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Research Article

Evaluation of clinical samples by various serological, biochemical and Polymerase Chain Reaction techniques for brucellosis

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Abstract

The present study included, the samples such as 24 whole blood, 24 sera, 24 milk and 24 vaginal samples from cattle with various clinical problems, were collected and used to detect etiological agent, antibody and genome for Brucellosis. All four different types of samples collected from each animal in this study consisted together a total of 96 samples. All samples were subjected to isolation, biochemical, serological and polymerase chain reaction diagnostic tests. From 19 cattle with different reproductive problems; uterine prolapse, retained placenta, abortion and combined with other problems such as reduction in milk yield, anorexia, diarrhoea, pregnancy and ketosis etc., tested showed none of the vaginal samples positive by culture method and all biochemical tests. Of 24 milk samples tested by Milk Ring Test, 3 samples (12.5%) were found positive for antibody to Brucellosis. Among 24 serum samples screened , one sample (4.16%) and 2 samples (8.33%) were found positive by Rapid Plate Agglutination Test and Standard Tube Agglutination Test respectively. Out of 24 blood DNA tested for the detection of IS711 gene and B4/B5 gene by polymerase Chain Reaction for Brucellosis, 15 DNA samples (62.5%) were detected as positive by both gene primners. A total of 24 milk DNA samples screened by polymerase Chain Reaction 2 samples showed positivity for IS711 gene and B4/B5 gene for Brucellosis. Polymerase chain reaction showed higher positivity compared to other test used in this study. Between the serological tests, STAT showed little higher response than RPT. Though culture method is gold standard test, in this study not even a single vaginal samples were found positive by culture technique for Brucellosis

Keywords: Cattle, Diagnostic Screening, Brucellosis, PCR.

Introduction

Brucellosis is an economically important disease of livestock population and it is a zoonotic disease affecting human beings as well. It is worldwide in distribution. Many researchers discussed about the endemic nature of this disease in India [1-3]. Brucellosis is caused by, three main etiological agent such as *B. abortus, B. melitensis and B. canis* affecting cattle, sheep, goats and peoples etc. Abortion, infertility, sterility and drop in milk production are the problems causes severe economic losses due to this disease.

Isolation of Brucella species by culture, detection of antigen and antibody by ELISA, demonstration of antibody by serologic test such as rapid plate agglutination test, milk ring test, standard tube agglutination test, and the detection of genome of Brucella by polymerase chain reaction techniques are the commonly used diagnostic tests being followed routinely to screen the animals for brucellosis. However, isolation by culture is time consuming and they may have possibility for contamination [4]. Demonstration of antibody needs to be in higher concentration at least for the detectable level. Unlike all these techniques, polymerase chain reaction (PCR) is a convenient test and has higher sensitivity and specificity [5, 6].

There were different primers tried to amplify the genes specific for brucellosis, however, there have been lack of diagnosis carried out by PCR using different sets of primers at a time for comparison. Among the sets of primers tested the B4/B5 primer set was found as the most effective as compared to all other primers tested [7]. Kanani and Patel [8, 9] also detected the sensitivity similar to the findings of Ghodasara [7] by PCR using the B4/B5 primer set. But still, culture isolation is considered the gold standard for the diagnosis of Brucellosis than polymerase chain reaction. And so, culture isolation followed by polymerase chain reaction is required for the confirmation of brucellosis. This study has been contemplated to detect the organism and antibody for brucellosis from different biological samples.

Materials and Methods

Selection of Animals

Cattle regardless of breeds, calving status, and with various reproductive and production problems were aimed mostly for collection of samples. Only female cattle used for this study. The breeds of cattle included in this study were Jersey cross, Non-descript and Holstein Friesian cross. All 24 cattle from which samples collected showed different clinical signs such as, respiratory distress, abortion once in its calving period, swollen udder, anorexia, reduced milk yield, uterus problem, ruminal acidosis, retained placenta, repeated diarrhea, other reproductive and production problems. The calving status was taken for the approximate assessment of age of the animal.

Sample Collection, Processing and Storage

Samples such as, whole blood, serum, vaginal swabs and milk were collected from cattle with various reproductive and production problems. Vaginal swabs were collected in 500µl sterile phosphate buffered saline solution and used for cultivation of Brucella and for performing PCR. Serum was separated from the coagulated blood samples and used for screening of antibody for brucellosis by Rapid Plate Agglutination Test (RPT) and Standard Tube Agglutination Test (STAT). Milk samples of 2ml were collected from each animal in a sterile tube and the Milk Ring Test (MRT) was performed. Further, the milk was defated and used for the isolation of genomic DNA and performing PCR. Whole blood samples were utilized for the extraction of genomic DNA and performing PCR for the amplification of Brucella genes. All isolated DNA samples, serum and culture samples were stored under refrigeration and further utilized for performing polymerase chain reaction in order to detect genes of Brucella.

Isolation and Identification of Brucella Organisms from vaginal culture

Isolation and identification of *Brucella abortus* was carried out as per OIE [10]. The tube containing vaginal swabs collected from each cattle (*n*=24) were added with nutrient broth for cultivation of organism. The growth was further inoculated into the supplement Brucella selective agar petridish plates and incubated at 37˚C until further growth and the growth period varied from 3 days to one week. The culture was then used for performing biochemical tests such as H_2S production tests, Urease Tests and Carbol fucshin test.

Biochemical Tests

Lead Acetate Test

Brucella selective media: In 100 ml of distilled water, 7 grams of *Brucella* selective media was dissolved and autoclaved for the preparation of agar slant. Lead acetate strip was used to see the change of color in the presence of H_2S production by the Brucella organism.

Fuchsin dye Test

In 100 ml of distilled water, 7 grams of *Brucella* selective media was dissolved and autoclaved. To which 0.4 mg (40 µg/ml) of fuchsin powder was added and kept in a water bath for 20 minutes until the content in flask was dissolved. About 4ml this was used to prepare slant for culture inoculation.

Urease Test

In 100 ml of distilled water 4 grams of Christensen's urea agar was mixed and autoclaved . In a 4 ml of this agar slant made in a tube vaginal culture was inoculated to observe the ammonia production.

Serum Sample

Blood samples from 24 cattle were collected in a plain glass tubes (IMPROVE, guiangzhou-improve medical instrument, Co-Ltd.,) containing no anticoagulant (3ml/animal) for serum separation and for performing serological techniques such as, Rapid Plate Agglutination Test, Milk Ring Test and Standard Tube Agglutination Test.

Blood samples

Blood samples from 24 cattle were collected in a citrated glass tubes (IMPROVE, guiangzhou-improve medical instrument, Co-Ltd.,) (3ml/animal) for DNA extraction and performing PCR.

Milk Samples

Udder and teat canals of cattle were cleaned with water and dried thoroughly and 5 ml of milk from each animals was collected from 24 cattle.

Genomic DNA

Genomic DNA for the detection of Brucellosis genes by PCR was extracted from whole blood, defated milk and vaginal culture.

Polymerase Chain Reaction (PCR)

Extraction of Genomic DNA from vaginal and milk

EZ-10 spin column genomic DNA minipreps Kit (Bio-Basic Inc. Canada) was used for the extraction of genomic DNA from nasal, ocular, vaginal swabs, milk and serum samples using the procedure detailed by manufacturer's for the DNA extraction from animal tissues with little modification. The procedure is as follows;

1. In a microfuge tube 300µl sample solutions (milk pellet suspension/ vaginal culture pellet suspension) supernatant and 300µl ACL digestion solution which contains 20µl Proteinase K solution incubated at 56^{o}C in a water bath for half hour. The samples were then cooled at room temperature and spun at 12,000 rpm for two minute and the supernatant was collected.
2. To the 300µl supernatant solution 200µl AB solution was added to the EZ-10 spin column and kept at rest for 5 minutes followed by spinning at 4,000 rpm for 2 minutes.
3. Flow through was discarded and washed with 500µl wash buffer spun at 10,000 rpm for 3 minutes. The washing step was repeated twice followed by one empty spin at 13,000 rpm for 5 minutes.
4. Further, the column was transferred in to the new collection tube and the DNA was eluded with 50µl elution buffer followed by spinning at 13,000 rpm for 3 minutes.
5. The genomic DNA was stored at -20^{o}C until further use.
6.

Extraction of Genomic DNA from whole blood

1. Half ml of blood was added in 2.0 ml collection tube and centrifugation at 3000 rpm for three minutes at 4°C. Discarded the supernatant.
2. 0.8 ml of TBP buffer was added to the collection tube and vortex mixer gently. Spun at 3000 rpm for three minutes. Discarded the supernatant. Repeat if the blood pellet looks reddish colour.
3. 0.5 ml of TBM buffer was added to the collection tube. The tube was mixed vigorously in vortex and added three µl proteinase K and incubated at 55°C for 30 minutes.
4. The supernatant was mixed with 260 µl of absolute Ethanol and transferred the mixture to MX-10 column and spun at 8,000 rpm for one minute. Discarded the flow-through in the collection tube.
5. 500µl of wash solution was added and spun at 8,000 rpm for one minute and discarded flow-through.
6. Spun at 8,000 rpm for additional minute to remove residual amount of wash solution.
7. The column was placed into a clean 1.5 ml tube, and 30-50 µl of elution buffer was added into the center part of membrane in the column. Then spun at 10,000 rpm for one minute to elute DNA from the column.

Composition of Mixture for PCR

The extracted genomic DNA was used as a template to perform PCR with minor modifications. PCR was

performed out in the final reaction volume of 25µl in a 200µl capacity microfuge PCR tubes is as follows:
2X PCR Master mix 13.0 µl, Dnase-Rnase free water 7.0µl (Nuclease Free Water), Forward primer (pmole/µl) 1.0µl, Reverse primer (pmole/µl) 1.0µl, DNA template 3.0µl

Thermal Cyclic Conditions for PCR

PCR was performed in a total reaction volume of 25µl, in Master Cycler (eppendorf) with following thermal cyclic conditions with a little changes in thermal cycling conditions and timings [9, 11].

Primers	Cyclic Conditions				
	Initial denaturation	Denaturation	Annealing	Extension	Final Extension
Forward Reverse	94°C 5min	95°C 1min	57°C 1min	72°C 1min	72°C 6min

Two sets of primers for amplification of Brucellosis genes, IS711 and B4/B5 by PCR were purchased Synergy, Chennai.

S. No	Name of the Oligos	Sequences (5' 3')	Fragment Size	Product Length	References
1.	IS711 Forward	gacgaacggaatttttccaatccc	24	498bp	Bricker et al., (1994)
	IS711 Reverse	tgccgatcacttaagggccttcat	24		OIE, (2008)
2.	B4 Forward	tggctcggttgccaatatcaa	21	223bp	Bailey et al., (1992)
	B4 Reverse	cgcgcttgcctttcaggtctg			

Analysis of Polymerase Chain Reaction Amplified Products

The PCR products were analyzed on 1.2 percent agarose gel electrophoresis. Agarose gel was prepared in 1 X TAE buffer followed by heating and cooling at 50°C. About 5 µl of PCR products of each samples were run tested by gel electrophoresis along with 3µl of DNA molecular weight Marker (100bp) to check the size the amplicons.

Documentation of Results

The amplified products were visualized by a UV trans illuminator after staining the gel with ethidium bromide and the results were recorded and photographed using gel documentation system (Gel Doc Mega).

Results and Discussion

Samples included in this study were 24 whole blood, 24 serum, 24 milk and 24 vaginal swabs, collected from cattle in Chennai during the period between February and March 2013. Majorly, detection of antibody and genome for Brucellosis is very much important to determine the status of infection in cattle. Since, Brucellosis is endemic disease in India and could cause various reproductive problems, in cattle, sheep and goats, screening of such animals are highly essential. By using different nationally accepted diagnostic tests, in this study, collected samples were screened for Brucellosis. Cultivation of Brucella was accomplished by using the vaginal swabs samples in nutrient broth liquid media followed by culture in Brucella specific soli agar media. The specific culture was then subjected to Lead Acetate Test, Urease Test and Fuchsin Dye Test. The milk samples collected from cattle were utilized to screen antibody against Brucella by Milk Ring Test (MRT) and the serum samples were subjected to Rapid Plate Agglutination Test (RPT) and Standard Tube Agglutination Test (STAT) with a aim to detect antibody to Brucellosis. The whole blood and milk samples were used for the performance of polymerase chain reaction (PCR) in order to detect specific genes for Brucella.

Sample collection and Processing for conducting diagnosis for Brucellosis

Samples such as 24 whole blood, 24 sera, 24 milk and 24 vaginal samples from cattle with various problems collected were utilized for this study are shown in (Table 1 and Table 2). All four different types of samples were collected from each animals consisted together a total of 96 samples were subjected to isolation technique, biochemical tests, serological tests and polymerase chain reaction to detect the culture growth, to check the biochemical reactions, to demonstrate the presence of antibody, and to detect the genome for Brucellosis. From 19 of cattle with reproductive problems, uterine prolapse, retained placenta, abortion and combined with other problems such as reduction in milk yield, anorexia, diarrhoea, pregnancy and keotis etc; 15 cattle were found positive by blood DNA positive by polymerase chain reaction for both gene IS711 and B4/B5 that was targeted for Brucellosis.

Table 1 Clinical Signs observed in cows from which the samples for this study were collected

S.NO	CASE NUMBER	BREED	AGE	HISTORY
1.	205998	Jersey X	3 calving	Calved 7months back, anorexia, diarrhea.
2.	205824	Jersey X	4 calving	Diarrhea, reduced milk yield
3.	205943	HF X	3 calving	Calved 6 months back, diarrhea.
4.	205942	Jersey X	3 calving	Coffee brown coloured urine
5.	205940	HF X	3 calving	6 months pregnant, inappetance
6.	206012	ND	4 calving	Reduced milk yield
7.	205880	Jersey X	4 calving	Calved 12 months back, anorexia, reduced milk yield.
8.	205926	HF X	3 calving	Calved 2 months back, diarrhea.
9.	20568	Jersey X	5 calving	Calved 10 days before, retained placenta, frequently falling down
10.	205688	Jersey X	3 calving	Calved 2months back, retained placenta, reduced feed intake
11.	205882	Jersey X	3 calving	Reduced feed intake
12.	205590	Jersey X	2 calving	Calved 3 days back, respiratory distress, nasal discharge
13.	205747	Cow XB	5 calving	Aborted fetus
14.	205949	HF X	2 calving	Calved 2 months before, inappetance, reduced milk yield
15.	205673	Cow XB	3 calving	Uterus problem
16.	205740	Jersey X	5 calving	Retained placenta, uterus problem
17.	205884	Jersey X	3 calving	Secondary ketosis
18.	216283	ND	4 calving	Retained placenta
19.	206082	Jersey X	2 calving	Calved 3 months back, reduced feed intake and milk yield
20.	206005	Jersey X	4 calving	Calved 2 months back, reduced milk yield
21.	206130	HF X	3 calving	Anorexia, reduced milk yield
22.	216260	HF X	2 calving	Retained placenta
23.	216161	ND	2 calving	Not taking feed properly
24.	205919	HF X	3 calving	Anoraxia, Nasal discharge

Table. 2 Sample Collected from Cows and Screening by Different Diagnostic Test for Brucellosis

S. NO	CASE NUMBER	CLINICAL SIGNS	SAMPLES				PCR	
			SERUM		SWAB	MILK	MILK	BLOOD
			RBPT	STAT	CULTURE	MRT		
1.	205998	Calved 7months back, anorexia, diarrhea.	-	-	Negative	-	-	-
2.	205824	Diarrhea, reduced milk yield	-	-	Negative	-	-	+
3.	205943	Calved 6 months back, diarrhea.	-	-	Negative	-	-	-
4.	205942	Coffee brown coloured urine	-	-	Negative	+	-	-
5.	205940	6 months pregnant, inappetance	-	-	Negative	-	-	-
6.	206012	Reduced milk yield	-	-	Negative	-	-	+
7.	205880	Calved 12 months back, anorexia, reduced milk yield.	-	-	Negative	-	-	+
8.	205926	Calved 2 months back, diarrhea.	-	-	Negative	-	-	-
9.	20568	Calved 10 days before, retained placenta, frequently falling down	-	-	Negative	-	-	+
10.	205688	Calved 2months back, retained placenta, reduced feed intake	-	-	Negative	-	-	+
11.	205882	Reduced feed intake	-	-	Negative	-	-	-
12.	205590	Calved 3 days back, respiratory distress, nasal discharge	-	-	Negative	-	-	+
13.	205747	Aborted fetus	-	-	Negative	-	-	+
14.	205949	Calved 2 months before, in-appetence, reduced milk yield	-	-	Negative	-	-	-
15.	205673	Uterus problem	-	-	Negative	-	-	+
16.	205740	Retained placenta, uterus problem	-	-	Negative	-	-	+
17.	205884	Secondary ketosis	-	-	Negative	-	-	-
18.	216283	Retained placenta	+	-	Negative	-	-	+
19.	206082	Calved 3 months back, reduced feed intake and milk yield	-	-	Negative	-	+	-
20.	206005	Calved 2 months back, reduced milk yield	-	+	Negative	-	-	+
21.	206130	Anorexia, reduced milk yield	-	+	Negative	-	-	+
22.	216260	Retained placenta	-	-	Negative	+	-	+
23.	216261	Not taking feed properly	-	-	Negative	-	-	+
24.	205919	Anorexia, Nasal discharge	-	-	Negative	+	+	+

Isolation and identification of *Brucella* organisms

An attempt was made to isolate Brucella from 24 vaginal swab samples in a Brucella specific solid agar media. The colonies are supposed to be round, convex, smooth, margin, translucent and yellowish paint in colour on *Brucella* selective media, however, this study found colonies only in two of cultured samples (8.3%) and the remaining samples showed no growth. Culture smears were made and stained by Gram's stain and examined under microscope in 100X oil immersion magnification. Though culture is a confirmative gold standard test for the detection of Brucella, [12] it is difficult, time consuming and cumbersome technique for being a diagnostic test.

Biochemical Test

Using the grown culture the biochemical test such as Lead acetate test, Fuchsin Dye test and Urease Test was carried out for confirmation of H_2S production, characteristic colony growth and ammonia production respectively.

Lead acetate test

Out of two culture growth which was identified by culture on Brucella specific solid agar media two of them shown H_2S production. This two culture growth was found positive for Lead acetate strips test based on the turning of white strip in to black color in the presence of H_2S production by Brucella organism [12].

Fuchsin Dye test

Of two culture growth which was identified on Brucella specific solid agar media, 1/2 of the culture plate (50%) showed pink colored colonies specific to fuchsin dye because the Brucella organism utilizes fuchsin dye for their growth and it is indicated by the pink color colonies on the agar slant [12]. But in this study no pink color produced in two of the culture .

Urease Test

Of two culture growth, which was identified on Brucella specific solid agar media, none of the culture plate, zero 0%, showed pink colored colonies in the presence of urease [12]. The Brucella organism use this urease for their growth and splits urease into ammonia and show the colonies as pink. But in this study no pink colonies produced were found in two of the culture growth.

Serological detection of antibody by Rapid Plate Agglutination Test (RPT), Milk Ring Test (MRT), and Standard Tube Agglutination Test (STAT).

Milk Ring Test (MRT)

A strongly positive reaction was indicated by formation of dark pink ring at the top surface of white milk column (Fig. 1).

The test was considered to be negative when there was uniform pink colour of the milk was noticed. Milk of brucellosis infected animal contains antibodies against the organism that combine with abortus Bang ring colored antigen formed antigen-antibody complexes and rises to top along with cream layer when milk was incubated. In this study, 24 milk samples were tested of which only 3 samples (12%) found with antibody. This test is being used to test bulk milk antibody and individuals or in a herd against Brucellosis. [13] reported a sensitivity of 72 % by milk PCR.

Rapid Plate Agglutination Test (RPT)

Agglutination is the aggregation of antigen and antibody particles or cells. From 24 serum samples tested by STAT only one sample (4.16 %) showed seroposivity for Brucellosis antibody. Samples which have showed lattices were considered as agglutination and it was viewed by visual through naked eye (Fig. 2). [14] reported only very less percent of serologically positive reactors (4.98%) following testing of cows by RPT which was found more or less similar to the present s study.

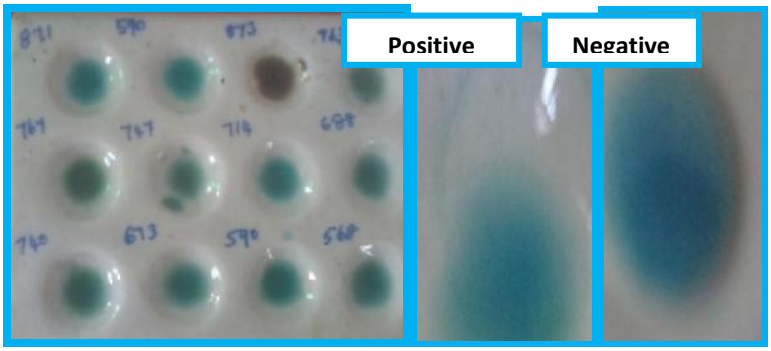
Standard Tube Agglutination Test (STAT)

To detect the antibody status against Brucellosis 24 serum samples were tested by Standard Tube Agglutination Test (STAT) , the highest serum dilution at which 50% agglutination and clearance of top solution seen in samples were considered positive (Fig. 3). 2 of 24 serum samples (8.33%) were found as sero-positive in this study. Wael et al (2012) detected 4.73 % cows serum as positive by serum agglutination test which was found lower as compared to the percent detected in this present study. Though the samples have been collected randomly, the higher sensitivity of this test might be due to sampled cows would have got infection at the time of collection.

Figure 1 MRT-Negative and Positive samples with controls

(+) **Positive Sample - a creamy ring is formed at the top (MRT)**
(-) Negative Sample – No ring formation

Figure 2 RPT positive and negative Reactions



(+) **Agglutination by RPT; (-) No Agglutination is formed**

Figure 3 STAT – Positive and Negative control tubes after incubation

1-7 Represents Agglutination for serum with an antibody titre

Polymerase Chain Reaction

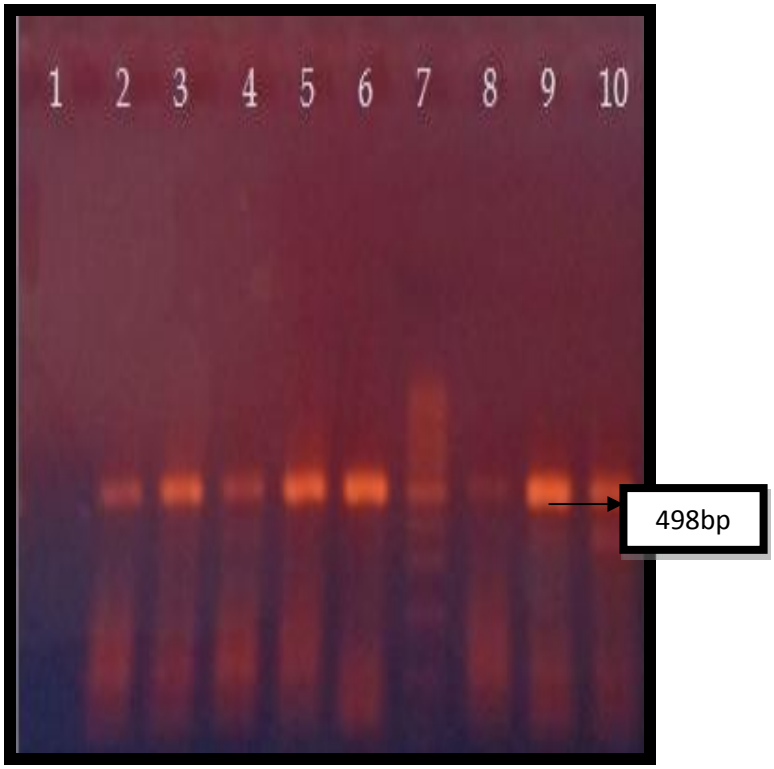
Polymerase Chain Reaction (PCR) for the detection of Brucella specific IS711//alkB **gene from whole blood sample DNA**

Polymerase chain reaction was performed on DNA isolated from whole blood (n=24) for the detection of *Brucella* genome. The test amplified the DNA fragment with size of 498 base pair (IS711/alkB primer pair) specific for Brucellosis from 15 of 24 (62.5%) DNA samples tested (Fig. 4). Wael et al (2012) identified among the samples tested by culture, serological tests, the detection by PCR was highly sensitive and they also cited that they had the results similar to Hamdy and Aminy [11]. The highest PCR results was attributed to the high sensitive nature of PCR to detect very less number of Brucella organisms present in the samples

Polymerase Chain Reaction (PCR) for the detection of Brucella specific gene, IS711/alkB , **from milk sample DNA**

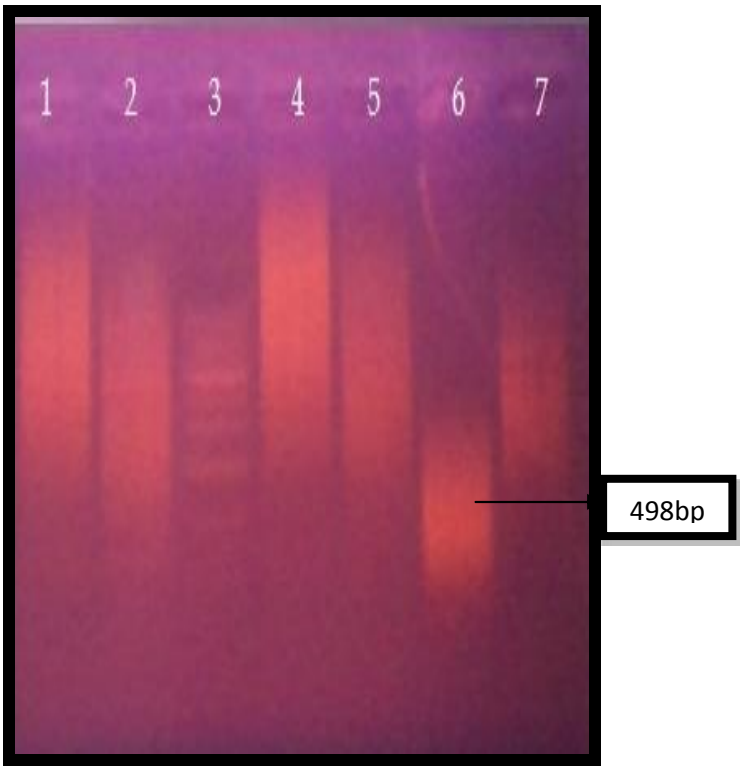
Polymerase chain reaction conducted on 24 milk DNA samples for the amplification of IS711//alkB gene fragments specific for Brucellosis. Of 24 samples tested only 2 milk samples (8.33%) DNA were found positive for 498 base pair specific for Brucellosis gene IS711 is shown in (Fig. 5). Using polymerase chain reaction technique, Wael et al (2012) detected 53.1% cows milk as positive for B. melitensis but this result is far higher than the positivity detected in milk DNA samples in this study.

Figure 4 Polymerase Chain Reaction (PCR) for the detection of Brucella specific IS711//alkB **gene from whole blood sample DNA**



Lane 1 : Negative control
Lane 2-6 : Positive B.abortus DNA control (498 bp)
Lane 7 : Molecular weight marker
Lane 8-10 : Positive B.abortus DNA control (498bp)

Figure 5 Polymerase Chain Reaction (PCR) for the detection of Brucella specific gene, IS711/alkB , **from milk sample DNA**



Lane 1 : Negative B.abortus DNA control
Lane 2 : Positive B.abortus DNA control
Lane 3 : Molecular weight marker
Lane 4-6 : Negative B.abortus DNA control
Lane 7 : Positive B.abortus DNA control

Polymerase Chain Reaction (PCR) for the detection of Brucella specific B4/B5 gene from whole blood sample DNA

Out of 24 sample DNA extracted from whole blood and targeted the gene B4/B5 specific for Brucella organism 15 DNA (62.5%) showed the 223bp fragments specific for B4/B5 gene of Brucella species by polymerase chain reaction and is shown in Figure 6. Among the sets of primers tested the B4/B5 primer set was found to be the most effective primers [7]. Kanani and Patel [8**,** 9] also detected the sensitivity similar to the findings of Ghodasara [7] by PCR using the B4/B5 primer set.

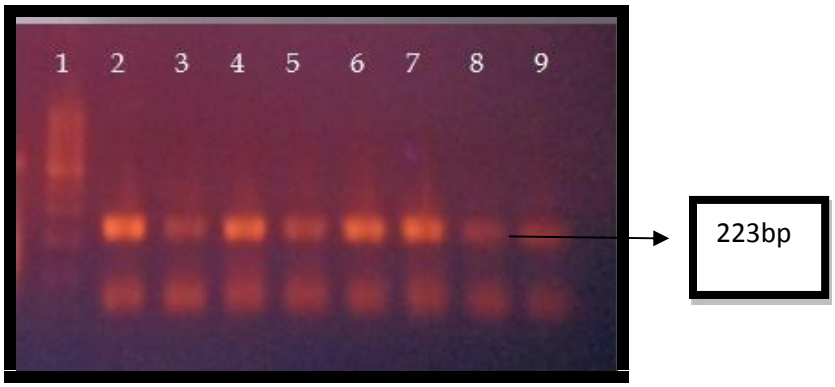
Polymerase Chain Reaction (PCR) for the detection of Brucella specific B4/B5 gene from milk sample DNA

Amplification of B4/B5 gene specific for Brucella organism from 24 milk DNA samples, Polymerase chain reaction was conducted and detected 2 out 24 milk DNA samples (8.33%) as positive for the fragment 223 base pair following analysis by 1.2% agarose gel electrophoresis is shown in Figure 5. Romero et al and O'Leary et al [6, 15] suggested the lower sensitivity of PCR assay than culture may be correlated to the presence of undetectable level of Brucella organism in the samples by PCR. Al-Mairiri et al [13] described that PCR detected 92 % milk sample as positive.

Polymerase Chain Reaction (PCR) for the detection of Brucella specific IS711 and B4/B5 gene from vaginal culture sample DNA

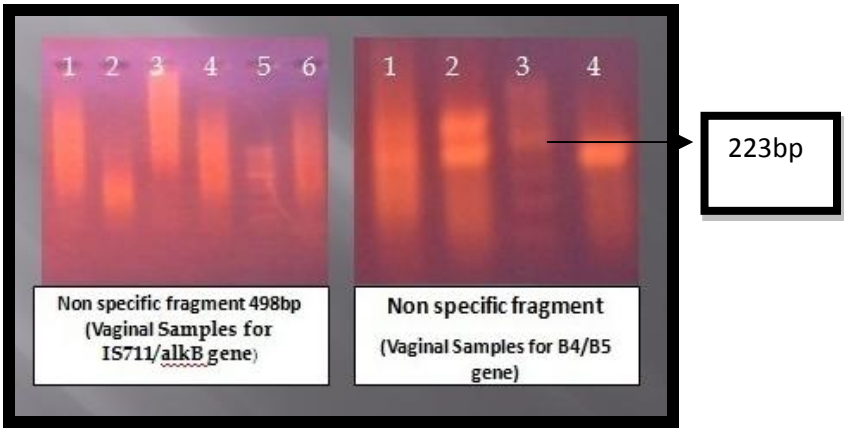
DNA extracted from 2 vaginal culture was subjected to Polymerase chain reaction for amplification of Brucella specific genes IS711 (498 base pair) and B4/B5 223 (base pair) out of which none showed exact size of the targeted fragment is as expected shown Figure 7.

Figure 6 Polymerase Chain Reaction (PCR) for the detection of Brucella specific B4/B5 gene from whole blood sample DNA



Lane 1 : Molecular weight marker
Lane 2-9 : Positive B.abortus DNA control

Figure 7 Polymerase Chain Reaction (PCR) for the detection of Brucella specific IS711 and B4/B5 gene from vaginal culture sample DNA



Lane 1-4 : Negative B.abortus DNA control
Lane 2 : Molecular weight marker
Lane 3 : Negative B.abortus DNA control
Lane 1-2 : Negative B.abortus DNA control
Lane 3 : Molecular Weight marker
Lane 3 : Negative B.abortus DNA control

Conclusion

The present study tested (n=96) 24 whole blood, 24 sera, 24 milk and 24 vaginal samples from cattle with various clinical problems. Culture method of vaginal swab samples (n=24) showed negative results for all samples which include all biochemical tests. Serological tests by Milk Ring Test, of 24 milk samples tested 12.5% (3 samples) showed positivity for antibody to Brucellosis. Rapid Plate Agglutination Test detected 4.16% (one serum sample) was found with antibody to Brucellosis. Standard Tube Agglutination Test showed 8.33% (2 serum samples) positivity. Polymerase Chain Reaction showed higher positivity of 62.5% (15 blood DNA samples) by IS711 and B4/B5 PCR tests for Brucellosis. The milk PCR for showed lower positivity of 8.33% (2 milk DNA samples) for IS711 and B4/B5 gene of Brucellosis. This study conclude that PCR is the best technique able to identify more number if positive samples than all other tests used to test Brucellosis.

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