UDC 619:616.98:578.823.1

CASES OF DETECTION OF SEROTYPE 14 BLUETONGUE VACCINE-LIKE VIRUS IN THE RF AND EUROPEAN COUNTRIES IN 2011–2012

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SUMMARY
Associated with isolation of vaccine-like attenuated bluetongue virus cases of 14 serotype bluetongue virus detection in European countries and the RF in 2011–2012 are analysed in the paper.

Key words: bluetongue, virus serotypes, live vaccines.

INTRODUCTION

A bluetongue problem continues to attract careful attention of veterinary specialists [1, 3, 4, 10, 28] due to the disease wide geographic spread in the world and, particularly, in Europe. Indeed, according to the data of the OIE and EU Animal Disease Notification System [11, 27] the occurrence of more than 2.6 thousand of serotype 1 and 4 bluetongue outbreaks was registered in European countries only at the end of September 2014 (Albania, Bulgaria, Bosnia and Herzegovina, Greece, Spain, Italy, Macedonia, Romania, Serbia and Turkey).

But in the first instance, the interest to the disease is associated with the unprecedented spread of bluetongue nosoarea from endemic regions of Africa and Asia northward when nosoarea border has shifted from usual 40° to 58° north latitude (Sweden, Norway). The introduction of bluetongue virus different serotypes into European countries was observed from October 1998 and in 2006 practically all countries of North-Western Europe were affected with serotype 8 virus. A peak of the disease manifestation intensity fell within 2007-2008 when more than 60 thousand outbreaks were detected. Widespread disease outbreaks were registered also in subsequent years, but as long ago as 2011 only 39 disease outbreaks were registered (Greece, Italy, Spain, Portugal, Cyprus).

The detailed epidemic analysis of the bluetongue situation for the given period was undertaken by V.V. Makarov et al. in a series of special publications on the subject including those within the covers of the journal «Veterinaria segodnya» [2, 6, 7, 8, 9, 10].

The present paper is aimed at the analysis of data of international information systems of official OIE, FAO, EU sources on cases of serotype 14 bluetongue virus registration in European countries and the Russian Federation in 2011-2012 as, from our point of view, these materials have found no proper reflection in national scientific publications.

RESULTS AND DISCUSSION

The detection in Russia of serotype 14 bluetongue virus was reported for the first time by GNU «VNIIVViM» (Pokrov) specialists carrying out bluetongue monitoring among imported into the country pedigree animals in the information presented at the annual conference EPIZONE in June 2012 as well as at the annual meeting of reference laboratories for bluetongue in Great Britain in December 2012 [14, 16].

The cattle consignment was imported on 29 August 2011 in OOO «Pedigree Centre Smolensky Gallovey» from Germany (Hagen). In the territory of Germany animals were tested on 8-20 August for bluetongue using ELISA and PCR with a negative result. Animals were transported via Poland and Belarus.

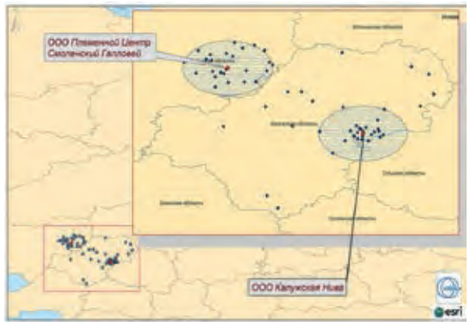

Fig. 1. Sites of detection of bluetongue-seropositive cattle and small cattle in the Smolensk and Kaluga Oblasts (VNIIVViM)

Fig. 2. Lithuanian regions where bluetongue seropositive animals were found [22]

Upon arrival in the Smolensk Oblast on 29 August animals were detained in quarantine and on 2 September 2011 their samples were tested at the GNU «VNIIVViM» using ELISA and PCR also with a negative result.

As a result of repeated serological testings dated 28 September 2011 five out of 71 cattle demonstrated specific antibodies to bluetongue virus and 4 cattle were positive by PCR. Subsequent serological testings of samples from positive animals showed a significant increase in antibody level during 4 months.

Bluetongue virus genome was detected in cow blood on 26 January 2012 but it was not identified in the course of typing with diagnostica of 1, 2, 4, 6, 8, 9, 11 and 16 serotypes.

Isolated RNA was sent to the F. Loeffler Institute (Germany) where serotype 14 bluetongue was diagnosed.

In January 2012 the virus was isolated in the VNIIVViM using Vero culture and lambs and typed by neutralization test as serotype 14; the information was sent to the OIE on 14 March 2012.

In June 2012 pathological materials were also sent to the EU Reference Laboratory for Bluetongue (Pirbright, Great Britain) and on 22 November 2012 it was confirmed that the virus belonged to serotype 14 [26]. The designated as RUS2011/01 strain had 99.9% identity with vaccine strain BTV-14RSArrr/14 included as a compound of the commercial polyvalent attenuated vaccine of RSA origin (Onderstepoort Biological Products (OBP – South Africa)) from a primary bottle A containing bluetongue viruses of 1, 4, 6, 12 and 14 serotypes.

Subsequent to the results of screening investigations for bluetongue held by the VNIIVViM in the Smolensk and Kaluga Oblasts in 2012 a great number of bluetongue-positive animals were found (Fig. 1).

Those findings enabled VNIIVViM researchers to put forward an assumption at the Conference of Reference Laboratories for Bluetongue in December 2012 that serotype 14 bluetongue virus circulated in the western part of the Russian Federation at least during the last two years especially as no systemic investigations for bluetongue were held among local cattle [16].

The epidemiological monitoring of cattle farms in the Smolensk and Kaluga Oblasts carried out by FGBI «ARRIAH» researchers demonstrated no bluetongue clinical manifestation.

Repeatedly addressing the European Commission Directorate-General for Health and Consumers the Rosselkhoznadzor Management expressed a serious concern due to detection in imported from Germany animals of serotype 14 bluetongue virus.

Practically at the same time the Baltic countries and Poland reported about the detection in their territories of serotype 14 bluetongue-positive animals.

The EU Commission for Animal Health and Welfare reported on its site [22] in December 2012 that Lithuania notified the EU Commission on 13 November 2012 about detection in its territory of serotype 14 bluetongue-positive cattle. Fifty one animals from 27 farms in different regions were tested. No animal demonstrated bluetongue clinical signs, all blood samples were negative by PCR but in 15 samples antibodies to bluetongue virus were detected. On 23–25 November 280 bovine blood samples were tested, at that 64 animals were seropositive but all samples were negative by PCR.

Lithuanian regions where bluetongue seropositive animals were found are shown in Fig. 2.

It is important to note that at the meeting of the EU Commission for Animal Health and Welfare on 4 December 2012 [21, 24] representatives of Spain reported that among imported from Lithuania cattle 9 animals were serotype 14 bluetongue-positive by PCR; data were confirmed by blood sample testings in Pirbright. Animals with a positive response were destroyed.

At the meeting of the EU Commission on 30 November 2012 Latvia informed that a significant number of bluetongue seropositive animals were found in the country starting September. Thus, out of 6,988 cattle 206 animals

Fig. 3. Geographical distribution of farms with bluetongue seropositive animals in Latvia [20]

were seropositive, 54 animals out of them were born in 2012. As a rule, 1-2 seropositive animals were found in herds, all animals were negative by PCR with respect to bluetongue, no clinical manifestation was observed as well. Blood from seropositive animals was sent to Pirbright where the fact of animal infection by serotype 14 bluetongue virus was confirmed.

The geographical distribution of farms with bluetongue seropositive animals in Latvia is shown in Fig. 3.

As is evident from Fig. 3, farms with bluetongue-seropositive animals are located practically in the whole territory of Latvia.

Estonia notified the EU Commission by a fax dated 22 November 2012 about detection of bluetongue-seropositive animals in the Saare region (Fig. 4) without any additional clarifications.

Poland [22] also reported suspected bluetongue among cattle to the EU in January 2012; it sent bovine blood samples to Pirbright. But no accurate results were obtained. At a later stage, as a result of monitoring investigations within the framework of the National Programme for Bluetongue Surveillance bluetongue virus genome was detected in the III rd quarter 2012 in animal blood from 3 farms in the region bordering Lithuania and Belarus (Fig. 5).

The Reference Laboratory in Pirbright [26] confirmed the infection of animals with serotype 14 bluetongue virus in Poland, Latvia and Lithuania. The virus had 100% identity with the reference vaccine strain BTV-14 South Africa included as a compound of the commercial polyvalent attenuated vaccine of Onderstepoort Biological Products origin (OBP – South Africa) and 99.9% identity with Russian strain RUS2011/01 isolated in the Smolensk Oblast.

Generally, the assessment of the situation on serotype 14 bluetongue virus in a number of countries including Russia and assumptions on a supposed source of infection are far from being conclusive.

According to the official data of the EU Reference Laboratory for Bluetongue (Pirbright) an epidemic virus of the aforementioned serotype circulates only in the USA, countries of Central and South America, Africa (table). The presence of antibodies to the virus of the given serotype was detected among animals in countries of Middle East, Pakistan, India.

As a matter of course a question was raised as to where from serotype 14 virus could occur in the territory of the Russian Federation. The simplest assumption was as follows: the virus was introduced with imported animals from Germany. But for all that, one cannot leave out of account the fact that imported in August 2011 animals were twice tested for bluetongue in Germany with a negative result and later they were tested with the same negative result while being in quarantine in the Smolensk Oblast. And antibodies to bluetongue virus were detected only in repeated testings held on 28 September.

The understandable question now arises of whether animals were affected in the territory of the Smolensk Oblast after delivery from Germany?

The Department of Environment, Food and Rural Affairs of Great Britain [15] carries out predictive investigations due to spread of animal contagious diseases. The Department made an assumption that vaccine virus could be most probably introduced with vaccinated animals from the region where serotype 14 virus was circulating. The USA were mentioned as such animal exporting country where a live vaccine from a virus of the given serotype could be used. It was emphasized that EU countries including Poland and Baltic countries did not import live animals from the USA but such animals were imported in recent years in Russia.

It is also necessary to note that in no instance the clinical manifestation of the disease was observed either in Russia or Poland and Baltic countries in cases of finding serotype 14 bluetongue-seropositive or PCR-positive animals.

Cases of circulation of bluetongue vaccine-like viruses were registered earlier in the course of the epidemic. Indeed, antibodies and later serotype 6 virus were detected in cattle in neighboring territories of the Netherlands and Germany in October-November 2008 in the course of the bluetongue epidemic caused by serotype 8 virus. Affected susceptible animals demonstrated bluetongue mild clinical signs [17, 18].

Later in Belgium serotype 6 virus was detected in calves imported from the Netherlands. The virus was homologous to South –African reference strain used in a live vaccine.

Belgium [19] reported in November 2008 about the similar situation but with circulation of serotype 11 bluetongue virus when in the course of bluetongue serotype 8 epidemic antibodies were detected in a cow with a dead newborn calve and later in 12 out of 7,294 animals on 8 out of 208 farms and serotype 11 virus genome was detected by PCR and the virus was to a high degree similar to the virus used in the South-African live vaccine against bluetongue.

The detection of circulation of bluetongue vaccine-like virus in non-vaccinated animals along with the possible backward mutation of attenuated strains or their reassortment with epidemic «wild» bluetongue viruses was a reason for approval of a special EU Directive No. 2012/5/EU of 14 March 2012 significantly restricting the use of «modified live vaccines» against bluetongue in Europe [14].

These provisions are consolidated in the OIE «Manual of diagnostic tests and vaccines for terrestrial animals» as amended in 2014 (Chapter C, point 2.3.2) recognizing the confirmed justification of concerns associated with a possibility of vaccine virus transmission by insect carriers with subsequent spread of virus in vector population and infection of intact ruminants [13].

As for the given serotype 14 virus spread in a number of European countries in 2011–2012, representatives of reference laboratories for bluetongue (Pirbright, Great Britain; Spain; VNIIVViM, Russia) are of the opinion that isolated strains are reassortants containing genome segments of different vaccine strains of South Africa origin [23].

At the same time VNIIVViM researchers consider the detection in 2011–2012 of 14 serotype virus circulation among animals in the Kaluga, Smolensk Oblasts and neighboring regions as an evidence of bluetongue **stationary affected areas** [5].

CONCLUSION

We are of the opinion that the fact of specific antibody detection in the course of monitoring investigations for bluetongue is not an evidence of animal affection with bluetongue. It is necessary to carry out an additional epidemiological investigation in order to establish the origin of these antibodies, nevertheless, while carrying out mass screening investigations researchers confine themselves on frequent occasions only to the first stage.

Trade-import operations for import in the country of pedigree and commercial ruminants should be performed under strict observance of «Terrestrial Animal Health Code» (OIE) [12] requiring submission of an international veterinary certificate confirming the fulfillment of all conditions for prevention of bluetongue introduction.

At the same time we think that in cases of import in the RF it is necessary to prohibit officially the purchase in foreign countries of ruminants for zoos and circus, pedigree, commercial ruminants, immunized with live attenuated vaccines against any bluetongue serotypes. The aforementioned measure will prevent the introduction and subsequent circulation of vaccine-like strains which will significantly decrease the efficacy of diagnostic investigations for bluetongue.

It is also necessary to take into consideration provisions of the aforementioned OIE Manual [13] that attenuated vaccine strains can be detected in semen from vaccinated bulls and rams and, moreover, if virus is not detected the vaccination with a live vaccine lowers semen quality.

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Fig. 4. Area of detection of bluetongue-seropositive animals in Estonia

Fig. 5. Area of serotype 14 bluetongue detection in Poland (Podlaskie regione)

Table

Region geography	Serotype																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Africa	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	-	I	I	-	I	-	I	-	-
Middle East	-	I	-	I	-	I	-	-	S	I	-	S	S	S	I	I	S	-	S	S	-	-	-	I	-	I
Pakistan + India	I	I	I	I	S	I	S	S	I	S	S	S	S	S	S	I	I	I	-	S	S	-	I	-	-	-
Australia	I	I	I	-	-	-	I	-	I	-	-	S	-	-	I	I	-	-	-	I	I	-	I	-	-	-
South-East Asia and Indonesia	I	I	I	I	-	-	I	-	I	I	S	I	S	-	-	I	I	S	S	S	I	-	I	-	-	-
China	I	I	I	I	-	-	-	-	-	-	-	I	-	-	I	I	-	-	-	-	I	-	-	-	-	-
USA	I	I	I	-	I	I	-	-	-	I	I	-	I	I	-	-	I	-	I	-	-	I	-	I	-	-
Central and South America	I	-	I	I	-	I	-	I	-	-	I	I	I	I	-	S	I	-	-	-	-	-	-	-	-	-
Europe*	I	I	I	I	-	I	-	I	I	I	R	-	-	-	-	I	-	-	-	-	-	-	-	-	R	-

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УДК 619:616.98:578.821.21:636.52/.58:615.371:616-085.37

ПРИМЕНЕНИЕ ТЕСТА «ЗАМЕДЛЕННОЙ РЕАКЦИИ СЕРЕЖЕК»

У КУР ДЛЯ ЭКСПРЕСС-ОЦЕНКИ НАПРЯЖЕННОСТИ ИММУНИТЕТА К ОСПЕ ПТИЦ

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РЕЗЮМЕ
Применили тест «замедленной реакции сережек» у цыплят, вакцинированных против оспы птиц. Определили модель связи величины относительного коэффициента воспаления и прививной дозы аттенуированного штамма «КЭМ-7» вируса оспы кур и произвели сравнение результатов теста «замедленной реакции сережек» с напряженностью иммунитета.

Ключевые слова: тест «замедленной реакции сережек», аттенуированный штамм «КЭМ-7» вируса оспы кур, напряженность иммунитета, метод «повторной прививки», клеточно-опосредованный иммунитет, гиперчувствительность замедленного типа.

UDC 619:616.98:578.821.21:636.52/.58:615.371:616-085.37

APPLICATION OF «DELAYED WATTLE REACTION»

ASSAY IN CHICKEN FOR RAPID ASSESSMENT OF ANTI FOWL POX IMMUNITY LEVEL

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SUMMARY
«Delayed wattle reaction» assay was applied in chicken vaccinated against fowl pox. Pattern of correlation between inflammation relative coefficient and inoculation dose of attenuated strain «KEM-7» of fowl pox virus was determined and results of «delayed wattle reaction» assay were compared with the level of immunity.

Key words: «delayed wattle reaction» assay, attenuated strain «KEM-7» of fowl pox virus, level of immunity, "re-inoculation" method, cell-mediated immunity, delayed hypersensitivity.