Romanian Biotechnological Letters *Vol. 18, No.3, 2013*

ORIGINAL PAPER

RISK OF SCRAPIE FOR ROMANIAN TURCANA SHEEP OF PRION PROTEIN GENOTYPE ARQ/ARQ

Received for publication, January 10, 2013
Accepted, March 15, 2013

IPATE IUDITH[1], STRASSER CONSTANTA[2], IONITA LUCIAN[2], STRATEANU AMALIA[1], STRASSER HORATIU[1], ENACHE LIVIU MIHAI[1]
[1] Romanian Academy- INCE-CSCBA
[2]Faculty of Veterinary Science Bucharest, Romania
Email: Ipate Iudith[1]- ipate.iudith@gmail.com,
Strasser Constanta[2]- strasserconstanta@yahoo.com

Abstract

The Romanian Turcana sheep it is a national breed with multiple quality: adaptability in rough climate, rusticity but with insufficient production of actual necessity. The research team collected samples from animals in Transylvania area to identify valuable genotypes resistance to scrapie disease. For classical scrapie the codon 171 is thought to be the major determinant of susceptibility, with glutamine (Q) and histidine (H) conferring susceptibility and arginine (R) resistance. The results of county analysis shows evidence 46 alanine (A) at codon 136 that confers resistance to scrapie prion structural changes. The presence of glutamine (Q) or histidine at codon 171 may send some characters of resistance to scrapie that was not detected in these samples. The analytical results from 126 samples the presence of glutamine (Q) in codon 171 of prion structural changes that confer resistance to scrapie prion. It is identify the case of scrapie with clinical signs with genotype ARQ/ARQ considered very low susceptibility to scrapie in Europe.

Key words: scrapie**,** genotype resistance, susceptibility

Introduction

Susceptibility to infection and incubation period in sheep has been shown to be affected by sheep genetics and breed. Transmission of the scrapie agent is not completely understood, and apparently healthy sheep infected with the agent can transmit disease. Scrapie is a disorder characterized by the deposition of the prion protein (PrP^{sc};associated with TSE) in the central nervous system and lymphoreticular system. Characteristic clinical signs of the disease are behavioural disturbances, pruritus and increased difficulty in locomotion. The long incubation period between exposure and clinical disease may allow animals to shed the agent for an extended period. The scrapie agent is thought to spread most commonly from the ewe to her offspring and other lambs through contact with the placenta and placental fluids, and sheep and goats are typically infected as young lambs or kids. Placental infectivity occurs in the incubation/preclinical stage of disease but is not constant with every pregnancy. Genetically susceptible lambs born to dams that develop clinical scrapie have a higher risk of developing the disease. Ram genetics will contribute to scrapie susceptibility in their offspring. Direct horizontal transmission likely accounts for scrapie cases in heavily infected flocks, where spread most likely occurs via an oral route. Infection also likely occurs via ocular exposure or contact with abraded skin or mucous membranes.Transmission to lambs through milk from infected ewes has been reported, as well as subsequent horizontal transmission among. Other infectious tissues have also been found, including: central nervous tissue, lymphoid tissue, peripheral nerves, blood, muscle, liver, nasal mucosa, and salivary glands.

For scrapie diagnostic 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. For classical scrapie the codons at positions 136 and 171 in the gene that code for amino acids in the prion protein (PrP) have been associated with scrapie susceptibility. Codon 171 is thought to be the major determinant of susceptibility, with glutamine (Q) and histidine (H) conferring susceptibility and arginine (R) resistance. The effect of lysine (K) at codon 171 on scrapie susceptibility is unknown due to its infrequent occurrence. Codon 136 affects susceptibility to the less common valine-dependent classical scrapie, with alanine (A) and valine (V) conferring resistance and susceptibility, respectively.All QQ sheep are susceptible to the more common valine-independent classical scrapie and can transmit the disease to susceptible flock mates. Conversely, ARR sheep are nearly completely resistant to this classical scrapie. These sheep are highly unlikely to carry or transmit scrapie. ARQ are rarely infected, and it is unknown whether infected ARQ sheep can transmit scrapie.

Materials and methods

Were collected samples, which revealed other valuable genotypes in the Transylvania area from Turcana sheep. The Romanian Turcana sheep it is a national breed with multiple quality: adaptability in rough climate, rusticity but with insufficient production of actual necessity. The original method Typi Fix - internationally patented from Prof.Brem - to discover DNA polymorphism and its applicability in the identification and traceability of meat species biodiversity. By this method one can follow the following aspects - determination of individual identity of animals in animal cells and finished products, control of paternal or maternal origin, detection of genes that influence the quality of raw material, analysis determination of genetic variability (*Fig.1*)**.**

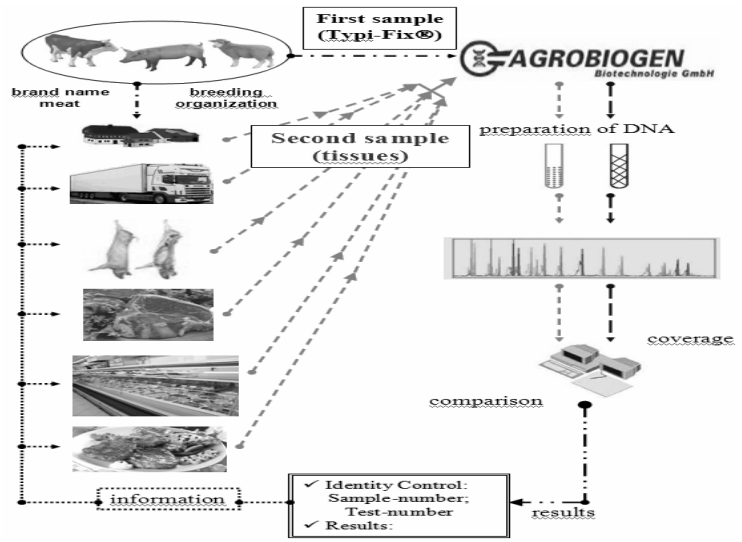


Fig.1 Traceability of products through the new method of Prof. G. Brem

The TypiFix™ ear tag system is a combination of a conventional ear tag with a simultaneous tissue sampling technology. By ear tagging the farm animals, the tissue samples are automatically collected and sealed in the TypiFix™ sample containers, where the tissue samples are preserved at ambient temperature and can be used for protein or DNA based

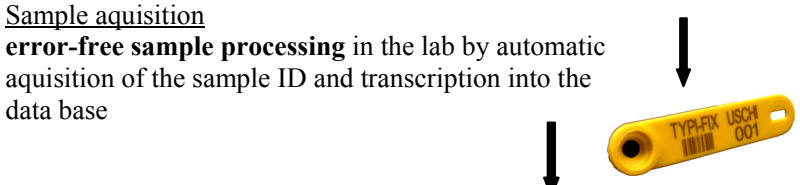
assays. The easy handling of the TypiFix™ ear tag system allows economic sampling of whole populations and is therefore an effective tool for analysis of genetic markers for paternity control, traceability and breeding traits. The Typi-Fix®-System is a procedure for the collection of DNA containing tissue samples avoiding all these hurdles and problems. With the Typi-Fix®-ear tags the animal is marked - in the usual convention - with a plastic ear tag. At the same time, however, a tissue sample is taken by the spike of the ear tag which immediately after the collection is packaged in a special plastic container (sample receiving container) labelled with the (bar coded) animals ear tag number. After collection the preservation and preparation of the DNA is initiated automatically by substances which are hold in stock in the sample receiving container. The identification number of the samples can be registered by a reading device (scanner). The sample container is connected to the ear tag by a plug and socket and is easily removed after the ear tag has been affixed and the tissue sample simultaneously collected. If desired, the sample container can also be used without the ear tag.

Tissue collection with TypiFixTM –System (Fig.2)

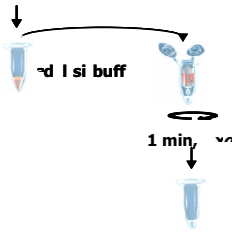
Sample collection of small tissue probes with the TypiFix™ system

Sample aquisition

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

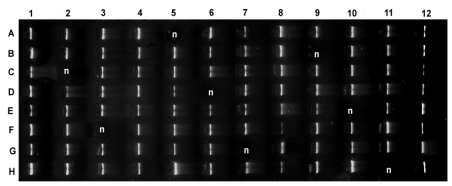


Purification of nucleic acids more than 5 x faster



DNA purification with DNA *FIX* columns
an extremely simplified and shortened one-step high-throughput separation procedure of genomic DNA from TypiFix™ samples.

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

Molecular genetic analysis PCR

Fig.2. Sample colletion with the TypiFix™ System for Scrapie-genotyping of sheep

DNA purification with DNA FIX columns an extremely simplified and shortened one-step high-throughput separation procedure of genomic DNA from TypiFix™ samples.

The sorbents retain protein and other contaminants, while the DNA passes the column in the exclusion volume. DNA isolation and purification can be automated through the use of a pipetting robot and a special one-step procedure (Nexttec technology). PCR reactions with these samples can also be prepared automatically. The results of the multiplex PCR analyses are linked with the scanned identification number and saved in the animal data bank. This aspect is very important for studying traceability and domestic animal biodiversity.

Gel electrophoresis of NCC™ purified DNA from 88 TypiFix™ eartag samples : 5 µl (total elution volume: 240 µL) of each sample were loaded on a 1% agarose/ EtBr gel. The

DNA concentration is about 10 ng/µl or greater = negative control. These higly-performant analyses are conducted at the well-known AGROBIOGEN laboratories in Germany, by Prof. Brem and his team, with their patented methods.

Tissue collection with TypiFixTM –System

DNA purification with DNA FIX columns an extremely simplified and shortened one-step high-throughput separation procedure of genomic DNA from TypiFix™ samples.

The sorbents retain protein and other contaminants, while the DNA passes the column in the exclusion volume. DNA isolation and purification can be automated through the use of a pipetting robot and a special one-step procedure (Nexttec technology). PCR reactions with these samples can also be prepared automatically. The results of the multiplex PCR analyses are linked with the scanned identification number and saved in the animal data bank. This aspect is very important for studying traceability and domestic animal biodiversity.

Gel electrophoresis of NCC™ purified DNA from 88 TypiFix™ eartag samples : 5 µl (total elution volume: 240 µL) of each sample were loaded on a 1% agarose/ EtBr gel. The DNA concentration is about 10 ng/µl or greater = negative control. These higly-performant analyses are conducted at the well-known AGROBIOGEN laboratories in Germany, by Prof. Brem and his team, with their patented methods.

Results and Discussion

The Animal Breeding and Genomics Centre (ABGC) has produced a report presenting general recommendations on how to continue breeding for scrapie resistance. The recommendations depend on the population size and the percentage of ARR in the population. In breeds with too few ARR animals, there is low selection intensity; otherwise there is a risk of excessive inbreeding in the population. In breeds with a great deal of double ARR, the selection can be stronger.

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 not convey the resistance, because has detected scrapie in thise breed sheep.

Table 1. DNA analyses of resistance/susceptibility genes for Scrapie (Partial results)

			Resuls		AS Codon		
No.	Lab no.	Typifix no.	animal ID	136	154	171	Genotype²
1	US080042	80	RO1072 726994	AA	RR	RQ	ARR/ARQ
2	US080043	81	RO1072 353152	AA	RR	QQ	ARQ/ARQ
3	US080044	82	RO1072 353154	AA	RR	RQ	ARR/ARQ
4	US080045	83	RO1074 538405	AA	RR	QQ	ARQ/ARQ
5	US080046	84	RO1074 538440	AA	RR	RQ	ARR/ARQ
6	US080047	85	RO1072 726972	AA	RR	RQ	ARR/ARQ

7	US080048	86	RO1072 726973	AA	RR	QQ	ARQ/ARQ
8	US080049	87	RO1072 727018	AA	RR	RQ	ARR/ARQ
9	US080050	88	RO1072 726932	AA	RR	RQ	ARR/ARQ
10	US080051	89	RO1072 726928	AA	RR	QQ	ARQ/ARQ
11	US080052	90	RO1074 538450	AA	RR	QQ	ARQ/ARQ
12	US080053	91	RO1074 538450	AA	RR	QQ	ARQ/ARQ
13	US080054	92	RO1074 538447	AA	RR	QQ	ARQ/ARQ
14	US080055	93	RO1074 538404	AA	RR	RQ	ARR/ARQ

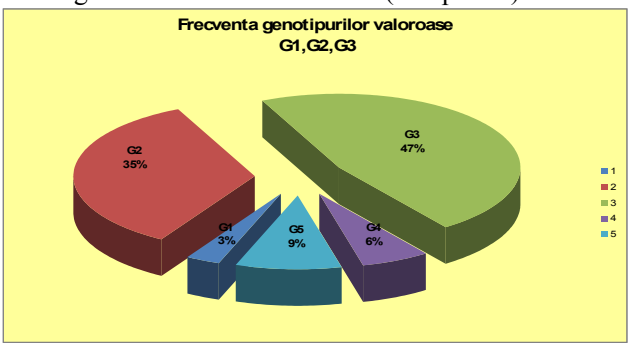
In results of analysis in Hateg (Tab 2) 41,4% the probes present the arginine (R) at codon 171 of the prion protein who confers resistance to the structural change of prion scrapie. But for genotype class (genotype classifications by the German Society of Animal breeding-DGfZ in Totesti (Tab.2) we detected 4 probes with genotype G4 (ARR/VRQ) and 5 probes with -G5 (VRQ/ARQ) who is susceptibility from scrapie disease.

Table 2. DNA analyses of resistance/susceptibility genes for Scrapie in Hateg –partially results

			Results				
			AA Codon[1]				Genotype-class[3]
No.	Animal ID	sex	136	154	171	PrP Genotype[2]	
1	RO146819946	m	VA	RR	RQ	ARR/VRQ	G4
2	RO FS 1	m	AA	RR	QQ	ARQ/ARQ	G3
3	RO 1075862576	m	AA	RR	QQ	ARQ/ARQ	G3
4	RO 146052131	m	AA	RR	RQ	ARR/ARQ	G2
5	RO 146819988	m	AA	RR	QQ	ARQ/ARQ	G3
6	RO 1041701026	m	AA	RR	QH	ARQ/ARH	G3
7	RO 1061463250	m	VA	RH	QQ	VRQ/AHQ	G5
8	RO 146052072	m	AA	RR	RQ	ARR/ARQ	G2
9	RO 146051967	m	AA	RR	RQ	ARR/ARQ	G2
10	RO FS 2	m	AA	RR	RQ	ARR/ARQ	G2
11	RO 146052082	m	AA	RR	QQ	ARQ/ARQ	G3
12	RO 1061459553	m	AA	RR	RQ	ARR/ARQ	G2
13	RO 1061463223	m	AA	RR	QQ	ARQ/ARQ	G3
14	RO 146052017	m	AA	RR	RQ	ARR/ARQ	G2
15	RO 146052023	m	AA	RR	RQ	ARR/ARQ	G2
16	RO 1044743731	m	VA	RR	QQ	VRQ/ARQ	G5
17	RO 1047102583	m	AA	RR	RQ	ARR/ARQ	G2
18	RO 146052050	m	AA	RR	QQ	ARQ/ARQ	G3
19	RO 106143340	m	AA	RR	QQ	ARQ/ARQ	G3

20	10614459607	m	AA	RR	QQ	ARQ/ARQ	G3
21	RO 146052035	m	AA	RR	RQ	ARR/ARQ	G2
22	RO FS 3- 022	m	VA	RR	QQ	VRQ/ARQ	G5
23	RO 146052034	m	AA	RR	QQ	ARQ/ARQ	G3

The best genotype class G1 (ARR/ARR) and G2 (ARR/ARH) it's presents in the probes of Totesti in percentage the 3% G1 and 25% G2 (Graphic 1).



Graphic 1. Valuable Genotypes frequency in Totesti

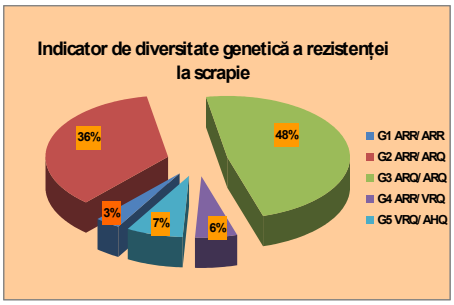
In case of Turdas (Tab.3) analysis shows that 13% (6% G4 + 7% G5) of the number of breeding flock should be removed because the chance of triggering an epidemic is high, which means that if the owners do not apply measures to remove and isolate these animals are able to lose the whole flock .

Table 3. Prion Protein Gene Analyses in Turdas resistance/susceptibility genes for Scrapie

	Results				
	AA Codon[1]				Genotype-class[3]
Owner- Animal ID-breed	136	154	171	PrP Genotype[2]	
Ferma Turdas -1090449202 Turcana sheep	AA	RR	RQ	ARR/ARQ	G2
Ferma Turdas -1101791385 Turcana sheep	AA	RR	RQ	ARR/ARQ	G2
Ferma Turdas -1090449204 Turcana sheep	AA	RH	QQ	ARQ/ARQ	G3
Ferma Turdas -1101791384 Turcana sheep	AA	RR	QQ	ARQ/ARQ	G3
Ferma Turdas -1101791383 Turcana sheep	AA	RR	RQ	ARR/ARQ	G2
Ferma Turdas -1101791357 Turcana sheep	AA	RR	QQ	ARQ/ARQ	G3
Ferma Turdas -1101791362 Turcana sheep	VA	RR	QQ	VRQ/ARQ	G5
Ferma Turdas -11017911783 Turcana sheep	AA	RR	QQ	ARQ/ARQ	G3

Ferma Turdas -1075859591 Turcana sheep	AA	RR	RR	ARR/ARR	G1
Ferma Turdas -1101791365 Turcana sheep	AA	RR	RR	ARR/ARR	G1
Ferma Turdas -1101791372 Turcana sheep	AA	RR	RQ	ARQ/ARQ	G3
Ferma Turdas -10466068035 Turcana sheep	AA	RR	RH	ARQ/ARQ	G3
Ferma Turdas -1101791370 Turcana sheep	AA	RR	RQ	ARR/ARQ	G2
Ferma Turdas -1101791395 Turcana sheep	AA	RR	QQ	ARQ/ARQ	G3

The frequency of G3 (ARQ/ARQ) in analysis the samples of Turdas it is 48%.(Graphic 2).



Graphic 2. Valuable Genotypes frequency in Turdas

In Europe ARQ are rarely infected, and it is unknown whether infected ARQ sheep can transmit scrapie. But unfortunately in the next six months, 4 sheep turcana breed of Turdas with genotypes G3 had specific clinical signs of scrapie and died. Histological analysis of laboratory confirmed disease.

Conclusion

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. The TypiFix™ ear tag system is simple, one-step collection and preservation of tissue samples. The TypiFix™ ear tag system is fast, fully-automated and economical preparation of DNA. This method is to be performed much more quickly and economically than is currently possible with the traditional methods of sample preparation.
3. In results of analysis in Hateg country 86, 8% the probes present the alanine (A) at codon 136 of the prion protein who confers resistance to the structural change of prion scrapie. In Hateg country (Tab.2) we detected 41,4% probes with genotype G4 (ARR/VRQ) and 5 probes with -G5 (VRQ/ARQ) who is susceptibility from scrapie disease.
4. In results of analysis in Turdas 82 % the probes present the alanine (A) at codon 136 of the prion protein who confers resistance to the structural change of prion scrapie. The analytical results from 56 samples the presence of glutamine (Q) in codon 171 of prion structural changes that confer resistance to scrapie prion. But classes G5-5 genotype 2 samples were detected with G4 genotype (ARR / VRQ) and 2 evidence-G5 (VRQ / ARQ), which are capable of prion disease.

5. In results of analysis in Turdas the frequency of G3 (ARQ/ARQ) in analysis the samples of Turdas it is 48%.
6. The 8.33% of 48% with genotype G3 presents specific clinical signs of scrapie and died. The Rumanian Turcana sheep with genotype G3 present the sensitivity from scrapie

Acknowledgements

This work was done by research conducted within the Romanian Academy Research Theme XI.4-"Research regarding the traceability in livestock eco-systems of animal's products in socio-economic conditions of Romania and Moldova."

References

1. BELT, P.B.G.M., MUILEMAN, I.H., SCHREUDER, B.E.C., RUIJTER, J.B., GIELKENS, A.L.J., SMITS, M.A., (1995) Identification of five allelic variants of the sheep PrP gene and their association with natural scrapie. Journal of General Virology 76, 509-517.
2. BREM G, Techniques and possibilities of traceability of food: genotyping of the domestic animal population as an innovative contribution to food safety**]** Dtsch Tierarztl *Wochenschr* 2004 Jul;111(7):273-6 [pmid:15366286]
3. CARLSON, G.A., KINGSBURY, D.T., GOODMAN, P.A., COLEMAN, S., MARSHALL, S.T., DEARMOND, S.J., WESTAWAY, D. AND PRUSINER, S.B. (1986) Linkage of prion protein and scrapie incubation time genes. Cell 46: 503-511.
4. Clark, A.M., Dawson, M., & Scott, A.C. (1994) Scrapie associated fibrils found in dead sheep. Veterinary Record 134, 650-651.
5. CLOUSCARD, C., BEAUDRY, P., ELSEN, J.M., MILAN, D., DUSSAUCY, M., BOUNNEAU, C., SCHELCHER, F., CHATELAIN, J. LAUNAY, J.J., LAPLANCHE, J.L. (1995) Different allelic effects of the codon 136 and 171 of the prion protein gene in sheep with natural scrapie. Journal of General Virology 76, 2097-2101.
6. EC, 2003a EC, Commission decision of 13 February 2003 laying down the minimum requirements for the establishment of breeding programmes for resistance to transmissible spongiform encephalopathies in sheep (2003/100/EC), Off. J. Eur. Union L41 (2003), pp. 41–45.
7. EC, 2003b EC, Commission Regulation (EC) No 1915/2003, Off. J. Eur. Union L283 (2003), pp. 29–33
8. Gibson, P. H., Somerville, R. A., Fraser, H., Foster, J. D. & Kimberlin, R. H. (1987) Scrapie Associated Fibrils in the diagnosis of Scrapie in Sheep. Veterinary Record 120, 125Ä127.
9. HADLOW, W. J. (1991) To a Better Understanding of Natural Scrapie. In: SubÄAcute Spongiform Encephalopathies, 117Ä130. (R. Bradley, M. Savey & B. Marchant, eds.) Kluwer Academic Publishers, Boston.
10. IUDITH IPATE, G. BREM, A.T. BOGDAN, MONIKA GUTSCHER, I. SEREGI, G. F. TOBA, L. ZOLDAG, A. Maroti-Agots, *Microsatelllite analysis for the study of biodiversity and traceability of the pig, Scientifical Papers, Animal Science and Biotechnologies***,** vol. 33-42(1,2), 2009Ed. Agroprint, Romania**,** ISSN: 1221-5287, pg.555
11. IUDITH IPATE, G. BREM, A. T. BOGDAN, MONIKA GUTSCHER, I. SEREGI, G. F. TOBA, S.IVANA, L. ZOLDAG, A. MAROTI-AGOTS, M. PARVU, N. POPESCU, *Genes assisted selection as instrument of selection the biodiversity and pooling the genetic diseases, Scientifical Papers, Animal Science and Biotechnologies*, vol. 33-42(1,2), 2009 Ed. Agroprint, Romania**,** ISSN: 1221-5287,pg. 563
12. RACE, R. E.,ERNEST, D. R., JENNY, A. L., TAYLOR, W. D., SUTTON, D. & CAUGHEY, B. (1992) Diagnostic implications of detection of proteinase-K resistant protein in spleen, lymph nodes and spleens of sheep. American Journal of Veterinary Research 53, 883-889.
13. SCHMERR, MJ., JENNY, A. L., BULGIN, M. S., MILLER, J. M., HAMIR, A. N., CUTLIP, R. C., & GOODWIN, K. R. (In print - 1999) Use of capillary electrophoresis and fluorescent peptides to detect the abnormal prion protein in the blood of animals that are infected with a transmissible spongiform encephalopathy. Journal of Chromatography A 853: 207-214.
14. WESTAWAY, D., ZULIANI, V., COOPER, C.M., DACOSTA, M., NEUMAN, S., JENNY, A.L., DETWILER, L. AND PRUSINER, S.B. (1994) Homozygosity for prion protein alleles encoding glutamine-171 renders sheep susceptible to natural scrapie. Genes and Development 8: 959-969.