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Brucella isolated in humans and animals in Latin America from 1968 to 2006

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SUMMARY

We report a retrospective analysis of 1933 *Brucella* strains isolated from humans and animals in Latin American countries between 1968 and 1991 and in Argentina between 1994 and 2006. During the first period 50% of strains were from humans, mainly from Argentina, Mexico and Peru but, while *B. suis* was the main cause of infection in Argentina, *B. melitensis* was responsible for most infections in the other countries. In Argentina in the later years, *B. melitensis* and *B. suis* were observed more frequently than in the first period while isolation of *B. abortus* decreased. Of 145 *B. melitensis* human isolates, eight gave susceptibility patterns to dyes and penicillin and two were *B. melitensis* biovar 3, which has never been reported in animals. Forty-six percent of *B. suis* isolated were resistant to dyes which is an atypical feature in this species.

INTRODUCTION

While in some countries the incidence of brucellosis is declining as a result of control measures in cattle, goats and sheep, the disease persists in the Mediterranean basin, the Middle East, the Arabian Gulf and some Latin American countries [1–3]. Brucellosis has been reported in Latin America since the first decade of the 20th century and remains to this day a major zoonosis despite campaigns for its control. Its persistence and wide distribution in different animal hosts is facilitated by the peculiar geographic, climatic and economic conditions of the area. Some determining factors which have not been sufficiently defined are the methods of cattle husbandry, nomadic or semi-nomadic goat herding and the incidence of porcine brucellosis. Control programmes are sometimes ineffective due to the lack of sustainable funding over time [4–6]. Isolation of a *Brucella* sp. in humans and animals provides irrefutable evidence of the infection [1, 7, 8] but in this region statutory diagnosis is achieved mainly by serological tests, and isolation of *Brucella* spp. from clinical specimens often relies on epidemiological investigations or look-back exercises.

At present the genus *Brucella* comprises six species which are classified by reactions in biochemical tests and preferred animal hosts [9]. However, the genus is highly homogeneous with all members showing >95% homology in DNA–DNA pairing studies [10, 11]. Recently, distinctive *Brucella* strains have been isolated from marine animals and a new species, named *B. maris*, has been proposed [12, 13]. Currently, *B. abortus*, *B. melitensis*, *B. suis*, *B. canis* and *B. maris* are known to be pathogenic for humans and may give rise to systemic infection involving any organ system and present with non-specific symptoms. Because of this protean clinical picture, the diagnosis of brucellosis may be confused with other infectious or non-infectious diseases leading to delay in treatment [14, 15].

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Table 1. *Geographical origin and species of origin of 1377* Brucella *strains isolated during 1968–1991 in Latin America*

Country	*n*	%	Humans	Cattle	Pigs	Goats	Sheep	Dogs	Grey weasels	Foxes	Horses	Capybaras	Buffaloes	Ferrets
Argentina	442	32·1	172	151	16	52	15	9	15	10	1			1
Brazil	37	2·7		18	5			10			3		1	
Colombia	91	6·6	13	69	5	1	3							
Cuba	70	5·1		35	27		2	1			5			
Chile	86	6·2	12	61	6	7								
Dominican Rep.	1	0·1		1										
Ecuador	3	0·2		3										
El Salvador	5	0·4		5										
Honduras	6	0·4	4	2										
Mexico	252	18·3	172	71	5	4								
Nicaragua	2	0·1		2										
Paraguay	5	0·4		1	1	3								
Peru	346	25·1	315	8	18	5								
Uruguay	17	1·2		5			11	1						
Venezuela	14	1	1	3	1			7				2		
Total	1377	100	689	435	84	72	31	28	15	10	9	2	1	1

Our laboratory has been a national centre for human brucellosis since 1994 and performs serological and bacteriological diagnosis of infection in patients with symptoms and/or history compatible with this disease. We report here a retrospective analysis of 1933 *Brucella* strains isolated from humans and animals in several Latin American countries from 1968 to 1991 and in Argentina from 1994 to 2006.

MATERIALS AND METHODS

Geographic distribution of the strains

From 1968 to 1991, 1377 strains isolated from humans and animals in Latin America were confirmed as *Brucella* spp. at the Pan American Zoonosis Centre (CEPANZO) in Buenos Aires, Argentina and stored at the National Laboratories and Institutes of Health Administration (ANLIS). The animal strains were isolated from domestic cattle, goats, pigs, sheep, dogs and horses, and wild animals such as the buffalo (*Bubalus bubalis*), fox (*Dusicyon gymnocercus antiquus*), grey weasel (*Didelphis marsupialis*), capybara (*Hydrochoerus hydroachaeris*) and ferret (*Galictis furax huranox*). Their origins were Argentina, Brazil, Chile, Colombia, Cuba, Dominican Republic, Ecuador, El Salvador, Honduras, Mexico, Nicaragua, Paraguay, Peru, Uruguay and Venezuela (Table 1). Most of the strains were from Argentina (32%), Peru (25%) and Mexico (18%), and were mostly recovered from humans, but those from Brazil, Chile, Colombia, and Cuba were predominantly from cattle.

Argentina has five geographical regions characterized by diversities in livestock and agriculture. Bovine and porcine species are mainly concentrated in the humid Pampa (HP); bovine and caprine in the Northwest (NW); bovine and ovine in the Northeast (NE); bovine, ovine and caprine in Cuyo (CU); and ovine and caprine in Patagonia (PAT) [16].

From 1994 to 2006, 367 strains from humans were collected at ANLIS, the headquarters of the newly implemented National Human Brucellosis Network (NHBN), a project aiming at standardizing serological and bacteriological diagnosis and the rapid treatment of patients throughout the country. Primary isolation from clinical specimens was performed in clinical diagnostic laboratories mainly from HP (66·7%), followed by NW (14·4%), CU (13·9%) and NE (3·8%) and sent to NHBN headquarters

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for typing. The NHBN project also isolates and characterizes *Brucella* spp. from animals for epidemiological purposes, and examined 189 strains from dogs between 1996 and 2006.

Type strains *B. abortus* 544, *B. melitensis* 16M, *B. suis* 1330, *B. canis* RM 6-66 and *B. ovis* 63/290, and vaccine strains *B. abortus* S19 and *B. melitensis* Rev. 1 were used as controls.

Culture samples

Animal strains were isolated from milk, vaginal swabs, semen, aborted foetuses, mammary, retropharyngeal or internal iliac lymph nodes, abscesses in reproductive organs, testes or epididymes, spleen and liver. Blood cultures were taken mainly from dogs and wild animals. Commercial dehydrated media or laboratory-prepared selective media were used for the isolation of *Brucella* from domestic and wild animals according to standard practice [17]. Human strains were isolated from blood cultures, bone marrow, CSF, liver tissue, abscesses, joint and prostatic fluid, knee cyst and lymph nodes. Routine isolation techniques such as monophasic and biphasic blood culture, lysis centrifugation [18] and automated blood culture systems (Bactec or BactAlert) were used [19, 20]. For monophasic blood culture, a commercial liquid medium Hemo Brucella (Britania SA, Buenos Aires, Argentina) or an in-house medium [21] were used, and for the biphasic medium, Hemoline (bioMérieux, Marcy l'Etoile, France) was employed.

Identification of *Brucella*

Isolates were identified as *Brucella* spp. in the clinical diagnostic laboratories on the basis of morphology, motility at 20 °C and 37 °C, reactions in triple sugar iron (TSI) agar, lactose fermentation on MacConkey agar, acid production from glucose, haemolysis on blood agar, production of catalase, oxidase, urease (Christensen method), citrate utilization, and nitrate reduction. At the reference laboratory colonies were tested for agglutination in acriflavine 1:1000 in distilled water to distinguish between smooth and rough forms. The strains submitted were usually in the smooth form except for *B. ovis* and *B. canis*. Cultures exhibiting rough colonies cannot be typed with monospecific sera or with smooth *Brucella* phages; in these cases smooth colony variants were selected for further typing [7, 17].

Typing of strains

Brucella species and biovars were determined by standard methods [8, 22] and included serum and CO_2 requirement, H_2S production, growth in the presence of Thionin (20 μg/ml) and Basic fuchsin (20 μg/ml), urease test (Bauer's method) and agglutination with polyclonal monospecific anti-A, -M, and -R antisera. They were also tested for susceptibility to *Brucella* BK, RC, Wb and Iz phages at routine test dilution (RTD) and Tb phage at RTD and 10 000 RTD [23]. *B. abortus* biovar (bv.) 1 was differentiated from S19 vaccine strains by its ability to grow on thionin blue (2 μg/ml), erythritol (1 mg/ml) and penicillin (5 IU/ml) and *B. melitensis* bv. 1 was distinguished from vaccine Rev. 1 strains by growth patterns on Thionin, Basic fuchsin, penicillin and streptomycin (2·5 μg/ml); growth patterns on Safranin O (100 μg/ml) and Malachite green (2 μg/ml) were also investigated [7].

The oxidative metabolic patterns of the strains from the first period of sampling were determined at CEPANZO, using the Warburg apparatus, Braun V 85 model, series B [17]. PCR–RFLP analysis of the *omp31* gene using *Ava*II and *Sal*I restriction enzymes was also performed to confirm the identification of *B. canis* [24, 25]. After typing, the strains were maintained lyophilized at 4 °C or in cryo-preservation medium at −70 °C.

RESULTS

Period 1968–1991

Of the 1377 strains of *Brucella* spp. isolated in Latin America during the period 1968–1991 from humans and animals, *B. melitensis* was the most frequently isolated species from humans, followed by *B. suis* and *B. abortus* (Table 2). *B. abortus* was the main cause of infection in cattle, *B. melitensis* in goats, *B. suis* in pigs and *B. ovis* in sheep; *B. canis* and *B. suis* were both isolated from dogs. *B. abortus* was found in buffaloes, foxes, capybaras and ferrets, while *B. abortus* and *B. suis* were isolated from horses and grey weasels.

Table 3 shows that *B. melitensis* bv. 1, *B. abortus* bv. 1 and bv. 4, and *B. suis* bv. 1 were the main biovars isolated. Thirty isolates were confirmed as *B. abortus* S19 and 85 isolates of *B. suis* bv. 1 were resistant to dyes in a manner atypical for this species (*B. suis* 1a). These strains grew on Thionin, but also on Basic fuchsin, Safranin O, Thionin blue, Malachite green and penicillin [4, 26, 27].

Table 2. *Sources and species of 1377* Brucella *strains isolated in Latin American countries* (*1968–1991*)

Source	*B. melitensis*	*B. abortus*	*B. suis*	*B. ovis*	*B. canis*	*n*	%
Humans	505 (73·3%)	74 (10·7%)	110 (16%)			689	50
Cattle	3	424 (97·5%)	8			435	31·6
Goats	71 (98·6%)		1			72	5·2
Pigs	3		81 (96·4%)			84	6·1
Dogs			13		15	28	2·0
Sheep	3	2	4	22 (71%)		31	2·2
Horses		4	5			9	0·6
Buffaloes		1				1	0·1
Foxes		10				10	0·7
Grey weasels		12	3			15	1·1
Capybaras		2				2	0·1
Ferrets		1				1	0·1
Total	585	530	225	22	15	1377	100

Table 3. Brucella *species and biovars isolated in Latin America* (*1968–1991*)

		B. melitensis			*B. abortus*					*B. suis*			
Country	*n*	1	2	3	1	2	3	4	S19	1	1a*	*B. canis*	*B. ovis*
Argentina	442	83			199	5		7	11	72	45	9	11
Brazil	37				11	3	8				12	3	
Colombia	91				77	1		1		9	3		
Cuba	70			2	27	1		2		35	3		
Chile	86	13	5		49	1		10	2	6			
Dominican Rep.	1								1				
Ecuador	3				1			2					
El Salvador	5				1			4					
Honduras	6				2					4			
Mexico	252	150		14	18			51	12	7			
Nicaragua	2							2					
Paraguay	5	3			1					1			
Peru	346	298	14	3	5				4		22		
Uruguay	17				5							1	11
Venezuela	14				3			3		6		2	
Total		547	19	19	399	11	8	82	30	140	85		
	1377		585				530			225		15	22

* Resistant to dyes.

During this first period 172 *Brucella* spp. were isolated from humans in Argentina (data not shown) mainly from geographical regions HP and NW. Of these, 55 were *B. abortus* (50 bv. 1, three bv. 4, one bv. 2 and one S19), 90 *B. suis* (47 bv. 1 and 43 bv. 1a) and 27 *B. melitensis* bv. 1. One strain (classified as *B. melitensis* 1a) grew slowly, produced small colonies and was susceptible to penicillin and dyes, similar to the *B. melitensis* Rev. 1 vaccine, but it was inhibited by 2·5 μg/ml streptomycin [28].

Period 1994–2006

Among the 367 strains isolated from humans in Argentina during this period (Table 4), *B. abortus* was isolated from 75 cases country-wide, the majority being biovar 1 (86·6%). Almost all of the 145 *B. melitensis* isolated were biovar 1 (93·1%), followed by biovar 1a (4·8%), biovar 3 (1·4%) and biovar 2a (0·7%), based on differences in the quantitative distribution of the 'A' and 'M' antigens. In contrast, the

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Table 4. Brucella *strains isolated from humans in Argentina 1994–2006*

Province/Geographical region	*n*	%	*B. melitensis*				*B. abortus*			*B. suis*		*B. canis*
			1	1a*	2a*	3	1	2	S19	1	1a†	
Buenos Aires/HP	215	58·6	32			2	60	4	5	56	53	3
Catamarca/NW	14	3·8	13	1								
Córdoba/HP	28	7·6	6		1		4	1		10	6	
Entre Ríos/NE	1	0·3								1		
Formosa/NE	2	0·5	2									
Jujuy/NW	4	1	1	3								
La Pampa/HP	2	0·5								1	1	
La Rioja/NW	5	1·4	4							1		
Mendoza/CU	42	11·4	42									
Misiones/NE	1	0·3								1		
Neuquen/PAT	3	0·8	2							1		
Río Negro/PAT	1	0·3								1		
San Juan/CU	9	2·4	9									
Santa Fe/NE	10	2·7	1				1			4	4	
Sgo.del Estero/NW	2	0·5		1						1		
Salta/NW	26	7·1	22	1						1	2	
Tucumán/NW	2	0·5	1	1								
Total			135	7	1	2	65	5	5	78	66	3
	367			145			75			144		3

NW, Northwest, NE, Northeast, CU, Cuyo, HP, Humid Pampa; PAT, Patagonia.
* Sensitive to dyes, penicillin and streptomycin.
† Resistant to dyes.

144 *B. suis* strains almost equally divided into biovars 1 and 1a, 54·2% and 45·8% respectively. All but four of the 189 *B. canis* strains were from mongrel dogs in Buenos Aires.

DISCUSSION

In Latin America, brucellosis is considered to be one of the most important zoonotic infections because of its impact on public health and the economy. Its distribution is closely related to the concentration of livestock [4–6]. The animal population in the region is mainly cattle followed by sheep, goats and pigs, but it appears that as a result of control programmes the incidence of bovine brucellosis has been reduced [29–33]. The main objective of this report was to present the geographical origins and sources of 1933 strains (isolated from humans and/or animals in both sampling periods, and 189 dogs) in 15 Latin American countries, especially Argentina. A reason for the low number of isolates recovered from domestic animals given the high prevalence of the disease in this group could be that although bacteriological diagnosis is recommended as a confirmatory test, it is inadequate for detection of the disease in large numbers of animals. The lack of laboratories with facilities for bacteriological diagnosis will also impact on this. The prevalence of bovine brucellosis varies considerably from one country to another with rates ranging from 0·5% to 10%, the incidence of brucellosis in pigs is not known with certainty and there are few countries reporting data on caprine and ovine brucellosis [4–6, 29–33].

Of the 1377 strains isolated in 1968–1991, 31·6% were from cattle (mainly from Argentina) most of them being *B. abortus* and, as a result of vaccination or excretion of the strains by vaccinated animals, *B. abortus* S19 strain was recovered from cattle, humans and a grey weasel. As expected pigs and goats were infected mainly with *B. suis* and *B. melitensis* respectively and sheep with *B. ovis*. However, *B. suis*, *B. melitensis* and *B. abortus* were also found in sheep, probably because of cross-contamination between animal species due to farming practices where continuous contact among the herds exists. *B. ovis* strains were recovered only from Argentina and Uruguay where epididymitis in rams is a major problem. In dogs *B. canis* and *B. suis* were identified and the high number of *B. suis* might be explained by contact with infected farm animals or their ingestion of

contaminated food. The need to consider the potential carrier role of this animal when kept in close proximity to other infected animals has been highlighted [1, 4]. Furthermore, the data show that biovar 1 of each species predominated over other biovars (*B. melitensis* 93·5%, *B. abortus* 75·3% and *B. suis* 62·2%).

During the first period, half of all *Brucella* spp. from human sources were from Argentina (90 *B. suis*, 55 *B. abortus* and 27 *B. melitensis*), Mexico and Peru, but, in the two latter countries *B. melitensis* was the main cause of infection. Currently the control strategy of goat brucellosis in Mexico has been redesigned in high-risk areas and the implementation of massive vaccination with Rev. 1 vaccine has resulted in a reduction of human cases [30].

In Argentina, cattle are the largest livestock population followed by sheep, goats and pigs. According to official reports the estimated prevalence of brucellosis in cattle ranged from 10% to 13% for farms with an individual rate of 4–5%. In other species, surveys found that in Buenos Aires 28·6% of sheep were not recommended for breeding owing to brucellosis. The individual prevalence in goats was 0·5–0·8% in the Northwest of the country and studies in pigs between 1960–1980 found a regional prevalence of 14·2–25% [4, 16]. However, there is no organized programme for monitoring porcine brucellosis but some industrial breeders screen animals by serological testing and slaughter to control the disease [16]. The use of Rev. 1 vaccine to control goat infection was authorized at the end of 2006 [34].

B. suis was isolated more frequently from humans during the first period (1968–1991). *B. suis* 1a, observed since 1980 [4], and also isolated in Brazil, Colombia, Cuba and Peru, represented almost half of *B. suis* in humans in both sampling periods. These strains were mainly from Buenos Aires, where cattle production is by far the most relevant followed by sheep farming [16]. Although *B. abortus* was the second most frequently isolated during the first period, only 69 cases were found in Buenos Aires and 75 cases in the entire country in 1994–2006. This epidemiological change was probably due to the success of cattle vaccination with vaccine strain *B. abortus* S19. In Argentina new legislation for the control and prevention of bovine brucellosis was introduced in 1993 resulting in a significant increase in vaccinated animals across the country [16]. This vaccine strain was isolated from only a single human case during the first period and from five cases during the second period, confirming its potential pathogenicity for humans.

B. melitensis was more frequently isolated in the second period, mostly in CU and the HP region. Of 145 *B. melitensis* strains recovered, eight from northwest provinces had atypical phenotypic characteristics and based on a difference in the quantitative distribution of the 'A' and 'M' antigens were classified into two biovar subtypes [28]. The two *B. melitensis* bv. 3 strains [35, 36] were the first to be isolated from humans in Argentina but it is the main biovar in Southern Europe and the Middle East. Almost all 189 *B. canis* strains isolated from dogs in 1996–2006 were from Buenos Aires (97·8%), with a peak in 1999–2000 and 2005–2006 coinciding with outbreaks in breeding kennels. This suggests that sanitary control in some kennels is not sufficiently effective [37]. Human infection caused by *B. canis* has been reported and currently tests are performed on patients for serum antibodies to rough *Brucella* strains [e.g. rapid slide agglutination test (RSAT) and IELISA using *B. canis* antigen], when brucellosis is suspected [25, 38].

In conclusion, although the true incidence of human brucellosis is unknown in Latin American countries, *B. melitensis* remains the principal cause of infection, while *B. suis* causes substantial morbidity in Argentina. The isolation of *Brucella* from humans reflects its presence in the animal population. Human infection is assumed to be frequently under-diagnosed because clinical symptoms may be confused and isolation procedures are not routinely applied, therefore care should be taken when considering the geographic distribution of the pathogen and its species. These data provide epidemiological information as guidelines for future control programmes, as well as informing of a new *B. melitensis* variant and a *B. suis* strain resistant to dyes in a manner atypical for this species.

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DECLARATION OF INTEREST

None.

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