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Antimicrobial resistance, phenotypic characteristics and phage types of *B. abortus* strains isolated from cattle and water buffalo (*Bubalus bubalis*) in Trinidad

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ABSTRACT

Strains of *Brucella abortus* isolated from cattle and domestic water buffalo (*Bubalus bubalis*) in Trinidad and Tobago were characterized as to their phenotypic features, phage types and resistance to antimicrobial agents using standard methods. A total of 86 isolates were recovered from the lymph nodes of 14 apparently healthy seropositive cattle and 17 water buffalo, skin lesions of 9 water buffalo and aborted tissues of 16 water buffalo. In addition 2 vaccine strains, *B. abortus* strains 19 (S19) and RB51 (SRB51) were tested. All (100.0%) strains of *B. abortus* tested grew in the presence of penicillin G, i-erythritol and basic fuchsin but none (0.0%) grew in the presence of thionin blue. All 88 (100.0%) strains of *B. abortus* were susceptible to bacteriophages TB and BK_2 but 84 (95.5%) were lysed by bacteriophage Wb. Of the 8 antimicrobial agents tested by the disc diffusion method, all 42 (100.0%) cattle and water buffalo carried resistant isolates and all 88 (100.0%) isolates of *B. abortus* exhibited resistance to one or more of the antimicrobial agents. All sources considered, resistance was high to azithromycin (100.0%), sulphamethoxazole/trimethoprim (98.9%) and moxifloxacin (80.7%) and low to streptomycin (5.7%), tetracycline (1.1%) and doxycycline (1.1%). The differences in prevalence of resistance of *B. abortus* isolates to antimicrobial agents were statistically significant ($P<0.05$; χ^2) but isolates from cattle and water buffalo had similar prevalence of resistance ($P>0.05$; χ^2). Resistance to antimicrobial agents used in the treatment of human brucellosis poses a public health hazard, but most of the strains had similar phenotypic characteristics and bacteriophage susceptibility patterns.

Key words: phage types, antibiotic resistance, *Brucella abortus*, cattle, water buffalo

Introduction

Brucellosis is a disease of zoonotic, public health and economic importance worldwide (CORBEL, 1997). Different species of the pathogen, which include *Brucella abortus, B.*

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melitensis, B. suis, and B. ovis, cause disease in several animal species (CORBEL and MORGAN, 1984). Abortion, stillbirths, neonatal deaths and infertility are some of the clinical manifestations caused by *Brucella* spp. (MORENO, 2002).

Amongst species of *Brucella*, several characteristics including the requirement of CO_2 for growth, growth in the presence of penicillin G, i-erythritol and basic fuchsin (GARCIA et al., 1988; OCHOLI et al., 2004), agglutination with monospecific antisera and susceptibility to bacteriophages have been used to classify the pathogen into biotypes (ALTON et al., 1988; GARCIA et al., 1988). It has also been documented that there are variations within biotypes of *B. abortus* although biovar 1 is considered to be most widespread (GARCIA et al., 1988; CORBEL, 1997). It has been suggested that some biotypes of *B. abortus* predominate in causing infection in livestock in certain geographical locations (CRAWFORD et al., 1979) but mixed infections by several biotypes have been reported (CORBEL, 1997; OCHOLI et al., 2004).

In Trinidad and Tobago, the Office of International Epizootics brucellosis-free status was lost when, in 1998, an outbreak of brucellosis was confirmed serologically and bacteriologically in water buffalo (*Bubalus bubalis*) and cattle (FOSGATE et al., 2002 and 2003a). Studies on the efficacy of *B. abortus* RB51 vaccine, which has been approved for use in cattle in the U.S.A. (POESTER et al., 2006), have been inconclusive on the local domestic water buffalo (*Bubalus bubalis*) population (FOSGATE et al., 2003b).

During the brucellosis outbreak, a total of 56 cattle and water buffalo herds had seropositive animals following the use of the buffered plate agglutination test (BPAT) and competitive enzyme-linked immunosorbent assay (c-ELISA). In addition, the milk ring test was used to screen 34 cattle farms, of which 8 farms had positive animals. A test and slaughter and limited RB51-vaccination of water buffalo policy was initiated, which resulted in a decrease to a prevalence of <2% by 2002 and infection was limited to the two largest water buffalo herds in the country (Ministry of Agriculture, Lands and Marine Resources (MALMR) (2005): Annual Report 2005.).

In view of the dearth of information on the characteristics of strains of *B. abortus* isolated from cattle and water buffalo in Trinidad and Tobago, this study was conducted to determine their frequency of resistance to antimicrobial agents, phenotypic characteristics and phage types.

Materials and methods

Sources of Brucella abortus isolates. Strains of *Brucella abortus* used originated from earlier studies on cattle and water buffalo (*Bubalus bubalis*) (FOSGATE et al., 2002 and 2003b; DIPTEE et al., 2006). All isolates were confirmed as *B. abortus* biovar 1 by the Central Veterinary Laboratory (CVL) at Weybridge, United Kingdom and stored in skimmed milk at -20 °C until used.

Table 1 displays the sources of 46 isolates of *B. abortus* isolated from 16 water buffalo dams and calves. The isolates originated from the mesenteric lymph nodes (MLN), uterine lymph nodes (ULN), uterine tissues (UT), supramammary (SUP) lymph nodes and heart tissues (HT), while from aborted calves, isolates were recovered from abomasal contents.

A total of 42 isolates of *B. abortus* were used from 40 water buffalo and cattle, comprising 8 from 8 apparently healthy slaughtered seropositive water buffalo calves experimentally inoculated subcutaneously with *B abortus* biovar 1 in a vaccination trial, 9 field isolates from 9 seropositive slaughtered water buffalo, 9 isolates from 9 water buffalo with skin lesions and 14 isolates from 14 slaughtered seropositive cattle (Table 2). In addition, two vaccine strains, *B. abortus* strains 19 (S19) and RB51 (SRB51) were used in the study.

Growth of B. abortus strains in the presence of dyes and antimicrobial agents. The ability of *B. abortus* strains to grow in the presence of thionin blue (20 µg per mL), i-erythritol (1 mg per mL), penicillin G (5 IU per mL) and basic fuchsin (20 µg per mL) using standard procedures (ALTON et al., 1988) and following the recommendations of the Central Veterinary Laboratory, Weybridge, U.K. was observed. All plates were inoculated under a Type II biohazard hood (Labconco Corporation, Missouri, U.S.A.). All incubations took place at 37 ± 1 ºC in an atmosphere containing 8% CO_2 (Formo Scientific Incorporation, Ohio, U.S.A.).

Detection of H_2S production. Strains of *B. abortus* were grown on Tryptose agar (Difco, Detroit, Michigan) slants with a lead acetate strip suspended over the slants and the tubes were then incubated at 37 ± 1 ºC in an atmosphere containing 8% CO_2. The strip was examined daily for blackening and replaced each day.

Determination of CO_2 requirement. Duplicate blood agar plates were streaked for isolation with *B. abortus* strains and incubated for 7 days at 37 ± 1 ºC aerobically (without CO_2) and in an atmosphere containing 8% CO_2.

Detection of urease production. A urea slant (Difco, Detroit, Michigan, USA) was inoculated with a loopful of pure *Brucella* culture of each strain and then incubated for 7 days at 37 ± 1 ºC in an atmosphere containing 8% CO_2. The appearance of a pink colour was considered indicative of urease production.

Phage Typing of B. abortus isolates. The procedure provided by the Central Veterinary Laboratory, Weybridge, U.K., was used for the phage typing of *B. abortus* with a slight modification. Each isolate of *B. abortus* was initially inoculated onto blood agar plates and incubated for 3-5 days at 37 ± 1 ºC in an atmosphere containing 8% CO_2. Thereafter, the culture was inoculated into 1 mL of trypticase soy broth, then incubated at 37 ± 1 ºC in an 8% CO_2 for 4 hours. Fifty microlitres (50 µL) of the bacterial suspension

was inoculated onto a serum dextrose agar (SDA) plate and spread evenly using a sterile spreader. The inoculum was left to dry for 30 min. Using a fine loop, one drop of the phages, Tb, Wb and BK_2, kindly provided by the CVL, Weybridge, U.K., was evenly placed on the lawn of *B. abortus* strain and allowed to dry under a Type II Biohazard hood at ambient temperature. The plates were thereafter incubated at 37 ± 1 ºC in 8% CO_2 for 3-5 days. Any evidence of complete lysis at the site where the specific phage was place was interpreted as the susceptibility of the strain *of B. abortus* to the phage.

Determination of antibiotic sensitivity of strains of B. abortus. The sensitivity of the isolates of *B. abortus* to antimicrobial agents was determined using the disc diffusion method. The antimicrobial agents used and their concentrations are as follows: azithromycin, AZM (15 mcg), ciprofloxacin, CIP (5 mcg), doxycycline, DO (30 mcg), moxifloxacin, MXF (5 mcg), rifampicin, RD (5 mcg), tetracycline, TE (30 mcg), streptomycin, S (10 mcg) and sulphamethoxazole/trimethoprim (23.75/1.25 mcg), SXT. The plates were incubated for 5 days at 37 ± 1 ºC in an atmosphere containing 8% CO_2. The resistance of isolates to the antimicrobial agents was determined using the zone sizes and the recommendations of ANONYMOUS (2002).

Statistical analysis. The frequency of detection of the various characteristics amongst strains of *B. abortus* was compared and analyzed, after processing the data using the Statistical Package for Social Sciences (SPSS) version 10. The chi-square test was used to determine statistically significant differences between the frequencies and sources using alpha at 0.05.

Results

All 88 (100.0%) isolates grew in the presence of penicillin G (5 IU/mL), i- erythritol (1 mg/mL) and basic fuchsin (20 μg/mL) but not in the presence of thionin blue (20 μg/mL), where none (0.0%) of the isolates grew. Again, all 88 (100.0%) isolates were susceptible to both bacteriophages TB and BK_2 but 84 (95.5%) were susceptible to phage WB (Table 3).

All cattle and water buffalo studied contained *B. abortus* isolates that were resistant to one or more of the eight antimicrobial agents tested (Table 4). Resistance was high to azithromycin (100.0%), sulphamethoxazole/trimethoprim (98.9%) and to moxifloxacin (80.7%) but low to rifampicin (6.8%), doxycycline (1.1%) and tetracycline (1.1%). The differences in resistance to antimicrobial agents were statistically significant ($P<0.05$; χ^2).

The differences in the prevalence of resistance to antimicrobial agents between *B. abortus* isolates from cattle and water buffalo and isolates recovered from tissues of apparently healthy animals compared with those from lesions or aborted animals were not significantly different ($P>0.05$; χ^2).

Table 1. Sources of *B. abortus* isolates from water buffalo that aborted following experimental challenge whose phenotypic phage and antibiotic characteristic were determined

Identification of strain of *Brucella abortus*	Number of isolates tested[1]	Source of isolates
829	5	[2]MLN (1), ABO (1), ULN (1), UT (1), SUP (1)
403	4	HT (1), MLN (1), ABO (1) SUP (1)
5233	2	ULN (1), ABO (1)
5319	5	ULN (1), MLN (1), UT (1), ABO (1), SUP (1)
832	2	ULN (1), SUP (1)
1948D	3	ABO (1), HT (1), MLN (1)
1969D	2	UT (1), SUP (1)
9000D	2	MLN (1), SUP (1)
841	4	MLN (1), ABO (1), HT (2)
1972	2	MLN (1), ABO (1)
833D	1	SUP (1)
1965	3	ABO (1), MLN (1), HT (1)
830	2	HT (1), MLN (1)
895	5	SUP (1), MLN (1), HT (1), ABO (1), UT (1)
829	1	HT (1)
5233	3	MLN (1), HT (1), SUP (1)
Total	46	MLN (11), ABO (9), ULN (4), UT (4), SUP (9), HT (9

[1]Pregnant water buffalo (*B. abortus* RB51-vaccinated and unvaccinated controls) challenged with a *B. abortus* biovar 1 strain Trinidad 1). [2]MLN--Mesenteric lymph nodes, ABO--Abomasal content, ULN--Uterine lymph node, UT--Uterine swab, SUP--Supramammary lymph node, HT--Heart tissue/swab.

Table 2. Sources of other isolates of *Brucella abortus* in the study to determine phage types and antimicrobial sensitiity

Animal Source	Type of sample	Number of isolates used	Identification of isolates of *B. abortus*	Source/ Reference
Water buffalo	Slaughter animal	8[a]	2959WB, 2628WB, 2647WB, 2975WB, 2975(Dir)WB, 2959(i), 2959 (ii), 2959 (iii)	Diptee et al., 2006
Water buffalo	Field strains	9[b]	14EC, 24AC, 2EC, 2CC, 10AC, 10 EC, 14EC,14AC, 20AC	Fosgate et al., 2002
Water buffalo	Field strains	9[c]	Biov 1/3WB, Biov 1/8W, Biov 1/1W, 1/2WB, 1/5WB, 1/4WB, 1/6WM, 1/7WB, B1WB	Veterinary Diagnostic Laboratory, MALMR
Cattle	Field strains	14[d]	26A/45B, 8C/9C, 44A/5C, 13B/24C, 44D/337C, 35B/7C, 43E/4C, 13E/27C, 8B/12C, SQ 1501, 41B/42C, 35C/17C, 41A/21C, 43B/20C	Fosgate et al., 2003b
Reference strain	Unknown	2[e]	S19, SRB51	NVSL, Iowa, U.S.A
Total		42		

[a]Isolated from the mesenteric lymph nodes (MLN) of apparently healthy water slaughtered seropositive water buffalo following SQ challenge with *B. abortus;* [b]Isolated from the MLN of naturally exposed water buffalo (*B. abortus* RB51-vaccinated and unvaccinated controls); [c]Isolated from skin lesions of naturally infected water buffalo; [d]Isolated from the inguinal or supramammary lymph nodes of apparently healthy slaughtered seropositive cattle; [e]One isolate each of *B. abortus* strain S19 and *B. abortus* strain RB51

Table 3. Phenotypic and phage type characteristics of *B. abortus* strains

				No. (%) of strains that were susceptible to phages:			No. (%) that grew in presence of:			
Animal source of *Brucella abortus* strains	Status of animal	Type of sample strain was culture from:	No. of strain[1] of *B. abortus* tested	TB	Wb	BK_2	[2]Pen G	i-Eryth.	Thio. B	Basic Fusc.
Water buffalo	Aborted animal following experimental I/V challenge with *B. abortus* strain Trinidad 1	[3]MLN, ABO, UTLN, UT, HT, SUP	46	46 (100.0)	45 (97.8)	46 (100.0)	46 (100.0)	46 (100.0)	0 (0.0)	46 (100.0)
Water buffalo	Apparently healthy seropositive after SQ slaughtered water buffalo	MLN	8	8 (100.0)	7 (87.5)	8 (100.0)	8 (100.0)	8 (100.0)	0 (0.0)	8 (100.0)
Water buffalo	Apparently healthy seropositive animal in a vaccine trial following natural exposure	MLN	9	9 (100.0)	8 (88.9)	9 (100.0)	9 (100.0)	9 (100.0)	0 (0.0)	9 (100.0)
Water buffalo	Animals with with skin lesions slaughtered	Skin lesions	9	9 (100.0)	9 (100.0)	9 (100.0)	9 (100.0)	9 (100.0)	0 (0.0)	9 (100.0)
Cattle	Apparently healthy seropositive slaughtered cattle	Inguinal and supramammary LN	14	14 (100.0)	13 (92.9)	14 (100.0)	14 (100.0)	14 (100.0)	0 (0.0)	14 (100.0)
Cattle	Unknown--S19	Unknown	1	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)
Cattle	Unknown--RB51	Unknown	1	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)
Total			88	88 (100.0)	84 (95.5)	88 (100.0)	88 (100.0)	88 (100.0)	0 (0.0)	88 (100.0)

[1]All strains of B. abortus were positive for urease, catalase and oxidase production and were all biovar 1; [2]Pen G-Penicillin G (5 IU/mL), i-Eryt.-i-Erythritol (1 mg/mL) ; Thio. B--Thionin blue (20 mg/mL) and Basic fusc.--Basic fuschin (20 mg/mL); [3]MLN--Mesenteric lymph nodes, ABO--Abomasal contents, UTLN--Uterine lymph nodes, UT--Uterine swabs, HT--Heart and SUB--Supramammary lymph nodes

Table 4. Antimicrobial sensitivity of *B. abortus* strains

Source of *Brucella abortus* strains	Status of animal	Type of sample strain was culture from:	Source	No. tested	No. (%) of strains/ animal resistant[2]	No. (%) resistant to:							
						[3]MXF	DO	CIP	RD	SXT	TE	AZM	S
Water buffalo	Aborted animal following experimental i/v challenge with *B. abortus* strain Trinidad 1	[1]MLN, ABO, UTLN, UT, HT, SUP	No. of animals	16	16 (100.0)	16 (100.0)	0 (0.0)	16 (100.0)	0 (0.0)	16 (100.0)	0 (0.0)	16 (100.0)	1 (6.3)
			No. of isolates	46	46 (100.0)	40 (87.0)	0 (0.0)	28 (60.9)	0 (0.0)	46 (100.0)	0 (0.0)	46 (100.0)	1 (2.2)
Water buffalo	Apparently healthy seropositive after SQ slaughtered water buffalo	MLN	No. of animals	4	4 (100.0)	4 (100.0)	0 (0.0)	4 (100.0)	1 (25.0)	4 (100.0)	0 (0.0)	4 (100.0)	1 (25.0)
			No. of isolates	8	8 (100.0)	8 (100.0)	0 (0.0)	7 (87.5)	1 (12.5)	8 (100.0)	0 (0.0)	8 (100.0)	1 (12.5)
Water buffalo	Apparently healthy seropositive animal in a vaccine trial following natural exposure	MLN	No. of animals	5	5 (100.0)	3 (60.0)	0 (0.0)	4 (80.0)	0 (0.0)	5 (100.0)	0 (0.0)	5 (100.0)	0 (0.0)
			No. of isolates	9	9 (100.0)	5 (55.6)	0 (0.0)	6 (66.7)	0 (0.0)	8 (88.9)	0 (0.0)	9 (100.0)	0 (0.0)
Water buffalo	Animals with with skin lesions slaughtered	Skin lesions	No. of animals	9	9 (100.0)	6 (66.7)	0 (0.0)	7 (77.8)	2 (22.2)	9 (100.0)	1 (11.1)	9 (100.0)	0 (0.0)
			No. of isolates	9	9 (100.0)	6 (66.7)	0 (0.0)	7 (77.8)	2 (22.2)	9 (100.0)	1 (11.1)	9 (100.0)	0 (0.0)
Cattle	Apparently healthy seropositive slaughtered cattle	Inguinal and supramammary LN	No. of animals	8	8 (100.0)	5 (62.5)	1 (12.5)	6 (75.0)	1 (12.5)	8 (100.0)	0 (0.0)	8 (100.0)	1 (12.5)
			No. of isolates	14	14 (100.0)	10 (71.4)	1 (7.1)	11 (78.6)	1 (7.1)	14 (100.0)	0 (0.0)	14 (100.0)	1 (7.1)
Cattle	Unknown--S19	Unknown	No. of isolates	1	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)	1 (100.0)
Cattle	Unknown--RB51	Unknown	No. of isolates	1	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)	1 (100.0)
Total			No. of animals	42	42 (100.0)	34 (81.0)	1 (2.4)	37 (88.1)	4 (9.5)	42 (100.0)	1 (2.4)	42 (100.0)	3 (7.1)
			No. of isolates	88	88 (100.0)	71 (80.7)	1 (1.1)	61 (69.3)	6 (6.8)	87 (98.9)	1 (1.1)	88 (100.0)	5 (5.7)

[1]MLN--Mesenteric lymph node, ABO--Abomasal contents, UTLN--Uterine lymph nodes, UT--Uterine swabs, HT--Heart and SUB--Supramammary lymph nodes; [2]Resistant to one or more of the antimicrobial agents; MXF--Moxifloxacin (5 mcg), DO--Doxycycline (30 mcg), CIP--Ciprofloxacin (5 mg), RD--Rifampicin (5 mg), SXT--Sulphamethoxazole/trimethoprim (23.75/1.25 mcg), TE-Tetracycline (30 mcg), AZM-Azitomycin (15 mcg), S-Streptomycin (10 mcg)

Discussion

It was not a surprise that all isolates of *B. abortus* recovered from various samples (lesions and normal tissues) from cattle and water buffalo across the livestock farms in Trinidad were all classified to belong to biotype 1. Similar findings have been reported elsewhere (CORBEL, 1997; OCHOLI et al., 2004). It has also been documented that most infections worldwide are caused primarily by *B. abortus* biotype 1, but geographic differences in the distribution of biotypes have also been detected (CRAWFORD et al., 1979; CORBEL, 1997).

The growth conditions, along with the ability of the isolates tested in the current study to grow in the presence of penicillin G, i-erythritol, basic fuchsin, but not in the presence of thionin blue, are in agreement with published reports for *B. abortus* biotype 1 by others (STINEBRING and KUNKEL, 1982; MEYER, 1985). Atypical reactions amongst *B. abortus* strains have also been noted by other authors (GARCIA et al., 1988). LUCERO et al. (2008) recently reported the observed changes in the susceptibility of *Brucella* spp. isolated from human and animal sources in Latin America.

The susceptibility of *B. abortus* isolates in the current study to bacteriophages TB, Wb and BK_2 is also in agreement with published reports for biotype 1 strains, which are normally susceptible to the three phages. It is however pertinent to mention that 4 (4.5%) of the isolates were resistant to bacteriophage Wb. This may be a reflection of the lytic activity of the strain of bacteriophage used in the current study since phage patterns of *B. abortus* strains are also used to classify the organism into biotypes (GARGANI and TOLARI, 1986).

Although chemotherapy is not routinely used in the control and eradication of brucellosis in animals, vaccination and test and slaughter policy respectively are preferred (CADMUS et al., 2008; MARTIN et al., 2009; POESTER et al., 2006), the prevalence of resistance detected amongst the isolates is important in the classification of the isolates studied. The fact that all 88 (100.0%) isolates from cattle and water buffalo studied were resistant to one or more of the eight antimicrobial agents tested is an indication that resistance to these agents is prevalent, albeit with varying prevalence. It is also interesting to note that a high percentage, 81.8% (72 of 88), of the isolates originated from water buffalo predominantly managed semi-intensively, with virtually no exposure to antimicrobial agents normally associated with chemotherapy practised for various clinical conditions in intensively managed cattle farms. Furthermore, there was no statistically significant difference in the prevalence of resistance to antimicrobial agents between isolates recovered from cattle and water buffalo and between those from apparently normal animals/tissues and from lesions. These findings indicate that the prevalence of resistance detected represents the normal distribution of resistance to antimicrobial agents amongst local *B. abortus* strains in cattle and water buffalo in the country. It has been reported that antimicrobial

resistance could be an intrinsic and inherent characteristic of bacteria (McDERMOTT et al., 2003; SHELDON, 2005).

Considering the fact that brucellosis is an important zoonoses, the choice of antimicrobial agents used in the present study was based on eight antimicrobial agents that have been reportedly used to treat human brucellosis (LOPEZ-MERINO et al., 2004). Of the eight antimicrobial agents tested, resistance was generally high to azithromycin (100.0%), sulphamethoxazole-trimethoprim (98.9%), moxifloxacin (80.7%) and ciprofloxacin (69.3%). On the other hand, the prevalence of resistance was comparatively low to rifampicin (6.8%), streptomycin (5.7%), tetracycline (1.1%) and doxycycline (1.1%). These findings agree or conflict with published reports elsewhere. LOPEZ-MERINO et al., (2004) in a study of *B. abortus* strains isolated from human and animal products reported that they were generally very susceptible to fluoroquinolones (ciprofloxacin and moxifloxacin) and tetracycline but resistance was high to rifampicin, streptomycin and sulphamethoxazole/trimethoprim (SXT). In the current study, resistance was however high to ciprofloxacin, monofloxacin and SXT but low to rifampicin. Resistance to rifampicin amongst *B. abortus* strains has also been reported by others (BAYKAM et al., 2004). Rifampicin is considered to be one of the most potent and broad spectrum antimicrobial agents against bacterial pathogens and is used for the treatment of human brucellosis (CASCIO et al., 2003; MARIANELLI et al., 2007) and it has been reported that isolates that are rifampicin-resistant are less virulent than rifampicin-sensitive strains of *B. abortus*. The ability of the antimicrobial agent to convert smooth virulent strains of *B. abortus* to a stable, rough attenuated strain has been used to develop the *B. abortus* strain RB51, used to produce vaccine to prevent brucellosis in cattle in the U.S.A. (SCHURIG et al., 1991).

The relatively high prevalence of resistance to ciprofloxacin in the present study is considerably higher than reported by other workers (BODUR et al., 2003; LOPEZ-MERINO et al., 2004; SENGOZ et al., 2006).

Similarly, the finding that all 88 isolates of *B. abortus* tested were resistant to azithromycin is a surprise because this macrolide is not used in veterinary practice in the country and it has been shown to be effective against *B. abortus* by others (WILLIAMS, 1991; LANG et al., 1994). DOMINGO et al. (1995) however reported that in mice experimentally infected with *Brucella* spp., although azithromycin was able to reduce infection significantly, it was not able to cure the animals as effectively as the classic regimen of doxycycline, which was also found to be effective in the current study. SOLERA et al. (2001) also reported that azithromycin was not particularly effective against brucellae.

Earlier studies conducted on the efficacy of antimicrobial agents in cows naturally infected by *B. abortus* strains have demonstrated the efficacy of oxytetracycline and

streptomycin (NICOLETTI et al., 1985; RADWAN et al., 1993), in agreement with the findings in the current study, where a relatively low prevalence of resistance was exhibited to both antimicrobial agents.

It was concluded that isolates of *B. abortus* recovered from various sources in Trinidad displayed similar growth requirements, phenotypic characteristics and phage susceptibility patterns. The high prevalence of resistance displayed to antimicrobial agents commonly used in the treatment of human brucellosis can however not be ignored because of the zoonotic nature of the disease.

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ADESIYUN, A. A., K. BAIRD, A. STEWART-JOHNSON: Otpornost na antimikrobne tvari, fenotipska obilježja i fagotipovi sojeva vrste *B. abortus* izdvojenih iz goveda i azijskih bivola (*Bubalus bubalis*) u Trinidadu. Vet. arhiv 81, 391-404, 2011.

SAŽETAK

Određivana su fenotipska obilježja, fagotipovi i otpornost na antimikrobna sredstva sojeva bakterije *Brucella abortus* izdvojenih iz goveda i azijskog bivola (*Bubalus bubalis*) u Trinidadu i Tobagu. Ukupno je 86 izolata bilo izdvojeno iz limfnih čvorova 14 klinički zdravih serološki pozitivnih goveda i 17 indijskih bivola, iz ozljeda kože devet bivola te tkiva pobačenih plodova 16 bivola. Analizirana su bila i dva cjepna soja bakterije *B. abortus*, soj 19 (S19) i RB51 (SRB51). Svi analizirani sojevi razmnožavali su se u prisutnosti penicilina G, i-eritritola i bazičnog fuksina, a nijedan se nije razmožavao u prisutnosti tioninskog modrila. Svih 88 sojeva bilo je osjetljivo na bakteriofage TB i BK_2, a 84 (95,5%) bili su lizirani bakteriofagom Wb. Sva pretražena goveda i bivoli nosili su sojeve rezistentne na jednu ili više antimikrobnih tvari. Uzročnici su bili

testirani na 8 antimikrobnih tvari disk-difuzijskim postupkom. Velika otpornost ustanovljena je na azitromicin (100,0%), sulfametoksazol/trimetoprim (98,9%) i moksifloksacin (80,7%). Slaba otpornost bila je ustanovljena na streptomicin (5,7%), tetraciklin (1,1%) i doksiciklin (1,1%). Razlike u prevalenciji rezistencije izolata *B. abortus* na antimikrobne tvari bile su statistički značajne ($P<0,05$; χ^2), ali su izolati iz goveda i bivola imali sličnu prevalenciju otpornosti ($P<0,05$; χ^2). Otpornost na antimikrobne tvari rabljene za liječenje bruceloze u ljudi ima javnozdravstveno značenje. Većina izolata ima slična fenotipska obilježja i sličnu osjetljivort na bakteriofage.

Ključne riječi: fagotipovi, otpornost na antibiotike, *Brucella abortus*, govedo, indijski bivol