Leptospira isolates from rural workers, abattoirs and infected bovine herds in Uruguay.

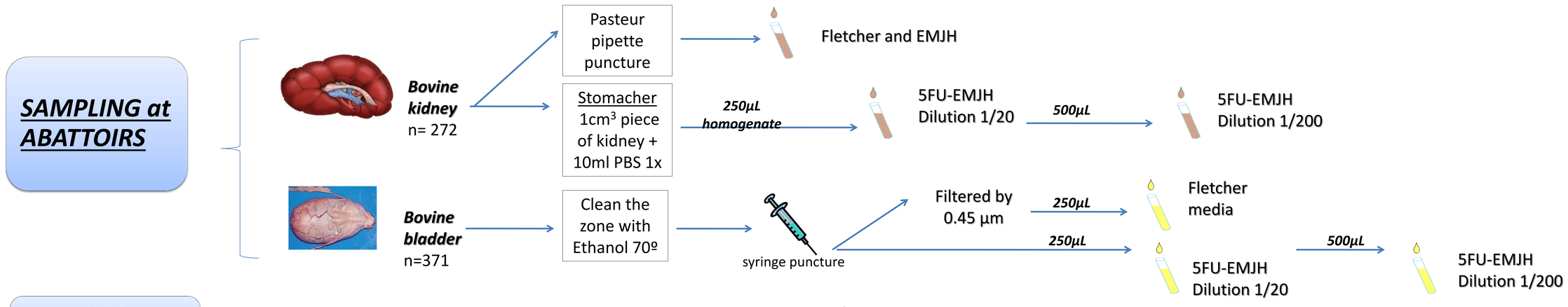
Meny P[1]*, Menéndez C[1], Ashfield N[1], Rios C[1],Quintero J[1], Schelotto F[1], Varela G[1], Zarantonelli L[2], Nieves C[2], Buschiazzo A[2], Suanes A[3], Buroni F[3],Salaberry X[3],Rivero R[3], Briano C[3], Dutra F[3], Fraga M[4] and Riet-Correa F[4].

[1]- Universidad de la República, UdelaR , Uruguay. [2]- Institut Pasteur, Montevideo.[3]- National Veterinary Laboratories DILAVE.[4]- National Institute for Agriculture and livestock Research INIA.

Introduction. Cattle population in Uruguay includes 12 million bovine animals[1]; human inhabitants are 3,300,000.Leptospirosis is an important cause of reproductive failure of cows and calf illness. Estimated figures of seroprevalence are 20% for individual animals and higher than 50% for herds[2]. Bovine reservoir of *Leptospira* is a frequent source of human infection, annually affecting 12/100,000 persons, mainly young rural workers[3]. Prevention of animal and human leptospirosis is a national priority in sanitary and economic terms.

Objectives. To know actual spread of human and animal *Leptospira* variants, for contributing to found and strengthen preventive measures.

Methods. Early blood cultures from humans with clinical and epidemiological suspected diagnosis in urban or rural settings; random sampling from kidneys and bladder urines of cows and steers at abattoirs throughout the country; field urine sampling in presumptively infected herds.

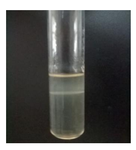


2. PCR : Primers were used to amplify the partial *LipL32 gene, 16S rDNA gene* and *23S rDNA gene*

3. MLVA: Human and bovine isolates showing positive 16S and LipL32 PCR results were further studied through MLVA procedure of molecular typing, seeking for individual VNTR profiles. Five pairs of primers were used, directed to VNTR 4, VNTR 7, VNTR 10, VNTR LB4 and VNTR LB5 loci amplification (Salaün et al. 2006)

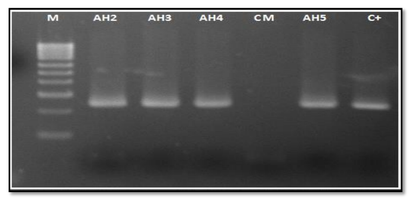
4. PFGE: *Not*I restriction enzyme was used for the DNA digestion. (Galloway & Levett, 2008)

5. SEROTYPING: To determine the probable serogroup, the human and bovine isolates were studied with the microscopic agglutination test (MAT) using a panel of 24 rabbit anti-*Leptospira* sera that was representative of all pathogenic serogroups.



Typical halo of Leptospira`s culture in Fletcher media

PCR technique



Agarose gel electrophoresis of PCR products obtained with LipL32 primers. Human Isolated : AH2-AH5, CM: reagent control, C+: positive control (*L. interrogans* serovar Pomona), M: DNA size marker, 100 bp DNA ladder.

Careful record of animal or human identification, clinical and epidemiological data of studied individuals and groups.

Detailed record of laboratory results

Results: Human isolates (2% aprox) were obtained from aged and severely ill rural patients. Diversity is higher than apparently limited variety of bovine isolates.

	ISOLATE ID (source)	MAT RESULT	PCR LipL 32/ 16S rDNA	MLVA ANALYSIS	Serology	PFGE ANALYSIS	Sequencing	Presumptive identification
HUMAN ISOLATES	AH1 dairy	1st pos 2nd pos	pos/pos	ND	ND	ND	*L. interrogans*	*L. interrogans* Pomona Pomona
	AH2 dairy & abattoir	only one neg sample	pos/pos	5-0-10	Sg. Pomona	*L.interrogans* Pomona Kennewicki	*L. interrogans*	*L. interrogans* Pomona Kennewicki
	AH3 rural work	only one neg sample	pos/pos	1-10-3	Sg. Canicola	*L. interrogans* Canicola Canicola/ Portlandvere	*L. interrogans*	*L. interrogans* Canicola Canicola/ Portlandvere
	AH4 dairy	1st neg 2nd pos	pos/pos	3-2-11	ND	ND	*L. interrogans*	*L. interrogans* Sejroe Wolffi/ Romanica
	AH5 abattoir	only one pos sample	pos/pos	1-5-4	ND	ND	*L. kirschneri*	*L. kirschneri* Australis Ramisi
	AH6 Rice work	1st neg 2nd pos	pos/pos	0-1-4	Sg. Pomona	*L. kirschneri* Pomona Mozdok	*L. kirschneri*	*L. kirschneri* Pomona Mozdok
	AH7 dairy herd	1st neg 2nd pos	pos/pos	1-NP-1-4-6	Sg. Ballum	*L. borgpetersenii* Ballum Ballum	*L. borgpetersenii*	*L. borgpetersenii* Ballum Ballum

	ISOLATE ID (source)	PCR LipL32/ 16S rDNA	MLVA ANALYSIS	Serotyping	Sequencing	Presumptive identification
BOVINE ISOLATES	AB1 (cow´s urine)	pos/pos	5-0-10	Sg. Pomona	*L. interrogans*	*L. interrogans* Pomona Kennewicki
	AB2 (steer´s urine)	pos/pos	NP		*L. noguchii*	
	AB3 (steer´s urine)	pos/pos	5-1-10	Sg. Pomona	*L. interrogans*	*L. interrogans* Pomona Kennewicki
	AB4 (steer´s kidney)	pos/pos	5-1-10	Sg. Pomona	*L. interrogans*	*L. interrogans* Pomona Kennewicki
	AB5 (cow´s kidney)	pos/pos	NP-NP-NP-NP-4	Sg. Autumnalis	*L. noguchii*	
	AB6 (steer´s kidney)	pos/pos	NP-NP-4-4-3	Sg. Sejroe	*L. borgpetersenii*	*L. borgpetersenii* Sejroe Hardjobovis
	AB7 (steer´s kidney)	pos/pos	NP	Sg. Autumnalis	*L. noguchii*	
	AB8 (cow´s kidney)	pos/pos	NP-NP-4-5-5	Sg. Sejroe	*L. borgpetersenii*	*L. borgpetersenii* Sejroe Hardjobovis
	AB9 (calf´s urine)	pos/pos	5-1-10	Sg. Pomona	*L. interrogans*	*L. interrogans* Pomona Kennewicki
	AB10 (steer´s kidney)	pos/pos	?	ND	*L. borgpetersenii*	

ENVIRONMENTAL ISOLATES	ISOLATE ID (source)	PCR LipL32/ 16S rDNA	PCR Lbi	Sequencing[10]	Identification
(slums, bovine herd fields, etc)	AA1	neg/pos	neg	*L. meyeri*	*Leptospira meyeri*
	AA2- AA7	All neg/pos	All pos	ND	All *Leptospira biflexa*

ND: not done
NP: no PCR product

Conclusion. The preliminary data of this work suggest a diversity of human circulating strains, which is important to compare with the isolates of animal origin, especially bovines, to obtain relevant epidemiological information in terms of prevention. Other mammalian species that make part of the animal reservoir of this zoonosis are being explored. This research contributes to the local epidemiological knowledge about leptospirosis, prevention of the disease by vaccines, and improvements in diagnostic procedures.

References
1 Ministry of Cattle breeding, Agriculture and Fishery MGAP, Uruguay. Policy and programs bureau.2016 yearbook.Available from http://www.mgap.gub.uy/unidad-ejecutora/oficina-de-programacion-y-politicas-agropecuarias/publicaciones/anuarios-diea/anuario2016
2 Repiso MV, Gil A, Bañales PM, D'Anatro N, Fernández L, Guarino H, et al. Prevalence of main infectious diseases affecting reproductive performance of meat cattle and characterization of breeding settings of Uruguay. *Veterinaria* 2005;40(157):5-28
3 Schelotto F, Hernández E, González S, Del Monte A, Ifrán S, Flores K, et al. A ten year follow up of human leptospirosis in Uruguay: a non-resolved health problem. *Rev Inst Med Trop Sao Paulo*. 2012; 54: 69-75

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