AGRICULTURAL BIOLOGY,
2017, V. 52, № 6, pp. 1157-1165
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.32/.38:636.082.12:577.21

doi: 10.15389/agrobiology.2017.6.1157rus
doi: 10.15389/agrobiology.2017.6.1157eng

CHARACTERISTICS OF ALLELE POOL OF THE ROMANOV SHEEP BREED FOR THE PRION PROTEIN GENE ASSOCIATED WITH GENETIC SUSTAINABILITY TO SCRAPIE

E.A. GLADYR[1], T.E. DENISKOVA[1], V.A. BAGIROV[1], O.V. KOSTYUNINA[1], N.N. MAKAROVA[2], G. BREM[1, 3], N.A. ZINOVIEVA[1]

[1]*L.K. Ernst Federal Science Center for Animal Husbandry,* Federal Agency of Scientific Organizations, 60, pos. Dubrovitsy, Podolsk District, Moscow Province, 142132 Russia, e-mail elenagladyr@mail.ru (corresponding author), tandeniss@rambler.ru, vugarbagirov@mail.ru, kostolan@yandex.ru, n_zinovieva@mail.ru (corresponding author);
[2]*LLC «Agrivolga»,* 11, ul. Spasskaya, Uglich, Yaroslavl Province, 152615 Russia, e-mail N.Makarova@agrivolga.ru;
[3]*Institut für Tierzucht und Genetik, University of Veterinary Medicine (VMU),* Veterinärplatz, A-1210, Vienna, Austria, e-mail gottfried.brem@vetmeduni.ac.at
ORCID:
Gladyr E.A. orcid.org/0000-0002-5210-8932
Deniskova T.E. orcid.org/0000-0002-5809-1262
Bagirov V.A. orcid.org/0000-0001-8385-2433
Kostyunina O.V. orcid.org/0000 0001 8206 3221
Makarova N.N. orcid.org/0000-0002-4742-9985
Brem G. orcid.org/0000-0002-7522-0708
Zinovieva N.A. orcid.org/0000-0003-4017-6863
The authors declare no conflict of interests
Acknowledgements:
The equipment of the Center for Bioresources and Bioengineering of Agricultural Animals was used in the study. Supported financially by Russian Science Foundation, project № 14-36-00039, and Federal Agency of Scientific Organizations, theme № 0600-2014-0004.3
Received October 4, 2017

Abstract

The Romanov is a unique indigenous sheep breed of Russia, belonging to the group of Northern short-tailed sheep. The breed is known all over the world, due to out-of-season breeding ability, phenomenal fecundity (up to 10 lambs) and unsurpassed quality of sheepskins. Presently the gene pool of the breed is actively involved in creation of new types of modern prolific sheep and it is considered as an important genetic reserve for the sheep breeding of the future. Diseases resistance is the most important selection trait in sheep. One of the diseases which can cause serious economic loses is spongiform encephalopathy of sheep, also known as scrapie. Scrapie is a fatal neurodegenerative disease of sheep and goats, belonging to the class of transmissible spongiform encephalopathies (TSE), which also includes bovine spongiform encephalopathy (BSE). Three polymorphisms in amino acid codons 136 (A/V), 154 (R/H), and 171 (R/Q/H) of the *PRPN* gene are associated with resistance or susceptibility of sheep to classical scrapie. Depending on the *PRNP* genotype, there are five classes of genetic sustainability to Scrapie (G1-G5). The ARR allele is desirable regarding the resistance to scrapie. However, discovery of atypical scrapie (Nor98) showed a possibility of transmitting BSE to animals of different sustainability classes, including G1 (ARR/ARR genotype). It is shown, that L/F amino acid substitution at position 141 provides resistance to atypical scrapie. The aim of our work was to study the allele pool of the Romanov sheep by the *PRNP* gene, associated with sustainability to both classical and atypical forms of scrapie. The material for the work was tissue samples of 364 clinically healthy Romanov animals including three modern populations of the Yaroslavl region and one population introduced for breeding in the Kamchatka. Genomic DNA was isolated using the Nexttec columns (Nexttec Biotechnologie GmbH, Germany). Identification of the alleles in the codons 136 (A/T/V), 141 (L/F), 154 (R/H) and 171 (Q/R/H/K) was performed by pyrosequencing on the PSQ96MA device (Quiagen, USA). We found four alleles, 136/154/171 — ARR, ARQ, AHQ and VRQ, and nine haplotypes of PRNP as ARR/ARR, ARR/ARQ, ARR/AHQ, ARQ/ARQ, AHQ/ARQ, AHQ/AHQ, ARR/VRQ, VRQ/AHQ and ARQ/VRQ, relating to all five classes of genetic sustainability to the classical Scrapie. The allele of wild type ARQ (the frequency from 0.704 to 0.933) and the genotype ARQ/ARQ (sustainability class G3) were the prevalent. In all the studied groups, a desirable ARR allele was identified with frequencies varied from 0.022 to 0.089 and averaged 0.066. The undesirable VRQ allele was found in three of the four groups, while its frequency was relatively low — from 0.011 to 0.022. The study of the *PRNP* polymorphism by four codons 136/141/154/171 revealed the presence of five different alleles — ALRR, ALRQ, ALHQ, VLRQ, AFRQ and ten genotypes. We detected an animal carrying a sensitive to the atypical scrapie allele F at position 141 of *PRNP* (genotype VLRQ/AFRQ) with the allele frequency of 0.001. The

results will be applied in the development of breeding programs for Romanovs, as well as in strategic planning of conservation of the genetic diversity of this unique Russian Northern short-tailed sheep.

Keywords: prion protein gene (*PRNP*), allele pool, the Romanov sheep, genetic sustainability, scrapie

The relevant objective of modern biological science is conservation of a unique genetic pool of Russian autochthone breeds with their harmonious integration into the selection process, considering world trends in animal husbandry [1-3]. Livestock products obtained from such breeds are of interest for nutritional and process industries. Romanov is unique Russian authentic sheep breed, representing one of the offspring of northern short-tailed sheep [4, 5]. The originality of the Romanov breed is attributable to the combination of unsurpassed wool sheepskins qualities with phenomenal fecundity (up to 10 lambs) and polyestricity [6]. History of breed creation originates from the XVII century, it is first mentioned in 1802 [6]. A hundred years ago, coarse-wool sheep breeding, which was conserved in seminatural peasant farms, not only fill the peasants' needs in wool and meat, but also gave products for export [7]. At the beginning of the last century, recognized zootechnical scientist P.N. Kuleshov, who was concerned about the reducing number of sheep population in Russia, suggested that sheep areas should be outlined, among which the "sheep region of the short-tailed sheep with Romanov's in the center" [5] was placed first. At present, the gene pool of Romanov sheep is considered as an important reserve for the creation of new herds, lines, types of multiparous sheep for different use.

Transmissible spongiform encephalopathy of sheep, also known as scrapie, is one of the diseases which can cause serious economic damage to sheep breeding. Scrapie is a fatal neurodegenerative disease that affects sheep and goats, and relates to the transmissible spongiform encephalopathies (TSEs). This group also includes bovine spongiform encephalopathy, BSE. The cause of TSE is the presence of infectious pathogens, prions, which do not have nucleic acids and, apparently, are composed entirely of modified protein (PrPSc). Normal cellular PrP (PrP^C) is converted into PrP^{Sc} through a post-translational process, which results in a high content of β-sheets [8]. It has been established that the scrapie resistance in sheep is mainly conditioned by polymorphism of prion protein gene *PRNP* which encodes the normal PrP^C. Three mutations in amino acid codons 136 (A/V), 154 (R/H) and 171 (R/Q/H) are associated with resistance or sheep susceptibility to classical scrapie [9-13].

Five amino acids (AA) encoded by three notable codons 136/154/171 explain the formation of 15 possible genotypes on *PRNP* [14, 15]. The haplotype $A^{136}R^{154}R^{171}$, marked as ARR, is desirable in terms of sustainability to scrapie. Depending on *PRNP* genotype, there are five classes of genetic sustainability to scrapie, from G1 to G5 according to the sustainability decreasing. The most preferred genotype ARR/ARR is related to the G1 class. In the past 25 years, among thousands of genotyped G1 sheep, no cases of classic scrapie have been registered [16, 17]. However, the successful transfer of BSE prions to sheep of the ARR/ARR genotype by intracerebral inoculation showed that the resistance of this genotype toward the TSE agents was not absolute [18]. Two cases of encephalopathy in sheep of ARR/ARR genotype, with clinical symptoms similar to classical scrapie were diagnosed in Gearmany [15].

Animals of ARR/AHQ, ARR/ARH, and ARR/ARQ genotypes are genetically resistant to scrapie. In order to avoid susceptibility of progeny to this disease, they can be used only under controlled combinations of parents (G2). Individuals with genotypes ARQ/ARQ (wild type), ARQ/ARH, ARQ/AHQ, AHQ/AHQ, ARH/ARH and AHQ/ARH (G3) have low genetic resistance, but, when mating with G1 animals, produce resistant offspring. The genotypes

ARR/VRQ (G4) and VRQ/AHQ, VRQ/ARH, VRQ/ARQ, VRQ/VRQ (G5) are susceptible to scrapie and should be excluded from reproduction. It has been shown that other amino acid polymorphisms may influence the resistance to prion protein and BSE, in particular at positions 101, 112, 143, 172, 175 and 176, most of which show frequencies lower than 5 % [19]. Octapeptide-repeat polymorphism, that is, different number of repeated N-end sequence of eight amino acids, P(Q/H)GGGWGQ, was reported. The repeat number in cattle, sheep and goats varies both between and within species, ranging from two to five [20, 21]. Polymorphisms of *PRNP* in three positions in the promoter region (C5354A, T5382C and C5622G) were revealed, in this, two latter polymorphisms can affect significantly the transcription factors [22, 23].

The discovery of so-called atypical scrapie showed the possibility of transmitting BSE to animals of different resistance classes, including G1 (ARR/ARR genotype). For the first time, an atypical scrapie was found in Norwegian sheep in 2003 and was named Nor98 [24]. High incidence of atypical scrapie was further identified in Germany and France [25]. In 2005, in Great Britain, 37 % of scrapie-affected sheep was accounted for atypical cases [26]. Atypical and classical scrapie forms differ in number of important features. Atypical cases are characteristic for a later age (4 years and older), often in the infected herd only a few scrapie-positive sheep can be identified [27]. As compared to the classic scrapie, an atypical form only in rare cases (or not at all) can cause neuropil vacuolation or the presence of immunohistochemically detectable PrP^{Sc} in brain [24]. Abnormal PrP in atypical cases is characterized by higher sensitivity to enzymatic cleavage in comparison to the classical form [28]. It is shown that at Nor98 atypical infection, the role of the fourth amino acid in the AA codons 136/141/154/172 at position 141 (L/F) increases. Most cases of atypical scrapie had been identified among animals with low sensitivity to classical scrapie (classes G1-G3) [28-30].

With the detection of strong genetic resistance of the certain sheep *PRNP* genotypes to classical scrapie and the identification of the nature of atypical scrapie [24], it became possible to use *PRNP* polymorphism as an additional criterion in breeding programs for achieving a balance between genetic diversity of populations and prevention of scrapie diseases.

A study on the limited sample of Romanov sheep showed a low genetic resistance to the classical scrapie [31]. However, the surveyed herds were mostly of secondary origin and were formed based on a limited number of lines. Consequently, the obtained results do not allow us to characterize the breed allele pool. To date, resistance of Romanov sheep to the atypical scrapie has not been studied.

Animals from the Yaroslavl gene pool herd can be the most vivid model reflecting the entire genetic diversity of the Romanov breed allele pool. For verification of the hypothesis that the allele pool of the secondarily naturalized herds of this breed, formed by delivery of limited number of lines and undergone selection pressure of other factors, is not typical for the breed as a whole, these sheep must be studied.

This is the first report on genetic polymorphism of the Romanov sheep (*Ovis aries*) populations in the AA position 141 of the *PRNP* gene and their susceptibility to the atypical prion protein Nor98.

The aim of this paper was to study the allele pool of Romanov sheep on prion protein gene, which is associated with resistance to classical and atypical scrapie, on the historical territories of the breed origin and in the places of its secondary naturalization.

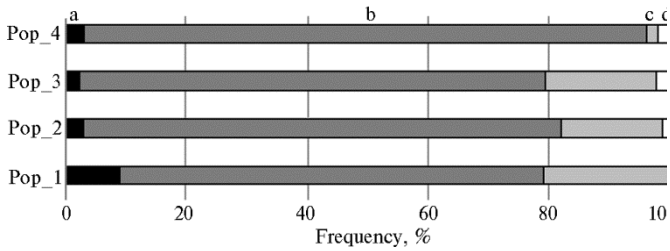
Techniques. The biomaterial was tissue samples (ear biopsy, blood) of 364

pre-healthy sheep of Romanov breed archived in 2013-2016. There were three populations from the Yaroslavl region, Pop_1 (n = 16, Avangard LLC), Pop_2 (n = 98, Agrofima Farmer LLC) and Pop_3 (n = 46, Zarechie LLC) and one population introduced for breeding in the Kamchatka Territory, Pop_4 (n = 52, OAO Kamchatagropromservis).

DNA was extracted using Nexttec columns (Nexttec GmbH, Germany) in accordance with the manufacturer's recommendations. The PCR was performed by the method of N.A. Zinovieva et al. [32]. The reactions were carried out according to the protocol of E.A. Gladyr et al. [33]. The alleles in the codons 136 (A/T/V), 141 (L/F), 154 (R/H) and 171 (Q/R/H/K) was identified by pyrosequencing on a PSQ96MA device (Qiagen, USA).

The data statistical processing was carried out using PSQ96MA SNP Software v.2.0, Microsoft Excel, and GenAlEx 6.501.

Results. Genotyping showed the presence in Romanov sheep of four haplotypes (ARR, ARQ, AHQ, VRQ) and nine genotypes on *PRNP* (ARR/ARR, ARR/ARQ, ARR/AHQ, ARQ/ARQ, AHQ/ARQ, AHQ/AHQ, ARR/VRQ, VRQ/AHQ, ARQ/VRQ) of all five cases of genetic resistance to classical scrapie. At the same time, there were differences in the distribution of haplotypes and genotypes between groups. The wild type ARQ haplotype was the most common with the frequency of occurrence from 0.704 in Pop_1 to 0.933 in Pop_4. In all groups, there was a desired ARR haplotype the frequency of which ranged from 0.022 in Pop_3 to 0.089 in Pop_1 and averaged 0.066. The undesired haplotype VRQ was found in three of the four groups except of Pop_1, at relatively low frequency, from 0.011 (Pop_2) to 0.022 (Pop_3) (Fig.).



Distribution of *PRNP* haplotypes ARR (a)**, ARQ** (b)**, AHQ** (c)**, and VRQ** (d) **associated with resistance to classical scrapie in Romanov sheep populations:** Pop_1 from Avangard LLC, Pop_2 from Agrofima Farmer LLC, Pop_3 from Zarechie LLC (Yaroslavl Province); Pop_4 from OAO Kamchatagropromservis (Kamchatka Territory).

The wild type ARQ/ARQ genotype of class G3 was the most frequent in all the populations (Table). Probably, it is due to the long-term pure breeding aimed solely to affirm unique adaptive and productive qualities. The frequency of ARR/ARR genotype which is the most resistant to the classic scrapie, was very low. A portion of animals carrying G2 genotypes differed 8 times and more between herds even within the same region. Apparently, it was related to the predominant use of ram producers of different lines.

In general, the studied Romanov sheep was characterized by relatively low frequencies of genotypes of the genetic classes G4 and G5 which cause a high risk of classic scrapie. Such genotypes were detected in three of four surveyed populations with a frequency of 2.2 to 4.1 %

We were the first to estimate the genetic status of the Romanov breed in terms of predisposition to the infection with atypical prion protein Nor98. The assay identified five different haplotypes, ALRR, ALRQ, ALHQ, VLRQ and AFRQ, and ten genotypes on *PRNP* (136/141/154/171), ALRR/ALRR, ALRR/ALRQ, ALRR/ALHQ, ALRQ/ALRQ, ALHQ/ALRQ, ALHQ/ALHQ, ALRR/VLRQ, VLRQ/ALHQ, VLRQ/ALRQ and VLRQ/AFRQ. The frequency of haplotypes and genotypes for four codons mostly had profiles which were the same as those for codons 136/154/171. Only in Pop_4, one animal carried allele F associated with sensitivity to atypical scrapie (VLRQ/AFRQ genotype) at position

141 of *PRNP*, so that this allele frequency was 0.001. It is important to underline that allele F^{141} was identified in the genotype VRQ/ARQ (class G5) in combination with the haplotype VRQ, the most susceptible to the classical scrapie.

Genotype distribution on prion protein gene *PRNP* (G) and on the classes of resistance to classical scrapie (C) in Romanov sheep

Genotype 136/154/171	Frequency									
	Pop_1		Pop_2		Pop_3		Pop_4		В среднем	
	G	C	G	C	G	C	G	C	G	C
					Class G1					
ARR/ARR	–	–	0.010	0.010	–	–	–	–	0.003	0.003
					Class G2					
ARR/ARQ	0.143	0.208	0.061	0.061	0.022	0.022	0.058	0.058	0.093	0.123
ARR/AHQ	0.066		–		–		–		0.030	
					Class G3					
ARQ/ARQ	0.446	0.792	0.592	0.888	0.609	0.934	0.865	0.904	0.566	0.852
AHQ/ARQ	0.316		0.265		0.282		0.039		0.258	
AHQ/AHQ	0.030		0.031		0.043		–		0.028	
					Class G4					
ARR/VRQ	–	–	–	–	0.022	0.022	–	–	0.003	0.003
					Class G5					
VRQ/AHQ	–	–	0.031	0.041	–	0.022	–	0.038	0.008	0.019
VRQ/ARQ	–		0,010		0,022		0,038		0,011	

Note. For description of populations, read *Techniques* section. Dashes mean the absence of the genotype in the populations.

As known, the susceptibility of sheep to classical scrapie directly depends on certain non-synonymous single nucleotide polymorphisms within prion protein gene *PRNP* located on chromosome 13 [34-36]. Modern molecular technologies make it possible to study genotype in the first days of animal's life and in order to early control over the spread of hereditary defects. Conservation, use and development of gene pools of local breeds for their effective integration in modern livestock husbandry with a view to obtain new breeding forms and to increase the range of native breeds are also in progress. The aim of most sheep breeding programs is to monitor a breed gene pool by gradually replacing genotypes which are characterized by hyper susceptibility to scrapie, using rams of ARR/ARR genotypes. Accumulation of the haplotype ARR in the breeds and populations is necessary for preventive protection against the classical pathogenic prion that causes scrapie. This fact is confirmed by research carried out in Canada in 2008-2012 on 184 Romanov sheep, which showed the plasticity of the breed gene pool and made it possible to bring the frequency of ARR haplotype and ARR/ARR genotype to 0.592 and 0.359, respectively, by selection [37]. At the same time, the high frequency of wild haplotype in aboriginal and domesticated breeds, which was noted in some papers [38, 39], remains an actual problem.

Thus, our data made it possible to evaluate the genetic resistance to classical and atypical scrapie in allele pool of Romanov sheep bred in the territories of historical origin and in the locations of introduction. The average frequency of undesirable $V^{136}R^{154}Q^{171}$ haplotype and F^{141} allele associated with susceptibility to atypical scrapie was 0.011 and 0.001, respectively, that is a positive factor for the further improvement and conservation of Romanov sheep breed. In the studied populations, a clear shortage of animals with G1 genotype was revealed and the frequency of haplotype ARR resistant to classic scrapie averaged 0.066. This creates the prerequisites for strategic breeding programs to accumulate genotypes resistant to pathogenic prion in the gene pool of Romanov sheep breed. Breeding for genetic resistance to scrapie and increasing proportion of the carriers of ARR allele and ARR/ARR genotype are necessary to prevent classical and atypical scrapie in Russian populations. The allele pool of the entire Romanov breeding stock must be studied to estimate genetic diversity of this unique northern Russian sheep.

REFERENCES

1. Ernst L.K., Zinov'eva N.A. *Biologicheskie problemy zhivotnovodstva v XXI veke* [Challenges in biology of XXI century]. Moscow, 2008 (in Russ.).
2. Stolpovskii Yu.A. Concept and principles of genetic monitoring for the purpose of preservation in situ of domestical animals kinds. *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2010, 6: 3-8 (in Russ.).
3. Deniskova T.E., Selionova M.I., Gladyr' E.A., Dotsev A.V., Bobrysheva G.T., Kostyunina O.V., Brem G., Zinovieva N.A. Variability of microsatellites in sheep breeds raced in Russia. *Agricultural Biology*, 2016, 51(6): 801-810 (doi: 10.15389/agrobiology.2016.6.801eng) (in Engl.).
4. Ivanov M.F. *Ovtsevodstvo* [Sheep farming]. Moscow, 1933 (in Russ.).
5. Kuleshov P.N. *Ovtsevodstvo Rossii* [Sheep breeding in Russia]. Petrograd, 1916 (in Russ.).
6. Ernst L.K., Dmitriev N.G., Paronyan I.A. *Geneticheskie resursy sel'skokhozyaistvennykh zhivotnykh v Rossii i sopredel'nykh stranakh* [Gene pool of farm animals in Russia and neighboring countries]. St. Petersburg, 1994 (in Russ.).
7. Dankvert A.G., Dankvert S.A. *Istoriya plemennogo zhivotnovodstva Rossii* [History of pedigree animal breeding in Russia]. Moscow, 2004 (in Russ.).
8. Prusiner S.B. Prions. *PNAS*, 1998, 95(23): 13363-13383.
9. Hunter N., Goldmann W., Foster J. D., Cairns D., Smith G. Natural scrapie and PrP genotype: case-control studies in British sheep. *Vet. Rec.*, 1997, 141: 137-140 (doi: 10.1136/vr.141.6.137).
10. Smits M.A., Barillet F., Harders F., Boscher M.Y., Vellema P., Aguerre X., Hellinga M., McLean A.R., Baylis M., Elsen J.M. Genetics of scrapie susceptibility and selection for resistance. *Proc. 51st Annual Meeting of the European Association for Animal Production*. The Hague, Netherlands, 2000: Paper S.4.4.
11. Bossers A., Belt P.B.G.M., Raymond G.J., Caughey B., de Vries R., Smits M.A. Scrapie susceptibility-linked polymorphisms modulate the in vitro conversion of sheep prion protein to protease-resistant forms. *PNAS*, 1997, 94(10): 4931-4936 (doi: 10.1073/pnas.94.10.4931).
12. Ishiguro N., Shinagawa M., Onoe S., Yamanouchi K., Saito T. Rapid analysis of allelic variants of the sheep *PrP* gene by oligonucleotide probes. *Microbiol. Immunol.*, 1998, 42: 579-582 (doi: 10.1111/j.1348-0421.1998.tb02327.x).
13. Buschmann A., Biacabe A.G., Ziegler U., Bencsik A., Madec J.Y., Erhardt G. Atypical scrapie cases in Germany and France are identified by discrepant reaction patterns in BSE rapid tests. *J. Virol. Methods*, 2004, 117: 27-36 (doi: 10.1016/j.jviromet.2003.11.017).
14. *DEFRA. National scrapie plan for great britain*. London, 2001: 1-28.
15. Groschup M.H., Lacroux C., Buschmann F., Luhken G., Mathey J., Eiden M., Lugan S., Hoffmann C., Espinosa J.C., Baron T., Torres J.M., Erhardt G., Andreoletti O. Classic scrapie in sheep with the ARR/ARR prion genotype in Germany and France. *Emerg. Infect. Dis.*, 2007, 13(8): 1201-1207 (doi: 10.3201/eid1308.070077).
16. Renaville R. *Résistance génétique a la «Tremblante» (aussi appelée scrapie) chez le mouton*. FIOW Gembloux, Belgique, 2002.
17. Vaccari G., Di Bari M.A., Morelli L., Nonno R. Chiappini B., Antonucci G., Marcon S., Esposito E., Fazzi, P., Palazzini N., Troiano P., Petrella A., Guardo G.-D., Agrimi U. Identification of an allelic variant of the goat *PrP* gene associated with resistance to scrapie. *J. Gen. Virol.*, 2006, 87: 1395-1402 (doi: 10.1099/vir.0.81485-0).
18. Houston F., Goldmann W., Chong A., Jeffrey M., Gonzalez L., Foster J. Prion diseases: BSE in sheep bred for resistance to infection. *Nature*, 2003, 423: 498 (doi: 10.1038/423498a).
19. Acín C., Martín-Burriel I., Goldmann W., Lyahyai J., Monzón M., Bolea R., Smith A., Rodellar C., Badiola J.J., Zaragoza P. Prion protein gene polymorphisms in healthy and scrapie-affected Spanish sheep. *J. Gen. Virol.*, 2004, 85: 2103-2110 (doi: 10.1099/vir.0.80047-0).
20. Goldmann W., Chong A., Foster J., Hope J., Hunter N. The shortest known prion protein gene allele occurs in goats, has only three octapeptide repeats and is non-pathogenic. *J. Gen. Virol.*, 1998, 79(12): 3173-3176 (doi: 10.1099/0022-1317-79-12-3173).
21. Schläpfer J., Saitbekova N., Gaillard C., Dolf G. A new allelic variant in the bovine prion protein gene (*PRNP*) coding region. *Anim. Genet.*, 1999, 30(5): 386-387 (doi: 10.1046/j.1365-2052.1999.00526-5.x).
22. O'Neill G.T., Donnelly K., Marshall E., Cairns D., Goldmann W., Hunter N. Characterization of ovine *PrP* gene promoter activity in N2a neuroblastoma and ovine foetal brain cell lines. *J. Anim. Breed. Genet.*, 2003, 120(2): 114-123 (doi: 10.1046/j.1439-0388.2003.00381.x).
23. O'Neill G.T., Cairns D., Toovey L., Goldmann W., Hunter N. New ovine PrP gene haplotypes as a result of single nucleotide polymorphisms in the *PrP* gene promoter. *J. Anim. Breed. Genet.*, 2005, 122(2): 86-94 (doi: 10.1111/j.1439-0388.2005.00520.x).
24. Benestad S.L., Sarradin P., Thu B., Schönheit J., Tranulis M.A.,

Bratberg B. Cases of scrapie with unusual features in Norway and designation of a new type, Nor98. *Vet. Rec.*, 2003, 153: 202-208 (doi: 10.1136/vr.153.7.202).
25. Buschmann A., Biacabe A.G., Ziegler U., Bencsik A., Madec J.Y., Erhardt G., Luhken G., Baron T., Groschup M.H. Atypical scrapie cases in Germany and France are identified by discrepant reaction patterns in BSE rapid tests. *J. Virol. Methods*, 2004, 117: 27-36 (doi: 10.1016/j.jviromet.2003.11.017).
26. Everest S.J., Thorne L., Barnicle D.A., Edwards J.C., Elliott H., Jackman R., Hope J. Atypical prion protein in sheep brain collected during the British scrapie-surveillance programme. *J. Gen. Virol.*, 2006, 87(2): 471-477 (doi: 10.1099/vir.0.81539-0).
27. Luhken G., Buschmann A., Brandt H., Eiden M., Groschup M.H., Erhardt G. Epidemiological and genetical differences between classical and atypical scrapie cases. *Vet. Res.*, 2007, 38: 65-80 (doi: 10.1051/vetres:2006046).
28. Le Dur A., Béringue V., Andréoletti O., Reine F., Lan Laï T., Baron T., Bratberg B., Vilotte J.-L., Sarradin P., Benestad S.L., Laude H. A newly identified type of scrapie agent can naturally infect sheep with resistant PrP genotypes. *PNAS*, 2005, 102(44): 16031-16036 (doi: 10.1073/pnas.0502296102).
29. Langeveld J.P.M., Jacobs J.G., Hunter N., van Keulen L.J.M., Lantier F., van Zijderveld F.G., Bossers A. Prion type-dependent deposition of *PRNP* allelic products in heterozygous sheep. *J. Virol.*, 2016, 90(2): 805-812 (doi: 10.1128/JVI.02316-15).
30. Gonzalez L., Jeffrey M., Dagleish M.P., Goldmann W., Siso S., Eaton S.L., Martin S., Finlayson J., Stewart P., Steele P., Pang Y., Hamilton S., Reid H.W., Chianini F. Susceptibility to scrapie and disease phenotype in sheep: cross-PRNP genotype experimental transmissions with natural sources. *Vet. Res.*, 2012, 43: 55 (doi: 10.1186/1297-9716-43-55).
31. Gladyr' E.A., Zinov'eva N.A., Burylova S.S., Selionova M.I., Moiseikina L.G., Ernst L.K., Brem G. *Dostizheniya nauki i tekhniki APK*, 2012, 11: 34-37 (in Russ.).
32. Zinov'eva N.A., Popov A.N., Ernst L.K., Marzanov N.S., Bochkarev V.V., Strekozov N.I., Brem G. *Metodicheskie rekomendatsii po ispol'zovaniyu metoda polimeraznoi tsepnoi reaktsii v zhivotnovodstve* [PCR in livestock farming. Manual guid].Dubrovitsy, 1998 (in Russ.).
33. Gladyr' E.A., Zinov'eva N.A., Brem G. *Sposob genodiagnostiki ustoichivosti ovets k skrepi. A.S. 2303067 (RF) MPK C12N 15/00, C12N 15/12. FGBNU FNTS VIZH im. L.K. Ernsta (Vserossiiskii gosudarstvennyi nauchno-issledovatel'skii institut zhivotnovodstva (VIZH) (RF). № 2005128754/13. Zayavl. 16.05.2005. Opubl. 20.07.2007. Byul. № 20* [Gene typing of sheep resistance to scrapie. Patent RF № 2005128754/13. Appl. 16.05.2005. Publ. 20.07.2007. Bul. № 20] (in Russ.).
34. Belt P.B.G.M., Muileman I.H., Schreuder B.E.C., Bos-de Ruijter J., Gielkens A.L., Smits M.A. Identification of five allelic variants of the sheep *PrP* gene and their association with natural scrapie. *J. Gen. Virol.*, 1995, 76: 509-517 (doi: 10.1099/0022-1317-76-3-509).
35. Goldmann W., Hunte N., Benson G., Foster J.D., Hope J. Different scrapie-associated fibril proteins (PrP) are encoded by lines of sheep selected for different alleles of the *Sip* gene. *J. Gen. Virol.*, 1991, 72: 2411-2417 (doi: 10.1099/0022-1317-72-10-2411).
36. Hunter N., Goldmann W., Smith G., Hope J. The association of a codon 136 *PrP* gene variant with the occurrence of natural scrapie. *Arch. Virol.*, 1994, 137: 171-177 (doi: 10.1007/BF01311184).
37. Cameron S., Bell-Rogers P., McDowall R., Rebelo A.R., Cai H.Y. Prion protein genotypes of sheep as determined from 3343 samples submitted from Ontario and other provinces of Canada from 2005 to 2012. *Can. J. Vet. Res.*, 2014, 78: 260-266.
38. Otelea M.R., Zaulet M.M., Dudu A., Otelea F., Baraitareanu S., Danes D. The scrapie genetic susceptibility of some sheep breeds in southeast Romanian area and genotype profiles of sheep scrapie infected. *Rom. Biotech. Lett.*, 2011, 16(4): 6419-6429.
39. Garcia-Crespo D., Juste RA, Hurtado A. Selection of ovine housekeeping genes for normalisation by real-time RT-PCR; analysis of *PrP* gene expression and genetic susceptibility to scrapie. *BMC Vet. Res.*, 2005, 1: 3 (doi: 10.1186/1746-6148-1-3).