Rabies is an acute viral disease which affects the central nervous system of mammals and is characterized with encephalomyelitis and paralysis with inevitably fatal outcome. According to the classification of the International Committee on Taxonomy of Viruses, the agent belongs to the order Mononegavirales, the family Rhabdoviridae and the genus Lyssavirus.[16]

Rabies is estimated to cause more than 50,000 human deaths and kill more than a million of animals per year. Russia is currently infected with the disease. Rabies cases in various mammal species, notably, cattle, sheep, goats, pigs, horses, dogs, cats and wild carnivores, etc. are still reported in the country.[13]. Wild carnivores contacting with domestic and farm animals and transmitting the pathogen to them are the main source and natural reservoirs of rabies.[6]. The highest level of rabies incidence in livestock is observed among cattle.[13]. Thus, 158 new outbreaks were reported in the Russian Federation in 2017 with the virus being identified in cattle.[14].

Rabies specific prophylaxis as well as assessment of its efficacy based on the level of accumulated anti-rabies virus neutralizing antibodies currently occupy one of the leading positions in the modern system of veterinary and sanitary measures against rabies.[4, 10, 12]. Proper and timely vaccination contains the spread of the disease and significantly reduces possible economic losses. Both live and inactivated vaccines are used for rabies prevention.[2, 7, 9, 11]. The paper presents the study results confirming efficacy and safety of three inactivated rabies vaccines based on the “ARRIAH” strain and intended for rabies prevention in cattle. The adsorbed and two emulsion rabies vaccines, formulated with different adjuvants demonstrated innocuity and safety, and induced post-vaccination immunity in all immunized animals at day 21 post inoculation. The mean titers of anti-rabies antibodies were as follows: for inactivated adsorbed vaccine – 4.02 ± 0.76 IU/cm^3; for inactivated emulsion vaccines – 16.30 ± 2.03 and 20.73 ± 3.39 IU/cm^3. As compared with the adsorbed vaccine, the emulsion vaccines formulated with Montanide ISA 206 and ISA 70 adjuvants induce stronger immunity.  

Key words: rabies, post-vaccination immunity, anti-rabies virus-neutralizing antibodies, inactivated adsorbed and emulsion vaccines against rabies, cattle.
Inactivated emulsion

**Adjuvant**

- AHO
- Montanide ISA 206
- Montanide ISA 70

**Adjuvant/antigen ratio**

- 90/10
- 50/50
- 70/30

**Emulsion type**

- W/O/W
- W/O

**Administration route**

- subcutaneous
- intramuscular

### MATERIALS AND METHODS

**Vaccines.** Experimental batches of vaccines against rabies were obtained on the basis of rabies virus of "ARRIAH" strain reproduced in a suspension cell subline from the Syrian baby hamster kidney (BHK-21/2-17b). Rabies virus was inactivated with the aminoethylenimine solution (AEEI). The solution of polyhexamethylene guanidine (PHMG) was used for purification of the suspension of the inactivated virus antigen from ballast proteins. Aluminium hydroxide gel (AHO) is used for formulation of adsorbed vaccines, oil adjuvants – for formulation of emulsion preparations [1, 8].

The aim of the work was to study efficacy and safety of use of inactivated vaccines against rabies based on "ARRIAH" strain formulated with different adjuvants in cattle.

### MATERIALS AND METHODS

**Vaccines.** Experimental batches of vaccines against rabies were obtained on the basis of rabies virus of "ARRIAH" strain reproduced in a suspension cell subline from the Syrian baby hamster kidney (BHK-21/2-17b). Rabies virus was inactivated with the aminoethylenimine solution (AEEI). The solution of polyhexamethylene guanidine (PHMG) was used for purification of the suspension of the inactivated virus antigen from ballast proteins. Aluminium hydroxide gel (FGBi "ARRIAH", Russia) and a range of Montanide oil adjuvants (SEPPIC, France) were used as adjuvants. Characteristics of test vaccine samples are shown in Table 1.

**Animals.** Forty-five Holsteins, weight 250–300 kg, aged 9–12 months, were used for studying post-vaccination immunity.

Innocuity and safety of vaccines against rabies were evaluated in 90 laboratory mice, weighing 10–12 g.

All experiments on animals were conducted in strict accordance with the interstate standards for keeping and care of laboratory animals GOST 33215-2014 and GOST 33216-2014, adopted by the Interstate Council for Standardization, Metrology and Certification, as well as according to the requirements of Directive 2010/63/EU of the European Parliament and Council of the European Union of 22 September 2012 on the protection of animals used for scientific purposes.

**Determination of infectivity titer of rabies virus.** Infectivity of rabies virus was determined with the use of highly sensitive monolayer of mouse neuroblastoma cell line (N2a) and subsequent dying with rabies immunoglobulin marked with fluorescein isothiocyanate (FITC). Viral titers were calculated with the Spearman-Karber method [15].

**Immunization of animals.** For the testing, cattle were divided into three groups by 10 animals each. Animals of group 1 were immunized with inactivated adsorbed vaccine subcutaneously in the inoculation dose of 5 cm³, group 2 – inactivated emulsion vaccine formulated with Montanide ISA 206 adjuvant in the dose of 2 cm³, group 3 – inactivated emulsion vaccine formulated with Montanide ISA 70 adjuvant in the dose of 2 cm³. All above-mentioned vaccine preparations were administered one time in the undiluted form (Table 2).
cell surface. The cell culture was kept at (37.0 ± 0.5) °C for four days. Then, the monolayer with rabies immunoglobulin marked with FITC was incubated, and the presence or absence of fluorescence specific to rabies virus was assessed. A lyophilized suspension of non-inactivated rabies virus of the “ARRIAH” strain with infectivity titer of 7.00 lg TCID50/cm² was used as a positive control. A lyophilized suspension of inactivated rabies virus served as negative control. The material is considered avirulent if no fluorescence specific to rabies virus was detected in any of the three consecutive passages.

**Evaluation of innocuity of vaccines against rabies in laboratory mice.** The adsorbed rabies vaccine was diluted with physiological saline solution four times, the resulting suspension was administered intracerebrally by 0.03 cm³ to 15 laboratory mice.

Emulsion vaccines were destroyed before administration until antigenic phase was isolated. For this purpose they were 2–3 times frozen and thawed, then centrifuged for 30 minutes at 1000 g. The antigen settling at the bottom of the tube was used for testing by intracerebral administration of 0.03 cm³ (15 animals per vaccine). The animals were observed for 21 days. The vaccine is considered to be avirulent if all animals were clinically healthy during the whole period of observation – without signs of rabies.

**Evaluation of safety of the vaccine in laboratory mice.** The vaccines were administered to 45 laboratory mice (15 animals per vaccine sample) intraperitoneally in the dose of 0.05 cm³ and clinical condition of the animals was observed for 21 days. The vaccine is considered safe if all animals are clinically healthy at the end of the observation period (without necrosis at the injection site).

**Evaluation of safety of the vaccine in cattle.** The vaccines were administered to 15 animals (5 animals per sample) in a triple dose (15 cm³ for adsorbed vaccines and 6 cm³ for emulsion vaccines). The clinical condition of the animals was monitored for 14 days. The vaccine was considered safe if all animals remained clinically healthy during the whole period of observation – without necrosis in the injection site.

**Statistical processing of data.** The research was conducted in three repetitions. The statistical processing of the data consisted in determination of arithmetic mean values of the rabies antibody titer and reliability of statistical difference between the mean values according to the Student-Fisher method [5]. The charting was performed using the StatSoft application package (version 6.0) and Microsoft Excel 2010.

### RESULTS AND DISCUSSION

At the first stage of the work, the obtained antigen of rabies virus was analyzed for innocuity in BHK-21/2-17b monolayer cell culture. According to the results of the study, in none of the three consecutive passages the presence of fluorescence specific to rabies virus was detected, which indicates a complete inactivation of the antigen. With that, in wells with positive control there were clear signs of rabies agent reproduction. The monolayer in wells with negative control was not exposed to rabies virus, specific fluorescence was absent.

At the next stage of the study, the innocuity and safety of the three proposed variants of rabies vaccines were evaluated in laboratory mice and cattle. All animals that were administered the product remained clinically healthy during the whole period of observation, without signs of rabies and without tissue necrosis at the injection site, so the vaccines produced were found to be innocuous and safe.

The next stage of the work dealt with studying post-vaccination immunity in cattle in response to administration of rabies vaccines based on “ARRIAH” strain with different adjuvants. Serums obtained before vaccination immunity in cattle in response to administration of rabies vaccines based on “ARRIAH” strain with different adjuvants (\(n_{\text{test}} = 3, p < 0.005\)).

### Table 3

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Characteristics of rabies vaccine</th>
<th>Animal No.</th>
<th>Rabies VNA titer 21 days post-vaccination, IU/cm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>inactivated adsorbed</td>
<td>AH0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>inactivated emulsion</td>
<td>Montanide ISA 206</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>inactivated emulsion</td>
<td>Montanide ISA 70</td>
<td></td>
</tr>
</tbody>
</table>

\[ M \pm m = 4.02 \pm 0.79^* \]

\[ M \pm m = 16.30 \pm 2.03^* \]

\[ M \pm m = 20.73 \pm 3.39^* \]

\(^*\) Calculation of \(M \pm m\) was made during analysis of VNA titer values which were within normal distribution in the range (\(X - 2\sigma; X + 2\sigma\)).

For animals of group 1 the value of 2.0 IU/cm³ was an exclusion, group 2 – 4.5; 5.9; 7.8 IU/cm³, group 3 – 10.3 and 13.5 IU/cm³.
Table 3 shows that inactivated vaccines against rabies from the “ARRIAH” strain on day 21 induced high levels of antibodies in immunized animals. After immunization with an adsorbed vaccine, VNA titers against rabies virus in 30% of cattle were 2.0–2.6 IU/cm³, in 30% – 3.4 IU/cm³ and 40% – 4.5–5.9 IU/cm³. After the administration of the emulsion vaccine based on Montanide ISA 206 adjuvant, in 30% of animals the rabies antibody titer was 4.5–7.8 IU/cm³, in another 30% it was 13.5–15.9 IU/cm³, in the remaining 40% it was 17.8–23.4 IU/cm³. Immunization with the vaccine based on the Montanide ISA 70 adjuvant induced VNA with titers of 10.3–13.5 IU/cm³ in 20% of animals, in 30% – 15.9–17.8 IU/cm³ and in 50% of cattle – 23.4–30.8 IU/cm³. Average titers of rabies antibodies were 4.02 ± 0.76; 16.30 ± 2.03; 20.73 ± 3.39 IU/cm³ for vaccines No. 1, 2, 3 respectively. Consequently, all experimental vaccines against rabies based on the “ARRIAH” strain induce post-vaccination immunity in cattle on day 21 after inoculation.

It should be noted that comparative analysis of the obtained results shows that emulsion vaccines against rabies induce a stronger immunity in comparison with the adsorbed preparation. Thus, the average value of VNA titer in group 2 was 4.05 times higher, and in group 3 it was 5.16 times higher than in group 1, where cattle was vaccinated with the adsorbed vaccine.

Comparison of mean values of rabies antibodies titers in cattle immunized with the two emulsion vaccines based on ISA 70 and ISA 206 adjuvants demonstrated that the difference between them was less significant than with the adsorbed vaccine and was 4.43 IU/cm³, or 1.27 times in favour of the preparation based on ISA 70, which can be explained by the peculiarities of interaction of the rabies virus antigen with the selected adjuvants.

**CONCLUSION**

The immunobiological properties of three inactivated vaccines against rabies based on “ARRIAH” strain formulated with different adjuvants (AHO, Montanide ISA 206 and ISA 70) were studied. It was found that the vaccines against rabies were innocuous and safe when tested on laboratory mice and cattle.

Post-vaccination immunity in cattle after administration of the proposed inactivated vaccines against rabies was assessed. It was found out that 21 days after the administration of the products formulated with AHO, Montanide ISA 206 and ISA 70 adjuvants, the average values of VNA titers were 4.02, 16.30 and 20.73 IU/cm³ respectively.

Experimental emulsion vaccines against rabies induce higher levels of VNA in cattle compared to the adsorbed vaccine. The average value of VNA titer in the group of animals immunized with the emulsion vaccine formulated with Montanide ISA 206 adjuvant was 4.05 times higher, and in the group of cattle vaccinated with Montanide ISA 70 – 5.16 times higher in comparison with animals vaccinated with the adsorbed vaccine. The emulsion vaccine based on oil adjuvant ISA 70 showed better VNA results than other experimental vaccines.

The research results confirmed that the developed products against rabies for cattle immunization are safe and effective.

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


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