and methods

Samples: The present study was conducted in cattle and camels, during survey in Northern State, Northern Sudan, from January upto September 2010. Hygroma fluids from edematous swellings in cattle and camels were aspirated for culture purposes. Using sterile disposable syringes, (20-30 ml) volumes of the aspirates were withdrawn, put in an ice box and transported to the laboratory as soon as possible. Vaginal swabs were taken from cases with retained placenta. A vagina scope was used to open the vulva and swabs were taken from the inner wall of the vagina and rotated gently in several directions using sterile cotton swabs kept in a glass tubes. The swabs were placed into thermo-flasks and transported to the laboratory for bacteriological examinations. Twenty milliliters volumes of bulk bovine milk samples were collected in sterile bijou bottles, each separately and transported in an ice box to the laboratory for investigations. Pieces of retained placenta were sampled and placed into sterile plastic containers each separately in an ice box put and transported to the laboratory for bacteriological investigations.

Culture Methods: Placental fragments, vaginal swabs, milk, and hygroma fluids were used to prepare slide smears which were stained by the modified Ziehl–Neelsen's method. Samples found positive for acid fast Brucella-like bacteria were cultured.

Identification of the isolates: Brucellae were isolated from milk, vaginal swabs (cattle isolates) and placental specimens (camel isolates). Isolates were identified as Brucella spp. on the

basis of morphology, staining of the organism, production of catalase, oxidase and urease.

Morphology: Brucella colonies were visible after (4-5) days incubation. They appeared round (1-2mm) in diameter, smooth, translucent and pale honey colour, when plates were viewed in the daylight through a transparent medium. Later the colonies became large and slightly darker. Colonies with these characteristics were suspected to be Brucella spp.

Staining of the organism: Smears were prepared from the suspected colonies and sained with Gram stain and Modified Ziehl -Neelsen (MZN). Organisms which appeared red against a blue background were suspected to be Brucella spp.

Catlase test: The test was done according to Cheasbrough (1984) as following: i. ml of hydrogen peroxide solution was poured into a test tube. ii. Using a sterile wooden stick, a culture of suspected organism was removed from the plate and immersed in the hydrogen peroxide solution. iii. Positive results: Active bubbling was observed, indicating catalase production. iv. Negative results: No release of bubbles was observed.

Oxidase test: The test was performed according to "Kovac's modification" as following: i. One to tow drops of oxidase reagent (0.5% testramethyl- phenylendiamine) were placed on a piece of Whatman Filter paper. ii. Using a disposable plastic loop, a loopful of the culture of the organism from the plate was mixed into the reagent on the filter paper. iii. Positive results: Blue color within 10 seconds. of incubation was observed. iv. Negative results: No Blue color within 10 seconds of inoculation was observed.

Urease Test (Christensen's Method) as following: i. Urea tubes were removed from storage and allowed to equilibrate to a room temperature (at least 30 minutes before setting up the test). ii. Using a sterile inoculating loop, a loopful of the test organism was streaked to the surface of Christensen's medium (urea agar). iii. Incubated at (35-37ºC) at an ambient atmosphere. iv. Timer was set for 15 minutes. v. After 15 minutes incubation, the inoculated agar was observed for a color change to pink in the inoculated area. vi. In case no color change was observed, the agar was placed in an incubator and observed again after 24hours incubation. vii. A positive result: development of pronounced pink color in the agar. viii. A negative result: no pink color.

Agglutination with monospecific Brucella antisera: Brucella isolates were tested by monospecific antisera of smooth and rough Brucella to determine the colonial phase of the isolates.

Typing of the Brucella isolates: Isolates that were identified as Brucella on the basis of the morphology and cultural characteristics were identified to the species and biovar levels. Further characterization was made on the basis of CO_2 requirement, H_2S production, and growth on dye plates.

Dye sensitivity tests: i. Basic fuchsin and thionin dyes at concentrations of 20ul/ml (1/50/000 w/v and 20 ul 1/ml (1/50/000 w/v) were incorporated into serum dextrose agar. ii. After the medium had been set, the plates were placed in the incubator overnight to check for sterility. iii. Suspensions of bacterial culture under test and reference cultures (Br.abortus S_{19}; and Br. melitensis 16M) were prepared by suspending a loopful of recently grown culture in 1.0 ml sterile normal saline, iv. Care was made in preparing suspensions of approximately uniform turbidity. v. A loopful was used to make five streaking for each test and reference culture. The loop was recharged after streaking. vi. The plates were labeled with the culture number and up to two cultures were tested per plate. vii. The plates were incubated at 37ºC for (3-5 days) and observed for growth.

Phage sensitivity: Preliminary step to phage-typing was to find the state of dissociation as described by Carbal and Thomas.

Procedure*:* i. Representive smooth strains sub-cultured on serum dextrose agar shapes were picked and suspended on Albimi Brucella broth to give a concentration of 10^{10} organisms/ml. ii. Volumes of 0.1 ml of this suspension were spread over moisture free surface of serum dextrose agar plates with swabs. The plates were allowed to dry for about one hour. iii. Nine drops of phage suspensions (usually 0.025 ml) were applied with appropriate pipettes to the surface of the previously Brucella-inoculated agar plates. iv. The drops were allowed to absorb in the agar for a few minutes the plates were inverted and incubated for up 24hours. v. At 37ºC temperature, inoculated plates were inspected after 24h. Intervals for signs of lysis or plagues. vi. For routine typing process, one representative strain of each group of Brucella was tested against Tb, Fi, Wb, BK and R phages.

Results and discussion

The results of this study are summarized in table 1 and 2. The isolates produced H_2S within 4 days of incubation. They grew on media containing basic fuchsin, but not thionin. The isolates were lysed by Wb, Tb, Bk and Fi phages. Based on these properties.

Brucellae have a wide range of host species, including humans. Consumption of contaminated foods and occupational contacts remain the major source of infection. Although brucellosis is a notifiable disease, official figures were lower than the a ctual one as under-diagnosis and under reporting of cases are recognized problems in many developing countries[5]. In the Sudan, the disease was first reported in (1904) and two species of Brucella have so far have been isolated, they are Br. abortus and Br. melitensis[6]. In Northern State, the disease is prevalent in Dongola, Daba and Marawe localities and new cases were reported annually[7]. Musa and Shigidi (2000)[8] identified two Br. melitensis strains which had been in Central Sudan in (1953) as biovar 3 while 21 Br. abortus strains which had been form different parts of the Sudan between 1957 and 1993 as Biovar 6. The seven isolates in this study were identified as Br. abortus biovar 1. Comparable findings were reported by Omer et al.[9] and Ali[10]. The results of this study can assist in understanding of the epidemiology of brucellosis in the country.

Table-1: Identification of Brucella isolates.

Area	Number of isolates	Species	Host	Origin
Southern area	One	Br.abortus	Camel	placental specimens
Middle area	Two	Br.abortus	Cattle	Milk
Middle area	Four	Br.abortus	Cattle	Vaginal swabs

Table-2: Characterization of the Brucella isolates.

sample	Growth characteristics					Monospecific serum			Phage at RTD				Result
	Urea	H2 S	CO2	BF	TH	A	M	R	Wb	Tb	BK	Fi	
Camel isolate Daba 1	+	+	-	+	-	+	-		CL	CL	CL	Plq	Br.abortus.biovar1
Cattle isolate													
Dongla 1	+	+	-	+	-	+	-		CL	CL	CL	Plq	Br. bortus.biovar1
Dongla 2	++	+	-	+	-	+	-		CL	CL	CL	Plq	Br.abortus.biovar1
Dongla 3	+	+	-	+	-	+	-		CL	CL	CL	Plq	Br.abortus.biovar1
Milk isolate M2	+	+	-	+	-	+	-		CL	CL	CL	Plq	Br.abortus.biovar1

Key: BF = Basic fuchsin at 20Ul/ML (1/ 50.000 w/v). TH = Thionin at 20 Ul/ ml (1/50.000 w/v). CL = Confluent Lysis. RTD = Routine test dilution. Plq= Plaque.

Conclusion

We concluded that the seven isolates were sensitive to the antibodies used for treatment of the disease and one isolate show in vitro resistance to Rifampicin.

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