Application of Polyvalent Hyperimmune Serum against Mastitis in Beef Cattle

Kairat Toksanbaevich Zhumanov\textsuperscript{v,1}, Kadir Biyashevich Biyashev\textsuperscript{1}, Birzhan Kadyrovich Biyashev\textsuperscript{1}, Abylay Rysbaevich Sansyzbai\textsuperscript{2}, Anda Valdovska\textsuperscript{1}

\textsuperscript{1}Kazakh National Agrarian University, 8, Abay Ave, Almaty 050002, Kazakhstan
\textsuperscript{2}Research Institute of Biological Security, Urban Settlement Guards, Korday District, Zhambyl Region, Kazakhstan

Abstract
Mastitis causes a huge loss in dairy cattle breeding; it significantly decreases the efficiency of ill animals and shortens their lifetime. Today the average duration of the efficient life of cows due to early forced disposal is 3-5 lactations, which is insufficient, because it does not cover the period of the highest milk efficiency of cows and increases expenses onto the reproducibility of milking herd. Mastitis causes great losses also in the milk industry: even small admixture of milk received from mastitis cows causes a decrease in quality of cheese and other dairy products.

Keywords
Mastitis garget; Milk of mastitis cows; Intoxications

Introduction
Different factors – infection, intoxication, trauma of udder, particularly at incorrect machine milking, chemical irritation, thermal induction (freezing), etc. – can be the causes of occurrence of cow mastitis.

The widespread literature data, published in foreign and domestic literature, certify that a big amount of different bacteria types can play a role in the etiology of udder inflammation occurrence. Specific etiologic significance is given to Staphylococcus, Streptococcus and \textit{E. coli}, whereas the other microorganisms play the secondary role [1-3].

Currently, the antibiotics are widely used to treat mastitis in veterinary practice.

The medications to treat cow mastitis are well known, including intracisternal introduction of medications, such as masticidum, flavurocidum, mastisanim A, mastisanim B, mastisanim E [4-7].

The disadvantage of well-known medications is that mastitis pathogens became highly resistant to a row of antibiotics due to their long-term usage, increasing of doses and break of frequency of administration. As a result, the majority of medications, produced based on antibiotics, has low therapeutic efficiency, and an inflammation process passes into subacute and chronic clinical course.

Until present, there is no effective way of seroprophylaxis of beef cattle mastitis.

We have not found the analogues on seroprophylaxis of animal mastitis in the available literature [8-10].

The aim and tasks of the research includes development of a method of receiving high-efficient curative serum against mastitis of beef cattle.

Materials and Methods
The main volume of the work has been done in the laboratory of anti-bacteriologic biotechnology of the biological safety academic department of Kazakh National Agricultural University. The material for laboratory studies has been received from cows of integrated agricultural production company "Alma-Ata" of Talgar rayon of Almatinskaya oblast.

The fundament of the bacteriological method is an elimination of pure culture of pathogen, which occurs at the first step of the research. An inoculation of the taken material is made for the elimination of pathogen's pure culture. The inoculation is done, as a rule, onto the solid mediums, which are chosen because of the characteristics of probable causative agent.

In bacteriological method, whenever possible, such mediums are used which allow to grow on it only a certain type of bacteria – the elective mediums, or the mediums, which allow to separate probable causative agent from other microorganisms or the other differential-diagnostic mediums.

To preserve blood from clotting we put as anticoagulant a 10% solution of sodium citrate with the calculation of 34 cm\textsuperscript{3} per 1 l of blood into sterile bottles for taking blood, which is mixed with the blood via smooth circular movements. Formed blood elements are separated during 10-15 min with the subsequent plasma defibrination with the addition of 30% solution of calcium chloride with the calculation of 1 cm\textsuperscript{3} per 1 l of blood during 25-30 min. The serum is preserved by 5% solution of chemically pure phenol until the end concentration of 0.5%, and it is pumped over into sterile sedimentation tank, where it is stored for 2 months, at +4-10\textdegree C.

To check the serum's sterility we do the inoculations of the serum onto beef-extract broth, beef-extract agar and beef-extract liver broth under Vaseline oil. The inoculations are cured during 10 days in thermostats at 37\textdegree C. The inoculations should be sterile.

The serum's safety is checked on white mice and Guinea pigs. The serum is injected subcutaneously: in the dose of 0.5 cm\textsuperscript{3} into 5 white mice and 10 cm\textsuperscript{3} into five Guinea pigs. The serum is considered safe, if the animals after the injection are alive within 10 days.

Received: Dec 8, 2015; Accepted: Dec 22, 2015; Published: Jan 23, 2016
Copyright: © 2016 Zhumanov et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

\*Corresponding author: Zhumanov KT, Kazakh National Agrarian University, 8, Abay Ave, Almaty 050002, Kazakhstan

© 2016 Zhumanov et al., Biol Med (Aligarh) 2015, 7:5
http://www.biolmedonline.com
Checking of the serum for anti-Staphylococcus, anti-Streptococcus and anti-Escherichia characteristics is done onto 20 Guinea pigs. The serum is injected under the skin of abdomen in the dose of 1 cm$^3$. In 24 h simultaneously in 12 control Guinea pigs the sterile doses of virulent cultures of S. aureus (5 – experimental and 3 – control), S. agalactiae (5 – experimental and 3 – control), S. pneumoniae (5 – experimental and 3 – control) and E. coli (5 – experimental and 3 – control) are injected. The serum is considered active if it protects from death more than 90% experimental animals. Control Guinea pigs should die within 10 days.

The experimental animals are being observed during 7 days after the death of controls.

Tree groups of Guinea pigs by 8 heads in each group are defined to determine the curative efficiency of polyvalent hyper immune serum.

**Results and Discussion**

The method to receive hyper immune polyvalent serum against beef cattle mastitis is as follows: the polyvalent antigen is made from master seed strain of S. aureus, S. agalactiae, S. pneumoniae and E. coli, then double grund-immunization with an interval of 5 days and hyper immunization of animals by 8-fold intramuscular injections with an interval of 3 days, at a total dose of antigens of $5 \times 10^9$ CFU are done.

The cultures of S. aureus, S. agalactiae, S. pneumoniae are deposited in the State Diagnosis “The National Center for Monitoring, Reference, Laboratory Diagnostics and Methodology in Veterinary” under the Committee of State Inspection in the Agro-Industrial Complex of the Agriculture Ministry of the Kazakhstan Republic under the registration numbers of M-66, K-112, A-14, the strain of E. coli 64G has been patented under №28311 dated 19.03.2014.

The hyper immune polyvalent serum against beef cattle mastitis is made as follows. The hyperimmunization of animals is conducted according to the following scheme:

- 1 cycle (preliminary grund-immunization of animals with the correspondent antigens),
- 2 cycle (preparatory period of hyperimmunization),
- 3 cycle (industrial period of hyperimmunization),

Clinically healthy oxen at the age from 3 to 5 years old with the weight of 350–400 kg were used for immunization. The selected animals are studied to reveal the infectious diseases according to the existent instruction on an order of procurement and sanitary processing of animals, used at the manufacture of bio preparations.

To create a grund-immunity (directed onto an increase of organism's specific reactivity) to animals – producers twice (with an interval of 5 days) intramuscularly into different parts of body the polyvalent antigen is injected (the mixture of inactivated cultures of Staphylococcus, Streptococcus and Escherichia) in doses of $2 \times 10^8$ CFU and $5 \times 10^8$ CFU (2 and 5 cm$^3$).

The preparatory period of hyperimmunization started on the 16th day after the second grunding via intramuscular injections of polyvalent antigen in increasing doses: 3, 4, 5, 6, 7, 8, 9 and 10 cm$^3$ (in $1 \times 10^3$-10$^9$ CFU) with an interval of 3 days between the injections.

At the given scheme, the preparatory period of hyperimmunization of animals-producers is decreased in comparison with the existent schemes of hyperimmunization from 90 days down to 30 days (three times less), and the volume of injected antigen is seven times less.

On the 30th day since the moment of antigen's injection the experimental blood collections from jugular vein into sterile vials were done. The received blood from each producer via serologic reactions was studied for the dynamics of accumulation of antibodies to Staphylococcus, Streptococcus and Escherichia.

### Table 1: Studying of the curative characteristics of hyperimmune serum on Guinea pigs at contamination with S. aureus

<table>
<thead>
<tr>
<th>Animals groups</th>
<th>Number of animals</th>
<th>Infective culture</th>
<th>Injection of tested serum</th>
<th>Dead</th>
<th>Survived</th>
<th>% of survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>20</td>
<td>S. aureus in dose of $10^8$ CFU</td>
<td>10 cm$^3$ s/c</td>
<td>19</td>
<td>1</td>
<td>95</td>
</tr>
<tr>
<td>Control group</td>
<td>5</td>
<td>S. aureus in dose of $10^8$ CFU</td>
<td>–</td>
<td>5</td>
<td>–</td>
<td>0</td>
</tr>
</tbody>
</table>

Control group’s animals died on 6th-7th day. The observation was done during 10 days.

### Table 2: Studying of the curative characteristics of hyperimmune serum on Guinea pigs at contamination with S. agalactiae

<table>
<thead>
<tr>
<th>Animals groups</th>
<th>Number of animals</th>
<th>Infective culture</th>
<th>Injection of tested serum</th>
<th>Dead</th>
<th>Survived</th>
<th>% of survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>20</td>
<td>S. agalactiae in dose of $10^9$ CFU</td>
<td>10 cm$^3$ s/c</td>
<td>1</td>
<td>19</td>
<td>95</td>
</tr>
<tr>
<td>Control group</td>
<td>5</td>
<td>S. agalactiae in dose of $10^9$ CFU</td>
<td>–</td>
<td>5</td>
<td>–</td>
<td>0</td>
</tr>
</tbody>
</table>

Control group’s animals died on 6th-7th day. The observation was done during 10 days.

### Table 3: Studying of the curative characteristics of hyperimmune serum on Guinea pigs at contamination with S. pneumoniae

<table>
<thead>
<tr>
<th>Animals groups</th>
<th>Number of animals</th>
<th>Infective culture</th>
<th>Injection of tested serum</th>
<th>Dead</th>
<th>Survived</th>
<th>% of survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>20</td>
<td>S. pneumonia in dose of $10^9$ CFU</td>
<td>10 cm$^3$ s/c</td>
<td>1</td>
<td>19</td>
<td>95</td>
</tr>
<tr>
<td>Control group</td>
<td>5</td>
<td>S. pneumonia in dose of $10^9$ CFU</td>
<td>–</td>
<td>5</td>
<td>–</td>
<td>0</td>
</tr>
</tbody>
</table>

Control group’s animals died on 6th-7th day. The observation was done during 10 days.

### Table 4: Studying of the curative characteristics of hyperimmune serum on Guinea pigs at contamination with E. coli

<table>
<thead>
<tr>
<th>Animals groups</th>
<th>Number of animals</th>
<th>Infective culture</th>
<th>Injection of tested serum</th>
<th>Dead</th>
<th>Survived</th>
<th>% of survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>20</td>
<td>E. coli in dose of $10^9$ CFU</td>
<td>10 cm$^3$ s/c</td>
<td>20</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Control group</td>
<td>5</td>
<td>E. coli in dose of $10^9$ CFU</td>
<td>–</td>
<td>5</td>
<td>–</td>
<td>0</td>
</tr>
</tbody>
</table>

Control group’s animals died on 6th-7th day. The observation was done during 10 days.
The industrial blood collection has been conducted at the presence of antibodies to Staphylococcus, Streptococcus and Escherichia of not less than 1:800-1:1,200 in the agglutination reaction in the blood serum.

The first collection of blood from the producers by the volume was not more than 0.6 l per 100 kg of animal’s weight, followed by 1.0-1.2 l per 100 kg of the weight. The blood from the producers was taken at normal temperature. Before the blood collection the animals were kept on a starvation diet during 12 h with unrestricted watering.

In 2 days after each blood collection the immunization with antigens in doses by 5 cm$^3$ ($5 \times 10^9$ CFU) was done intramuscularly. The next scheduled blood collection was done in 7-8 days after antigens injections.

The first group of animals was infected with the lethal dose of virulent culture of Staphylococcus aureus, the second with Streptococcus agalactiae, the third with Streptococcus pneumoniae and the fourth with E. coli. In 24 h the animals of the first group (5 heads), the second (5 heads), the third group (5 heads) and fourth (5 heads) were injected the tested serum in the dose of 10 cm$^3$. Three heads of Guinea pigs were kept in each group as the control. The percent of experimental Guinea pigs survived was 95%, with death of controls occurring on the 6th-7th day. The results are given in the Tables 1-4.

Conclusions

The experimental studies show that the polyvalent serum, received via the suggested method, is safe, sterile and areactogenic preparation, which possesses the significant curative and prophylactic action against main causative agents of beef cattle’s mastitis. The suggested method allows receiving of the high-efficient curative serum, improving of the hyperimmunization scheme, decreasing of the volumes of the injected antigen, cheapening of the manufacture technology and decreasing of the labor expenses.

References