UDC 619:615.9:636.085.19:543.9
DOI: 10.29326/2304-196X-2020-1-32-60-65

Mycotoxicological monitoring. Part 1. Complete mixed feed for pigs and poultry (2009–2018)

G. P. Kononenko[1], A. A. Burkin[2], Ye. V. Zotova[3]

All-Russia Research Institute of Veterinary Sanitation, Hygiene and Ecology – Branch of the Federal State-Financed Scientific Institution "Federal Scientific Centre – All-Russian Research Institute of Experimental Veterinary Medicine named after K. I. Scryabin and Ya. R. Kovalenko of the Russian Academy of Sciences", Moscow, Russia

[1] *ORCID 0000-0002-9144-615X, e-mail: kononenkogp@mail.ru*

[2] *ORCID 0000-0002-5674-2818, e-mail: aaburkin@mail.ru*

[3] *ORCID 0000-0002-1479-8602, e-mail: ezotova63@gmail.com*

SUMMARY

Results of the ten-year annual mycotoxicological testing of complete mixed feeds for pigs and poultry submitted by holdings and processing establishments located in the Northwestern, Central, Southern, Volga and Ural Federal Districts are presented. Competitive ELISA tests showed that the occurrence of T-2 toxin, deoxynivalenol, zearalenone, fumonisins of group B, alternariol, ochratoxin A, citrinin, mycophenolic acid, ergot alkaloids and emodin was about 5% and quantities thereof varied within one or three orders; quantities of T-2 toxin, deoxynivalenol, zearalenone, fumonisins, and ochratoxin A might exceed maximum admissible levels for feed grains. Diacetoxyscirpenol, aflatoxin B_1, sterigmatocystin and cyclopiazonic acid belonged to group of rare contaminants. Level of feed contamination with T-2 toxin and emodin was found to be consistently high during the said period; in some of the years occurrence of deoxynivalenol, fumonisins as well as ochratoxin A, citrinin, mycophenolic acid and ergot alkaloids increased. In 2016–2018, mixed feed contamination with alternariol increased whereas contamination with fumonisins steadily decreased and level of zearalenone occurrence remained consistently low. Evidence for a wide occurrence of emodin known as "diarrheic factor" as well as for sporadic increase in mixed feed contamination with alternariol, citrinin, mycophenolic acid, mycotoxins having the highly dangerous toxic impact and long-term adverse effects, was detected for the first time. These data confirmed the need for their inclusion into the regulated group of substances significant for public health. General features of pig and poultry feed contamination as well as usefulness of regional surveys for intoxication risk prediction are described. Special attention is paid to the importance of the projects for creation of common information resources that could become a unique scientific basis for innovations in feed poisoning prevention. Original monitoring data systematized and summarized in the paper are given in electronic format in section Additional materials.

Key words: complete mixed feed, mycotoxins, monitoring, enzyme-linked immunosorbent assay (ELISA).

For citation: Kononenko G. P., Burkin A. A., Zotova Ye. V. Mycotoxicological monotoring. Part 1. Complete mixed feed for pig and poultry (2009–2018). *Veterinary Science Today*. 2020; 1 (32): 60–65. DOI: 10.29326/2304-196X-2020-1-32-60-65.

Conflict of interest. The authors declare no conflict of interest.

For correspondence: Galina P. Kononenko, Doctor of Science (Biology), Professor, Head of Laboratory for Mycotoxicology and Feed Hygiene, ARRIVSHE – Branch of the FSFSI FSC ARRIEVM RAS, 123022, Russia, Moscow, Zvenigorodskoe shosse, 5; *e-mail: kononenkogp@mail.ru.*

УДК 619:615.9:636.085.19:543.9

Микотоксикологический мониторинг. Сообщение 1. Полнорационные комбикорма для свиней и птицы (2009–2018 гг.)

Г. П. Кононенко[1], А. А. Буркин[2], Е. В. Зотова[3]

Всероссийский научно-исследовательский институт ветеринарной санитарии, гигиены и экологии – филиал Федерального государственного бюджетного научного учреждения «Федеральный научный центр – Всероссийский научно-исследовательский институт экспериментальной ветеринарии имени К. И. Скрябина и Я. Р. Коваленко Российской академии наук» (ВНИИВСГЭ – филиал ФГБНУ ФНЦ ВИЭВ РАН), г. Москва, Россия

[1] *ORCID 0000-0002-9144-615X, e-mail: kononenkogp@mail.ru*

[2] *ORCID 0000-0002-5674-2818, e-mail: aaburkin@mail.ru*

[3] *ORCID 0000-0002-1479-8602, e-mail: ezotova63@gmail.com*

РЕЗЮМЕ

Представлены результаты 10-летнего ежегодного микотоксикологического обследования полнорационных комбикормов для свиней и сельскохозяйственной птицы, предоставленных хозяйствами и перерабатывающими предприятиями Северо-Западного, Центрального, Южного, Приволжского и Уральского федеральных округов. Методом конкурентного иммуноферментного анализа показано, что Т-2 токсин, дезоксиниваленол, зеараленон, фумонизины группы В, альтернариол, охратоксин А, цитринин, микофеноловая кислота, эргоалкалоиды и эмодин встречаются с частотой более 5%, их количества варьируют в пределах одного-трех порядков, и количества Т-2 токсина, дезоксиниваленола, зеараленона, фумонизинов и охратоксина А могут превышать регламенты допустимого содержания в зерне, предназначенном на кормовые цели. Диацетоксисцирпенол, афлатоксин B_1, стеригматоцистин и циклопиазоновая кислота относятся к группе редких контаминантов. Установлено, что высокая загрязненность комбикормов Т-2 токсином и эмодином сохраняет по годам устойчивый характер, в отдельные годы возрастает встречаемость дезоксиниваленола, фумонизинов, а также охратоксина А, цитринина, микофеноловой кислоты и эргоалкалоидов. В 2016–2018 гг. отмечено обострение ситуации по загрязненности комбикормов альтернариолом при положительной тенденции снижения контаминации фумонизинами и сохранения стабильно низкой встречаемости зеараленона. Факт обширной распространенности эмодина, известного как «диарейный фактор», а также спорадического, в отдельные периоды наблюдений, возрастания контаминации комбикормов альтернариолом, цитринином и микофеноловой кислотой – микотоксинами с особо опасными формами токсического действия и негативными отдаленными последствиями – выявлен впервые. Эта информация подтверждает необходимость их введения в группу нормируемых санитарно-значимых показателей. Обсуждаются общие черты контаминации комбикормов для свиней и птицы, а также целесообразность региональных обследований для прогнозирования рисков развития интоксикаций. Особое внимание уделяется актуальности выполнения проектов, направленных на формирование единых информационных ресурсов, которые могут стать уникальной научной базой для инноваций в сфере профилактики кормовых отравлений. Исходные данные мониторинга, систематизированные и обобщенные в данной работе, представлены в электронном виде в разделе «Дополнительные материалы».

Ключевые слова: полнорационные комбикорма, микотоксины, мониторинг, иммуноферментный анализ.

Для цитирования: Кононенко Г. П., Буркин А. А., Зотова Е. В. Микотоксикологический мониторинг. Сообщение 1. Полнорационные комбикорма для свиней и птицы (2009–2018 гг.). *Ветеринария сегодня*. 2020; 1 (32): 60–65. DOI: 10.29326/2304-196X-2020-1-32-60-65.

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Кононенко Галина Пантелеевна, доктор биологических наук, профессор, заведующий лабораторией микотоксикологии и санитарии кормов ВНИИВСГЭ – филиал ФГБНУ ФНЦ ВИЭВ РАН, 123022, Россия, г. Москва, Звенигородское шоссе, д. 5, *e-mail: kononenkogp@mail.ru.*

INTRODUCTION

Feed safety plays a crucial role in successful animal farming and processing industry development. Various responses of livestock sex/age groups and species to natural and anthropogenic factors as well as various diet compositions make feed material control extremely difficult. Completeness of the data on expected toxicants for each type of feed (their occurrence and levels) is a prerequisite for solving the said problem.

In our country, there is a long overdue need for the projects aimed at creation of common information resources that could become a unique scientific basis for innovations in feed poisoning prevention as well as top-requested reference manual for specialists engaged in feed production and animal feeding. Implementation of such projects requires establishment of principles that will be mandatory for the project participants as well as selection of optimal data entry system convenient for data storage, replenishment and multi-purpose use.

Complete mixed feed is a basis for modern pig and poultry industries. Summarizing and updating data on feed contamination with mycotoxins is one of the key problems remained unsolved. Creation of common information resource containing data on mycotoxicological tests of feed was firstly discussed at the 3rd Congress of Russian Toxicologists in 2008[1]. Feed and agricultural product monitoring is arranged and carried out by the Rosselkhoznadzor but access to the monitoring results is limited and data published in scientific-practical and scientific-production periodicals, Congress and Conference Proceedings are associated with small samplings often without any indications of tested sample number and origin or presented after being processed in accordance with conditional criteria [1, 2]. This impedes or makes data summarizing and free use impossible.

Recently, scientific periodicals have actively applied the experience gained in creation of replenishable electronic databases, for example, microorganism genotype collections [3]. A series of publications summarizing data of the analytical studies performed based on common approach that allows users to process the said data in accordance with their objectives could be an initial stage of the project for creation of mycotoxicological monitoring information resource in our country.

The goal of the paper is to summarize results of tests of complete mixed feed for pigs and poultry for mycotoxin contamination for 2009–2018 and provide original data contained in the electronic database.

MATERIALS AND METHODS

Average samples (1,338 samples) from complete mixed feed batches intended for various sex/age animal groups including SK-type feed for pigs (1,075 samples) and PK-type feed for poultry (263 samples), submitted to the laboratory by specialists of feed mills and veterinarians of pig and poultry establishments located in the Northwestern, Central, Southern, Volga and Ural Federal Districts, predominantly from the areas where such industries were intensively developed for the purpose of routine control, incoming control and diagnosis of mycotoxicoses in 2009–2018.

Group of target mycotoxins included T-2 toxin (T-2), diacetoxyscirpenol (DAS), deoxynivalenol (DON), zearalenone (ZEN), fumonisins of B group (FUM), alternariol (AOL), ochratoxin A (OA), citrinin (CIT), aflatoxin B_1 (AB_1), cyclopiazonic acid (CPA), mycophenolic acid (MPA), ergot alkaloids (EA) and emodin (EMO).

Samples preparation was carried out using unified method based on liquid extraction and indirect

[1] Burkin A. A., Kononenko G. P. Monitoring test methods for risk assessment of acute and chronic mycotoxicoses occurrence. *3rd Congress of Russian Toxicologists: Abstracts (December 2–5, 2008).* Ed. By G. G. Onishenko, B. A. Kurlyandsky. M.; 2008; 71–73. (in Russian)

Table 1
Mycotoxins in complete mixed feed for pigs (summarized data for 2009–2018)

Таблица 1
Микотоксины в полнорационных комбикормах для свиней (обобщенные данные 2009–2018 гг.)

Toxin	Occurrence n^+/n (%)	Level, µg/kg				
		Range		mean	median	90% percentile
		min	max			
T-2	949/1,075 (88.3)	4	500	31	20	66
DON	411/1,075 (38.2)	39	1,580	198	100	400
DAS	5/1,075 (0.5)	32	250	89	51	175.2
ZEN	125/1,075 (11.6)	18	151	36	26	64.6
FUM	277/1,043 (26.6)	40	6,300	421	190	975
AOL	377/1,043 (36.2)	20	998	61	38	106.6
OA	294/1,051 (30.0)	4	105	10	6	20
CIT	120/1,050 (11.4)	20	250	43	32	71
AB_1	0/1,051 (–)	–	–	–	–	–
STE	14/1,042 (1.3)	4	177	22	7	27.2
CPA	5/1,042 (0.5)	59	148	89	74	128.8
MPA	88/1,042 (8.5)	10	5,400	99	26	64.3
EA	135/1,043 (12.9)	5	3,970	92	14	65.8
EMO	760/915 (83.1)	20	1,255	78	51	151.3

n – number of tested samples;
n^+ – number of mycotoxin-containing samples.

competitive ELISA, validated in 1995–2008 and then included in official control methods[2]. Detection limits determined based on 85% antibody binding were as follows: 2 µg/kg (AB_1), 3 µg/kg (EA), 4 µg/kg (T-2, OA, STE), 20 µg/kg (ZEN, AOL, CIT, MPA, EMO) and 50 µg/kg (DAS, DON, FUM, CPA). The following coding pattern was used for filling-in record form in the database: mixed feed type (PK, SK), recorded year (1–10), detected mycotoxins.

Microsoft Excel 2016 and Statistica, Version 6 programmes were used for statistical processing including calculation of percentage of occurrence based on n^+/n ratio and following three values for positive samples – the arithmetical mean, the median and the 90% percentile.

RESULTS AND DISCUSSION

Thirteen out of fourteen target mycotoxins (except for AB_1) were detected in feed for pigs (Table 1). T-2 and EMO were detected more often than others, DAS, STE and CPA were detected in less than 5% of samples; detection rate for other mycotoxins varied from 8.5 to 38.2%. The quantity of toxins generally varied within one-two orders and in case of EA – within three orders. Median displacement to the smaller values as compared to the mean value was demonstrated for all contaminants that was indicative of unified dissymmetric distribution of contents where a half of accumulation levels was less than other ones. Moreover, the largest quantities were always higher than threshold concentrations for 90% of values (90% percentile), and the most strongly pronounced for MPA and EA. All this was indicative of possible abnormally high toxin accumulation levels in typical situations. The highest T-2, DON, ZEN, FUM and OA levels detected in mixed feed were higher than admissible levels for feed grains [4].

In poultry feed, AB_1 was detected in 1.1% of samples, DAS was not detected and therewith there was a clear similarity with pig feed in the T-2 and EMO prevalence, rare detection of STE and CPA, similar occurrence for other 10 mycotoxins – 9.5–47.9%, degree of variation in their levels and character of the median displacement to the mean values and 90% percentile to the highest levels (Table 2). Such similarity can be accounted for the common raw feed materials used for the feed formulas (wheat, barley, corn grains, sunflower meal and cake, soybean meal) as well as similar their proportions in the formulas despite of variations related to the animal category. The rate of fusariotoxins (T-2, DON, ZEN, FUM), AOL as well as OA, CIT, MPA, EA and EMO occurrence was more than 5% in complete mixed feed intended both for pigs and poultry.

Selective tests of mixed feed initiated by the ARRIVSHE in 1997 were described in the earlier publications as far as the data were gained. In 1997–2004 mycotoxin contamination level in 766 feed consignments used in commercial poultry industry was 34.6–79.5%, T-2 was the most prevalent in feed (38.5% of samples) at the level of 30–59 µg/kg,

[2] GOST 31653-2012 Feed. Immunoenzyme method for mycotoxin detection. M.: Standardinform; 2012. 11 p. (in Russian)

Table 2
Mycotoxins in complete mixed feed for poultry (summarized data for 2009–2018)

Таблица 2
Микотоксины в полнорационных комбикормах для сельскохозяйственной птицы (обобщенные данные 2009–2018 гг.)

Toxin	Occurrence n^+/n (%)	Level, µg/kg				
		range		mean value	median	90% percentile
		min	max			
T-2	208/263 (79.1)	4	280	25	12	63.3
DON	85/263 (32.3)	50	757	181	112	462.4
DAS	0/263 (–)	–	–	–	–	–
ZEN	45/263 (17.1)	20	334	37	25	54.6
FUM	95/263 (36.1)	50	5,000	350.5	165	585
AOL	126/263 (47.9)	20	595	85	47	200
OA	87/263 (33.1)	4	107	9	5	14.6
CIT	25/263 (9.5)	20	194	55	33	132
AB_1	3/263 (1.1)	2	12	6	4	10.4
STE	4/263 (1.5)	8	11	10	10.5	11
CPA	2/263 (0.8)	50	123	86.5	86.5	115.7
MPA	32/263 (12.2)	20	158	43	31.5	80.5
EA	35/263 (13.3)	3	5,000	311	15	159.6
EMO	149/201 (74.1)	14	536	76	50	162

n – number of tested samples;
n^+ – number of mycotoxin-containing samples.

in some samples – 550 µg/kg, DON (13.7%), ZEN (10.3%) and FUM (9.9%) were detected less frequently, rate of OA detection at the level of 10–33 µg/kg varied from 15.2 to 42.2%, AB1 was detected rarely (1.6%) and no CTE was detected[3]. Results for 2005–2009 supported conclusions made at the previous stage and provided new information that DAS occurence was far lower as compared to other fusariotoxins, CIT took an active part in contamination and CPA was absent[4,5], and allowed the first specific proposals on mycotoxicological control improvement to be formulated [5]. Later, the following brief reports were published: on frequent occurrence of EMO, antrachinonic toxin known as "diarrheic factor", within the range of dozens to thousands µg/kg in the set of 29 samples[6]; EA occurrence in 2008–2013 [6]; AOL occurrence in 2009–2014 and MPA occurrence in 2007–2014[7] including reporting of abnormal accumulation of this toxin (5,400 µg/kg) in dense caked fraction of mixed feed for piglets due to heavy infestation by highly toxicogenic *Penicillium* spp. fungi[8]. Detailed analysis of the whole data body allowing assessment of feed contamination with mycotoxins is presented for the first time.

Dynamics of annual variation in occurrence of 10 major contaminants is given in Figure. Level of T-2 and EMO contamination remained high that was indicative of wide occurrence of their producers. Significant variations in DON, FUM and AOL occurrence with multi-fold increase in particular years appeared to be associated with specific contamination of the grain supplied to regional feed mills since level of *Fusarium* spp. and *Alternaria* spp. infestation was different in various territories and years. In 2016–2018 situation on AOL contamination became aggravated. In general, decrease in feed contamination with FUM and consistently low feed contamination with ZEN can be noted as a positive trend for the last four years (Fig.).

Drastic increase in OA, CIT, MPA and EA detection in particular years could be accounted for known occurrence in endemic areas as well as combination of adverse factors

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[4] Kononenko G. P., Burkin A. A. Mycotoxicological control of raw feed materials and mixed feed. *Current aspects of veterinary pharmacology, toxicology and pharmacy: Proceedings of the Congress of Russian Pharmacologists and Toxicologists.* St-P.; 2011; 242–244. (in Russian)

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[6] Kononenko G. P., Burkin A. A. Emodin: contamination of grain feed. *Advances in Medical Mycology.* 2007; 9: 88–89. (in Russian)

[7] Burkin A. A., Kononenko G. P. Alternariol occurrence in biological objects. *Modern Mycology in Russia: Proceeding of the III International Mycological Forum (April 14-15, 2015).* Ed. by Yu. T. Dyakov, Yu. V. Sergeyev. M.: National Academy of Mycology; 2015; 5: 223–225. (in Russian)

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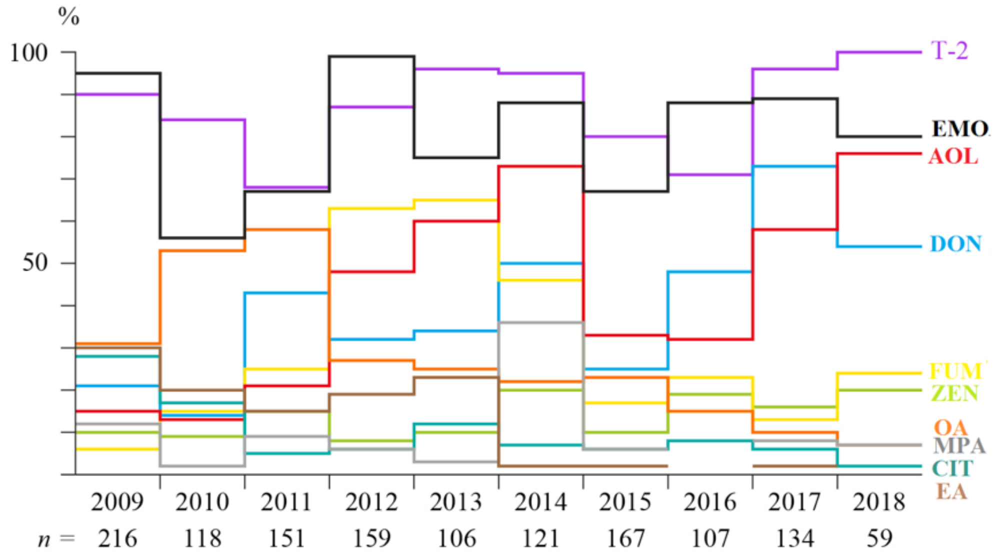

Fig. Dynamics of annual occurrence of T-2 toxin (T-2) deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FUM), alternariol (AOL), ochratoxin A (OA), citrinin (CIT), mycophenolic acid (MPA), ergot alkaloids (EA) and emodin (EMO) in mixed feed for pigs and poultry (summarized data)

Рис. Динамика ежегодной встречаемости Т-2 токсина (Т-2), дезоксиниваленола (ДОН), зеараленона (ЗЕН), фумонизинов (ФУМ), альтернариола (АОЛ), охратоксина А (ОА), цитринина (ЦИТ), микофеноловой кислоты (МФК), эргоалкалоидов (ЭА) и эмодина (ЭМО) в комбикормах для свиней и птицы (обобщенные данные)

during agricultural product harvesting, transportation and pre- and post-processing storage.

Original data of the 10-year monitoring of complete mixed feed for pigs and poultry systematized and summarized in the paper are given in electronic format in section Additional materials are given at: http://doi.org/10.29326/2304-196X-2020-1-32-60-65. Access to the entire database allows any other variants of the data processing, for example data on single and concomitant contamination, joint OA and CIT occurrence that is rather frequent [7, 8] and can enhance their nephrotoxic effect [9], correlation between T-2 and DAS amounts that are similar in toxicity level as well as calculation of excessive contamination cases when regulations on maximum admissible levels for mycotoxins are put into effect at the country level. It should be noted, that wide geographic area where samples used for the said study have been collected does not allow regional aspects to be taken into account. Nevertheless, data obtained for particular territories are very valuable since they provide an unique opportunity to gain understanding of feed grain contamination. Feed grain testing for risk prediction is very difficult due to large-scale harvesting and various soil and climate factors that can influence the mycotoxicological situation. Under these circumstances, feed mixes with large proportion of grain ingredients can be used as ecological markers. In some European countries tracing of mycotoxin contamination in food grains based on results of tests of bakery products sold through retail chains was tested, recognized to be cost-effective and has been put in international practice [10, 11, 12]. Unfortunately, there have been yet no specific regional projects on mixed feed assessment in our country and sample testing results are presented in aggregated form sometimes without indication of type of tested mixed feed in theses covering a wide range of related aspects [13, 14].

CONCLUSION

General features and peculiarities of mycotoxin contamination of complete mixed feed intended for pigs and poultry have been established during extensive monitoring carried out for the last 10 years with annual data collection. Obtained results confirmed importance of regular testing of such feed for their contamination with fusariotoxins, ochratoxin A, regulated in feed grains as well as ergot alkaloids. For mixed feed, it is recommended to include emodin, antrachinonic toxin known as diarrheic factor as well as alternariol, citrin and mycophenolic acid having highly dangerous toxic impacts and long-tern adverse effects into the list of substances significant for public health. Obtained data could be useful for assessment of the situation in feed producing industry and providing rationale for mycotoxin regulation in mixed feed and product control system improvement at processing establishments.

Additional materials to the paper (records forms with database) can be found at: http://doi.org/10.29326/2304-196X-2020-1-32-60-65.

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Received on 16.12.19
Approved for publication on 18.02.20

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Galina P. Kononenko, Doctor of Science (Biology), Professor, Head of Laboratory for Mycotoxicology and Feed Hygiene, ARRIVSHE – Branch of the FSFSI FSC ARRIEVM RAS, Moscow, Russia.

Кононенко Галина Пантелеевна, доктор биологических наук, профессор, заведующий лабораторией микотоксикологии и санитарии кормов ВНИИВСГЭ – филиал ФГБНУ ФНЦ ВИЭВ РАН, г. Москва, Россия.

Alexey A. Burkin, Candidate of Science (Medicine), Senior Researcher, ARRIVSHE – Branch of the FSFSI FSC ARRIEVM RAS, Moscow, Russia.

Буркин Алексей Анатольевич, кандидат медицинских наук, старший научный сотрудник ВНИИВСГЭ – филиал ФГБНУ ФНЦ ВИЭВ РАН, г. Москва, Россия.

Yelena V. Zotova, Candidate of Science (Veterinary Medicine), Senior Researcher, ARRIVSHE – Branch of the FSFSI FSC ARRIEVM RAS, Moscow, Russia.

Зотова Елена Владимировна, кандидат ветеринарных наук, старший научный сотрудник, ВНИИВСГЭ – филиал ФГБНУ ФНЦ ВИЭВ РАН, г. Москва, Россия.