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GENES ASSISTED SELECTION AS INSTRUMENT OF THE BIODIVERSITY AND POOLING THE GENETIC DISEASES

SELECTIA ASISTATA DE GENE (GAS) CA INSTRUMENT DE SELECTIE A BIODIVERSITATII SI PREVENIREA BOLILOR GENETICE

IUDITH IPATE [1], G. BREM[2], A.T.BOGDAN[1], MONIKA GUTSCHER[2], I. SEREGI, G.F.TOBA , S IVANA, L. ZOLDAG[3], A. MAROTI-AGOTS[3], M.PARVU[4], N. POPESCU[1]

[1]*Romanian Academy- CSCBA*
[2]*Faculty of Veterinary Science Viena*
[3] *Faculty of Veterinary Science Budapesta*
[4]*Faculty of Spiru Haret*

The objective it was analysis the prion protein for scrapie resistance genotyping as codon- amino acid at codon 136, 154, 171 from 5 known haplotypes resulting PrP Genotype. Tissue samples were collected at Institute for Research and Development Rusetu for study the scrapie resistance. Ovine scrapie is a member of the transmissible spongiform encephalopathies (TSEs), a heterogeneous family of fatal neurologic disorders characterized by deposition of an abnormal isoform (prion protein [PrP] PrP-Sc) of a cellular sialoglycoprotein in neural tissue.

One other objective it was analysis the stress resistance and F 18 + E.coli resistance of pigs. Tissue samples were collected at Institute for Research and Development Rusetu and from Romsuintest Peris.

The results are favorable for use the genes assisted selection as instrument of the biodiversity and for selection the animals that are the positives characters.

Keywords: scrapie resistance, stress resistance, genotype,

Introduction

Three major mutations are associated with sheep susceptibility or resistance to classic scrapie and BSE: at codons 136 (A or V), 154 (R or H), and 171 (R, Q, or H) . Animals with genotypes $V^{136}R^{154}Q^{171}$/VRQ, ARQ/VRQ, ARQ/ARQ, and VRQ/ARH PrP are most susceptible to scrapie. In the past 20 years, no TSE cases have been found in ARR/ARR sheep in Europe, although thousands of scrapie-diseased animals have been genotyped. However, 1 report, albeit heavily questioned, has been made in the literature of a possible case in an ARR/ARR sheep in Japan. Therefore, this genotype was considered to confer full resistance to BSE and

scrapie .To minimize the risk of humans acquiring TSE by consuming animal products, massive breeding programs involving PrP-genotyping of millions of sheep were initiated in the European Union (EU).In the literature concerning susceptibility to various strains of scrapie, three codons are routinely discussed: 171, 154 and 136 (reported 136, 154, 171/ 136,154,171). In North America, 171 and 136 are given primary importance in association with classical scrapie. Of all sheep diagnosed with scrapie from 1998–2008, 93% were of the genotype ARQ/ARQ. The presence of arginine (R) at codon 171 of the prion protein confers resistance to the prion protein undergoing the structural change.The presence of glutamine (Q) or histidine at site 171 may convey some resistance, because has not detected scrapie in the sheep . However, these genotypes are infrequent; thus, the lack of cases in this genetic subpopulation may be due to their lack of presence and exposure, rather than resistance. The coding for alanine (A) by codon 136 confers resistance to the prion protein undergoing the structural change associated with scrapie. The coding for valine (V) by codon 136 can produce susceptibility to structural change associated with scrapie. V at site 136 is linked with Q at site 171, such that R cannot be found at site 171 in combination with V at site 136.Data from all sheep diagnosed with scrapie between 1998–2008 is displayed below with the relative probability of a sheep with that particular genotype being susceptible to classical scrapie listed on the right side of the list:

Genotype (136, 171)	**Susceptibility to Scrapie**
• negligible	AR/AR
• very low	VQ/AR
• intermediate	VQ/AQ
• high	VV/QQ, AQ/AR, AQ/AQ

FAO recommend one list for use the markers by species. We used in the experiences this recommendation:

Ranked list of recommended markers by species:

Cattle	Buffalo	Yak	Goat	Sheep	Pig	Horse	Donkey	Chicken	Camelids
INRA063	CSSM033	AGLA293	SRCRSP5	MAF65	S0026	HMS07	HMS07	ADL0268	CMS9
INRA005	CSSM038	BM1824	MAF065	OarFCB193	S0155	HMS06	HMS06	MCW0206	CMS13
ETH225	CSSM043	BM2113	MAF70	OarJMP29	S0005	HTG07	HTG07	LEI0166	CMS15
ILSTS005	CSSM047	CSSM066	SRCRSP23	OarJMP58	Sw2410	AHT05	AHT05	MCW0295	CMS17
HEL5	CSSM036	ETH152	OarFCB48	OarFCB304	Sw830	HTG04	HMS03	MCW0081	CMS18
HEL1	CSSM019	ETH225	INRA023	BM8125	S0355	HMS02	HMS02	MCW0014	CMS25
INRA035	CSRM060	HEL1	SRCRSP9	OarFCB128	Sw24	ASB02	HTG06	MCW0183	CMS32
ETH152	CSSM029	HEL5	OarAE54	OarCP34	Sw632	HMS03	HTG10	ADL0278	CMS50
INRA023	CSSM041	HEL13	SRCRSP8	OarVH72	Swr1941	HTG06	AHT04	MCW0067	CMS121
ETH10	CSSM057	ILSTS008	SPS113	OarHH47	Sw936	HTG10	ASB17	MCW0104	CVRL01
HEL9	BRN	ILSTS028	INRABERN172	DYMS1	S0218	AHT04	ASB23	MCW0123	CVRL02
CSSM66	CSSM032	INRA005	OarFCB20	SRCRSP1	S0228	VHL20	LEX33	MCW0330	CVRL05
INRA032	CSSM008	MGTG4B	CSRD247	SRCRSP9	Sw122	ASB17	LEX34	MCW0165	CVRL06
ETH3	CSSM045	MGTG7	McM527	MCM140	Sw857	ASB23	SGCV28	MCW0069	CVRL07
BM2113	CSSM022	TGLA53	ILSTS087	MAF33	S0097	LEX33	LEX68	MCW0248	LCA66
BM1824	CSSM046	TGLA57	INRA063	MAF209	Sw240	UCDEQ425	COR058	MCW0111	VOLP03
HEL13	CSSM013	TGLA73	ILSTS011	INRA063	IGF1	LEX34	COR069	MCW0020	VOLP08
INRA037	ETH003	TGLA122	SRCRSP7	OarFCB20	Sw2406	SGCV28	VHL209	MCW0034	VOLP10
BM1818	CSSM061	TGLA126	ILSTS005	BM1329	Sw72	COR058	ASB02	LEI0234	VOLP32
ILSTS006	BMC1013	TGLA227	SRCRSP15	MAF214	S0226	COR069	HMS20	MCW0103	VOLP67
MM12	DRB3	ETH185	SRCRSP3	ILSTS11	S0090	VHL209	COR007	MCW0222	YWLL 08
CSRM60	CSSM062	ILSTS013	ILSTS029	OarFCB226	Sw2008	COR007	LEX54	MCW0016	YWLL 09
ETH185	CSSME070	ILSTS050	TGLA53	ILSTS28	Sw1067	LEX54	LEX73	MCW0037	
HAUT24	ETH121	SPS115	ETH10	MAF70	S0101	LEX73	COR022	MCW0098	
HAUT27	ILSTS033	BM861	MAF209	BM1824	Sw1828	COR022	LEX63	LEI0094	
TGLA227	ILSTS005	BYM-1	INRABERN185	HUJ616	S0143	LEX63	COR018	MCW0284	
TGLA126	ILSTS030	INRA189	BM6444	OarCP38	S0068	COR018	COR071	MCW0078	
TGLA122	ILSTS008		P19 (DYA)	ILSTS5	S0178	COR071	HMS45	LEI0192	
TGLA53	RM099		TCRVB6	OarAE129	Sw911	HMS45	NVHEQ054	ADL0112	
SPS115	HMH1R		DRBP1	SRCRSP5	S0002	COR082	COR082	MCW0216	
				MCM527					

Material and Methods

Tissue samples were collected at Institute for Research and Development Rusetu for study the scrapie resistance and for tested the stress resistance and F 18+E.coli resistance of pigs were collected the probes at Research and Development Rusetu and from Romsuintest Peris.

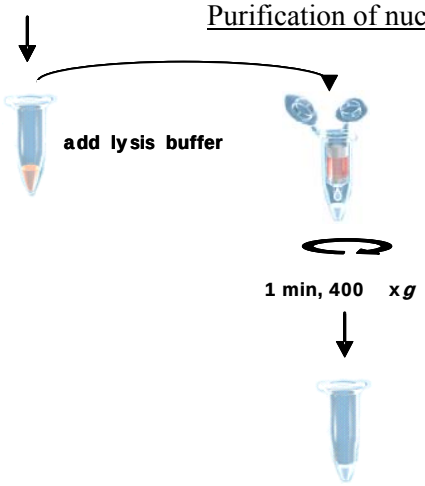
Sample colletion with the TypiFix™ System
for Scrapie-genotyping of sheep

Sample collection
of small tissue probes with the TypiFix™ system

Sample aquisition

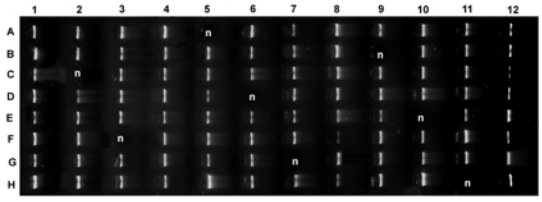
error-free sample processing in the lab by automatic aquisition of the sample ID and transcription into the data base

Purification of nucleic acids more than 5 x faster



DNA purification with dna*FIX* columns an extremly simplified and shortened one-step
high-throughput separation procedure of genomic DNA from TypiFix™ samples. The sorbents retain protein and other contaminantes, while the DNA passes the column in the exclusion volume

Reproducible DNA yield and quality



Gel electrophoresis of dnaFix purified DNA from 88 TypiFix™ samples 5 µl (total elution volume: 240 µL) of each sample were loaded on a 1% agarose/ EtBr gel. The DNA concentration is about 5 ng/µl or greater, n = negative control

Moleculargenetic analysis PCR

Results and Discussion

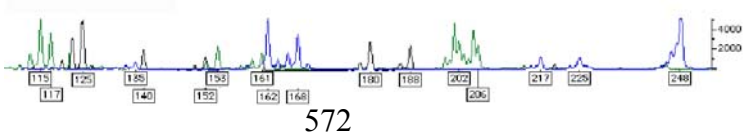
In the past, natural scrapie and bovine spongiform encephalopathy (BSE) infections have essentially not been diagnosed in sheep homozygous for the $A^{136}R^{154}R^{171}$ haplotype of the prion protein. This genotype was therefore assumed to confer resistance to BSE and classic scrapie under natural exposure conditions. It was reported the identification of 2 natural scrapie cases in ARR/ARR sheep that have biochemical and transmission characteristics similar to cases of classic scrapie, although the abnormally folded prion protein (PrP^{Sc}) was associated with a lower proteinase-K resistance. PrP^{Sc} was clearly distinct (Table 1).

No.	Lab no.	Typifix no.	animal ID	Resuls AS Codon 136	154	171	Genotype[2]
1	US080042	80	RO1072726994	AA	RR	RQ	ARR/ARQ
2	US080043	81	RO1072353152	AA	RR	QQ	ARQ/ARQ
3	US080044	82	RO1072353154	AA	RR	RQ	ARR/ARQ
4	US080045	83	RO1074538405	AA	RR	QQ	ARQ/ARQ
5	US080046	84	RO1074538440	AA	RR	RQ	ARR/ARQ
6	US080047	85	RO1072726972	AA	RR	RQ	ARR/ARQ
7	US080048	86	RO1072726973	AA	RR	QQ	ARQ/ARQ
8	US080049	87	RO1072727018	AA	RR	RQ	ARR/ARQ
9	US080050	88	RO1072726932	AA	RR	RQ	ARR/ARQ
10	US080051	89	RO1072726928	AA	RR	QQ	ARQ/ARQ
11	US080052	90	RO1074538450	AA	RR	QQ	ARQ/ARQ
12	US080053	91	RO1074538450	AA	RR	QQ	ARQ/ARQ
13	US080054	92	RO1074538447	AA	RR	QQ	ARQ/ARQ
14	US080055	93	RO1074538404	AA	RR	RQ	ARR/ARQ

As codon- amino acid at codon 136, 154, 171 from 5 known haplotypes resulting PrP Genotype.

Moleculargenetic analysis

PCR



Scrapie-Genotyping

In ours results of analysis (Table 1) half the probes present the arginine (R) at codon 171 of the prion protein who confers resistance to the prion protein undergoing the structural change. The coding for alanine (A) by codon 136 confers resistance to the prion protein undergoing the structural change associated with scrapie. All the probes have in the 136 codon the alanine.
The presence of glutamine (Q) or histidine at site 171 may convey some resistance, because has not detected scrapie in thise sheep.

In the next experience we tested the the stress resistance and F 18+E.coli resistance of pigs were collected the probes at Research and Development Rusetu and from Romsuintest Peris. (Table 2)

No.	Lab no.	Typifix no.		Stress resistance	E.coli resistance
1	US080007	1	Peris	MM	BB
2	US080008	51	Peris	MM	BA
3	US080009	52	Peris	LM	BA
4	US080010	53	Peris	LM	BB
5	US080011	54	Peris	MM	BB
6	US080012	55	Peris	MM	BB
7	US080013	56	Peris	failed	failed
8	US080014	57	Peris	MM	BA
9	US080015	58	Peris	LM	BA
10	US080016	59	Peris	MM	BB
11	US080017	60	Peris	LM	BB
12	US080018	61	Peris	MM	BB
13	US080019	62	Peris	LM	BB
14	US080039	94	Rusetu	MM	AA
15	US080040	95	Rusetu	MM	BA
16	US080041	96	Rusetu	MM	BB

MM = stress resistant, LM = stress resistant but genetic carrier, LL =not stress resistant

AA = E.coli resistance, BA = not resistant but genetic carrier, BB = not resistant
Each the animal tested of Rusetu Institute have the stress resistance gene (MM), because in this place the researcher created one breed based on rustic local breed.
Half of animals examined of Romsuintest have the stress resistance gene (MM) and the others animals have LM = stress resistant but genetic carrier. In ours analysis results not have one correlation with stress resistant genotype and E.coli resistance. One single exemplar has the genotype E.coli resistance, this animal is from Rusetu Institute.

In the future must to repeat the experience for find the animals who are the stress resistant genotype and E.coli resistance.

Conclusions

1. It was analysis the prion protein for scrapie resistance genotyping *as codon*- amino acid at codon 136, 154, 171 from 5 known haplotypes resulting PrP Genotype.

2. It was analysis the stress resistance and F 18 + E.coli resistance of pigs.

3.The results are favorable for use the genes assisted selection as instrument of the biodiversity and for selection the animals that are the positives characters.

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