"BUL BIO-NCIPD" COMPANY WITH TRADITIONS IN VACCINE PRODUCTION AND WITH LOOK AHEAD TO FUTURE

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I. TRADITIONS

http://bulbio.com

- **BB-NCIPD** Ltd. - commercial company, 100% state-owned, belongs to the Ministry of Health of Bulgaria, with over 130 years history.
- became a separate entity at the end of 2000 based on the production department of the National Center of Infectious and Parasitic Diseases that had a long history in the manufacture of biopreparations (see Historical notes).
- The production nomenclature covers more than 600 medicines, divided in two main groups:
  - human medicines
  - in-vitro diagnostic medicine products;
- have been implemented new technologies meeting the highest requirements of the international standards;
The vaccines of “BB-NCIPD” comply with the WHO and European Pharmacopoeia requirements.

The bio-products of BB-NCIPD Ltd. are exported in over 140 countries in the world and this export forms more than 40% of its revenues.

• The production of drugs for human medicine meet the Good Manufacturing Practice requirements. BB-NCIPD Ltd. holds production license (No. I-65/12.02.2003), issued by the Bulgarian Drug Agency, which approves it as a manufacturer, who meets the requirements of Human Medicines and Pharmacies Act.

• A system of quality control meets the requirements of ISO 9001:2008 (Certificate Lloyd's Register QA No. 368090).
Combating vaccine-preventable diseases is a major concern of the health care system in the advanced countries. The horizontal transmission of infection (from person to person) is difficult or even becomes impossible by preventing replication of the infectious agent through immunization.

The effects of reduction of immunization coverage can be dangerous and even tragic.

The immunization is widely recognized as the most successful and cost-effective health interventions ever implemented in public health practice. It prevents between 2 and 3 million deaths each year (http://www.who.int/campaigns/immunization-week/2014/event/en/).
A major producer of vaccines for mass application in Bulgaria is "BB-NCIPD."
Nowadays the vaccine-production of "BB-NCIPD" is concentrated in the area of bacterial vaccines that according their mechanism of action protect by the following diseases:
• With mucosal replication - pertussis;
• Production of toxins - diphtheria, tetanus;
• Replication in macrophages-TB.
The improved from the WHO, vaccines give the ability for its distribution worldwide. Produced are for the domestic and foreign markets \(\text{(Table 1)}\) diphtheria and tetanus toxoid alone or in combination with whole cell pertussis vaccine and the oldest historically among the vaccines, the BCG vaccine.
The vaccines are available to our clients with and without a preservative thiomersal in different cuts and also in Bulk as active substances.
<table>
<thead>
<tr>
<th>BCG vaccine, freeze-dried (live)</th>
<th>TETATOX tetanus vaccine (adsorbed)</th>
<th>DIFET diphtheria and tetanus vaccine (adsorbed)</th>
<th>DIFETOKOK diphtheria, tetanus and pertussis vaccine (adsorbed)</th>
<th>TETADIF tetanus and diphtheria vaccine (adsorbed)</th>
<th>ANTI-CHF VACCINE (inactivated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Suspension for injection</td>
<td>Suspension for injection</td>
<td>Suspension for injection</td>
<td>Suspension for injection</td>
<td>Solution for injection</td>
</tr>
<tr>
<td>Sizes</td>
<td>Boxes of 20 ampoules each containing 10 doses (plus diluent) Boxes of 20 ampoules each containing 20 doses (plus diluent)</td>
<td>1 ampoule of 1 dose of vaccine 1 vial of 10 doses of vaccine 1 vial of 20 doses of vaccine</td>
<td>1 ampoule of 1 dose of vaccine 1 vial of 10 doses of vaccine 1 vial of 20 doses of vaccine</td>
<td>1 ampoule of 1 dose of vaccine 1 vial of 10 doses of vaccine 1 vial of 20 doses of vaccine</td>
<td>50 ampoules of 1 ml of vaccine</td>
</tr>
<tr>
<td>Dose</td>
<td>For infants - 0.05 ml I/D Dose Above 1 year of age - 0.1 ml I/D Dose</td>
<td>0.5 ml 0.5 ml 0.5 ml</td>
<td>0.5 ml 0.5 ml 0.5 ml</td>
<td>0.5 ml 0.5 ml 0.5 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Contents</td>
<td>Live bacteria derived from a culture of the Bacillus of Calmette and Guepin (BCG), which contains dried suspension of live attenuated strain Micobacterium bovis (Sofia SL222)</td>
<td>Human vaccinating dose 0.5 ml contains: Purified Tetanus Toxoid not less than 40 IU Aluminium hydroxide (Al++)-not more than 1.25 mg Thiomersal-not more than 0.05 mg Sodium chloride-not more than 5.00 mg Water for injection-q.s. 0.5 ml</td>
<td>Human vaccinating dose 0.5 ml contains: Purified Diphtheria Toxoid-not less than 30 IU Purified Tetanus Toxoid-not less than 40 IU Aluminium hydroxide (Al++)-not more than 1.25 mg Thiomersal-not more than 0.05 mg Sodium chloride-not more than 5.00 mg Water for injection-q.s. 0.5 ml</td>
<td>Human vaccinating dose 0.5 ml contains: Purified Diphtheria Toxoid-not less than 30 IU Purified Tetanus Toxoid-not less than 40 IU Inactivated B. pertussis suspension -not less than 4 IU Aluminium hydroxide (Al++)-not more than 1.25 mg Thiomersal-not more than 0.05 mg Sodium chloride-not more than 5.00 mg Water for injection-q.s. 0.5 ml</td>
<td>CCHF antigen - brain suspension of newborn white mice - inactivated</td>
</tr>
<tr>
<td>Indications</td>
<td>For the primary immunization of infants and immunization or reimmunization of children and adults who have reacted negatively to the usual tuberculin tests</td>
<td>Specific prophylaxis of tetanus Combined protection against diphtheria and tetanus. Combined protection against diphtheria, tetanus and pertussis. Combined protection against tetanus and diphtheria. for children over 7 years of age and adults</td>
<td>Prophylaxis of CCHF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adminstr</td>
<td>Intradermally</td>
<td>Intramuscularly</td>
<td>Subcutaneously or intramuscularly.</td>
<td>Subcutaneously.</td>
<td>Subcutaneously</td>
</tr>
<tr>
<td>Storage</td>
<td>Between +2°C and +8°C. Protect from light.</td>
<td>Between +2°C and +8°C. Do not freeze.</td>
<td>Between +2°C and +8°C. Do not freeze.</td>
<td>Between +2°C and +8°C. Do not freeze!</td>
<td>Between +2°C and +8°C. Protect from light.</td>
</tr>
<tr>
<td>Usage</td>
<td>Tuberculin syringe and Mantoux-type needle are used for intradermal application. Special care should be taken to avoid subcutaneous injection. Any open ampoules remaining should be discarded.</td>
<td>Shake before use. Do not use a product that has been frozen.</td>
<td>Shake before use to obtain a homogenous suspension Do not use a product that has been frozen.</td>
<td>Shake before use! Do not use a product that has been frozen.</td>
<td>Opened ampoule should be used immediately.</td>
</tr>
<tr>
<td>Exp.</td>
<td>24 months</td>
<td>36 months</td>
<td>36 months</td>
<td>30 months</td>
<td>36 months</td>
</tr>
</tbody>
</table>

**BCG** vaccine is a vaccine derived from the Bacillus of Calmette and Guerin (BCG), which contains dried suspension of live attenuated strain Micobacterium bovis (Sofia SL222). It is used for the primary immunization of infants and immunization or reimmunization of children and adults who have reacted negatively to the usual tuberculin tests. It is stored frozen at between +2°C and +8°C and do not freeze. It is used subcutaneously or intramuscularly.

**TETATOX** is a tetanus vaccine that contains live bacteria derived from a culture of the Bacillus of Calmette and Guerin (BCG). It is used for the primary immunization of infants. It is stored frozen at between +2°C and +8°C and do not freeze. It is used subcutaneously.

**DIFET** is a diphtheria and tetanus vaccine. It is used for combined protection against diphtheria and tetanus. It is stored frozen at between +2°C and +8°C and do not freeze. It is used subcutaneously.

**DIFETOKOK** is a diphtheria, tetanus and pertussis vaccine. It is used for combined protection against diphtheria, tetanus and pertussis. It is stored frozen at between +2°C and +8°C and do not freeze. It is used subcutaneously.

**TETADIF** is a tetanus and diphtheria vaccine. It is used for combined protection against tetanus and diphtheria. For children over 7 years of age and adults. It is stored frozen at between +2°C and +8°C and do not freeze. It is used intramuscularly.

**ANTI-CHF VACCINE** is a CCHF antigen - brain suspension of newborn white mice - inactivated. It is used for prophylaxis of CCHF.
The immunization (reimmunization) with DIFTET, TETADIF, DIFTETKOK can be made simultaneously with other vaccines such as poliomyelitis, influenza, hepatitis B, measles, rubella and BCG. They can be associated also with immune globulins. The usage of different syringes, needles and injection side is needed.

When there are contraindications to pertussis component, diphtheria and tetanus vaccine is applied by the immunization schedule of DIFTETKOK vaccine.

For the needs of WHO, UNICEF and PAHO (Pan American Health Organization) are provided bacterial vaccines via our long-standing business partner Inter Vax, Canada. Produced is also PPD Tuberculin, ready to use for Mantoux’s intradermal test to assist in clinical diagnosis of tuberculosis.
The only virus vaccine that is produced in the company is inactivated vaccine against Crimean Hemorrhagic Fever (CHF). It contains inactivated virus as antigen strain of CHF V 42/81 and administered prophylactically population in endemic distribution of the causative regions. Two applications of the vaccine provide specific immunity and prevent disease CHF. We are currently running a joint project to develop a new recombinant DNA vaccine against CHF in partnership with Canada and Kazakhstan.
II. FUTURE DEVELOPMENTS

- Apart from improving animal health and productivity, veterinary vaccines have a **significant impact on public health** through reductions in the use of veterinary pharmaceuticals and hormones and their residues in the human food chain.
- According to the **European Pharmacopoeia 8.0.** Chapter "Vaccines for veterinary use", the bacterial strain is **permitted to be modified by genetic engineering**, such as the identity, purity and antigen activity of each bacterial culture used must be carefully controlled.
- A new approach in the **design of recombinant vaccines** are inactivated vaccines containing whole cells. Successfully manipulation of the bacterial genome could provide **surface-presented antigens of various pathogens**. The challenge here would be the use of **innovative methods for inactivation** of bacterial vaccines.
Treating the development vaccine with a **hybrid material containing silver nanoparticles** will inactivate the strain and as a result can be obtained recombinant “ghost” cells.

“Ghost” vaccines are an innovative idea to obtain better results of immunization due to the presence of fuller spectrum of saved antigenic determinants and development of protective immunity.

Development of a **vaccine for veterinary use of recombinant "ghost" cell carriers of the bacterial genomes of different pathogens** against causes of enteric disease is the basis of a draft proposal with potential awaiting development and implementation.

To achieve this main goal we should go a long way of experimental research.
The choice of the **components of a polyvalent vaccine against enteric diseases in animals** is a first important step in its development.

It is known that a **traditional production of non-living** (killed) **vaccine by heat treatment, irradiation or chemical treatment** of the pathogen often leads to **denaturation of significant structural components of the cell wall**, changing the antigenic character of the vaccine and due to the loss of important immunogenic epitopes cannot create a complete immunity.

Obtaining of "ghost" vaccine by **inactivating bacteria with hybrid material** based on silver nanoparticles stabilized by polyvinyl alcohol (PVA/AgNps) and **keeping the antigenic range and creating of complex protective immunity** is an innovative new approach to the application of whole cell inactivated vaccines.
Experimental study on the components in poly-valent "ghost" Salmonella vaccine for veterinary use

- Annually in many European countries and the United States are reported a large number of cases of Salmonella gastroenteritis. Approximately 80 deaths are recorded each year in the UK.
- There are also known data caused by a significant number of non-typhoidal Salmonella systemic and non-enteric forms of human infections.
- In a study performed for 5 year period in Bulgaria it was found that 21% of them are resistant to A and G, 17.64% are resistant to T, 14.28% to Nx and 10% -resistant to C.
- The emergence of multidrug-resistant Salmonella strains raises the question of strengthening the measures related to the prevention and protection at poultry.
About half of the **Salmonella outbreaks** are due to **contaminated poultry and poultry products.** The route to poultry infection is the **colonization of the hen house and its pets,** such as **rodents, insects and wild birds.** Salmonella in the feces of laying eggs **contaminate surface or penetrated through the cracks of light shells.**

At **hens with ovarian infection** was established that **S. Enteritidis** can reach the egg by **internal vertical transmission** via the reproductive tract to the yolk or albumin.

Historically **S. Typhimurium** is the most commonly reported serotype.

In 2001, the three most common Salmonella serotypes (more than 50% of all isolates) were **S. Typhimurium (22%), S. Enteritidis (18%), S. Newport (10%).**

**S. Newport** is one of the Salmonella serotypes causing diseases in **cattle.**
Alternative to the available at the market inactivated with formaldehyde Salmonella vaccines could be a vaccine derived from ghost cells resulting from treatment with the hybrid material PVA/AgNps. The aim of the first investigation was to establish the components of the poly-valent “ghost” Salmonella vaccine by inactivation of different Salmonella strains - two strains *S. Enteritidis*, *S. Newport Puerto Rico* and *S. Typhymurium*. Initially, MBC for different Salmonella strains was determined by macrodilution method (Figure 1). The MBC for both strains *S. enterica* serovar Enteritidis and *S. enterica* serovar Thyphimurium was established as lower than 0.027 mg/L. Only for *S. Newport- Puerto Rico* the MBC was - 0.108 mg/L (≈0.11 mg/L). The tested Salmonella strains were sensitive to silver, as tests with the same hybrid material showed that MBC values equal or more than 1.1 mg/L are sign for silver resistance.
Figure 1. MBC of PVA/AgNps determined by macrodilution method for:

a) S. Newport - Puerto Rico
b) S. Enteritidis ATCC 13076
c) S. Enteritidis and d) S. Typhimurium.
The **Maximal non-toxic concentration** (MNC) is the maximal concentration, that altered neither the morphology of monolayer nor the cell survival rate. **MNC was defined as 0.007 mg/L.** The concentration required to inhibit cell viability by 50% (**CD50**) was determined as **0.53 mg/L** in a dose-dependent manner(Figure 2).

![Figure 2. Cytotoxic effect of PVA/AgNps on the viability of mouse fibroblast (L20B) cell line at 24h and 48h](chart.png)
As the MBC from the respective strains was determined at $10^5$ - $10^6$ CFU bacterial load, therefore to inactivate one billionth bacterial, silver concentration of 30 mg /L suspension was applied.

From working cultures of the 4 control Salmonella strains – *S. Typhimurium, S. Newport- Puerto Rico, S. Enteritidis, S. Enteritidis ATCC 13076*, were prepared as antigens for immunization "ghost" Salmonella vaccines. The inactivation of the bacteria was confirmed with cultural method.

Bacterial suspension was standardized in densitometer to 3MF and used as an antigen for intravenous immunization of Californian rabbits with increasing antigenic load of 0.5 to 2 ml by established in the "BB-NCIPD" scheme - in vena marginalis in intervals of 3 to 4 days.
The specific titer of all obtained after immunization rabbit Salmonella antisera was determined in a Gruber’s reaction stage agglutination. The antisera were with O-titer 1:6400 with exception of the anti- S. Enteritidis serum, that has O titer 1:1600.

It was found a significant difference in the activity of sera, obtained from both strains S. Enteritidis (Table 2), therefore it was considered to incorporate both of them in the polyvalent Salmonella “ghost” vaccine for veterinary use.

<table>
<thead>
<tr>
<th></th>
<th>S. enterica serovar Enteritidis ATCC13076 1,9,12;gm;</th>
<th>S. enterica serovar Enteritidis 1,9,12;gm;</th>
<th>79 a S. enterica serovar Newport Puerto Rico 6,8 [20];1,2</th>
<th>S. enterica serovar Typhimurium 1,4,[5],12;i;1,5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti- S. Enteritidis ATCC13076 serum</td>
<td>++++</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Anti- S. Enteritidis serum</td>
<td>-</td>
<td>++++</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Anti- S. Newport Puerto Rico serum</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Anti- S. Typhimurium serum</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Legend: ++++ very good visible agglutinates in clear liquid; +++ good visible agglutinates in almost clear liquid; ++ visible agglutinates in turbid liquid; + slightly visible agglutinates in turbid liquid.
TEM analysis a month after completion of the immunization was performed (Figure 3) to one of those used in attempts antigens.

It was found that the presence of the PVA/AgNps for longer period in the antigen for the immunization results in complete lysis of the bacterial cells after apoptosis.

Therefore, an additional step consisting in washing of the antigen after inactivation with PVA/AgNps, in order to preserve the inactivated bacterial cells in the form of "ghost" cells is necessary.
1. *E.coli* O104

- The outbreak from *E.coli O104:H4* in Germany and other EU/EEA countries was one of the largest reported HUS (Hemolytic – uremic syndrome) outbreaks in the world.

- The enterroaggregative Verotoxin (Vtx-) producing *E.coli* strain (EAggEC)/VTEC) serotype O104:H4 has often been described as an enterrohaemorrhagic *E.coli* (EHEC).

- VTEC that produce Attaching and Effacing (AE) lesions on enterocytes are *EHEC.*
Interesting fact is that the primary sources and vehicles of typical EHEC infections in humans are ruminants, whereas no animal reservoir has been identified for enteroaggregative *E.coli*.

The VTEC sero-group O104 has been reported three times as *isolate from animals and food* by the EU member states:

- Two of the isolations were *from cattle* and the detected *serotypes* were O104:H12 and O 104:H21.
- VTEC serotype O104 was isolated also *from wild boar*.
- From *sheep and young cattle* was isolated O104:H7.
- In food VTEC O104 was isolated *from bovine carcasses and meat*. 
When **infecting humans VTEC** can also be responsible for **HUS** due to the production of Vtx.

**Serotype** *E.coli* O104:H21 was also agent of **sporadically outbreaks**.

Although **the main agent of two HUS cases in German** was *E.coli* O104:H4 VTEC strain.

The strategy of the present study was to **create “ghost” E.coli O104 cells using the hybrid material, synthesized according to reported in the literature method** (Figure4).

**Figure4:** a) TEM image- **spherical AgNps** with an average **diameter of 5.0 ± 1.0 nm.**

b) UV-Vis spectroscopy confirmed the presence of AgNps by appearance of strong absorption bands at 420 nm.
The determined MBCs of *E. coli* O104 was 0.054 mg/L, which demonstrated sensitivity to silver.

For the inactivation process, PVA/AgNps solution with silver concentration of 30 mg/L was used.

The process of inactivation was confirmed onto cultural method.

The value of therapeutic efficacy (TE) was 75.71.

MBC and the evidences of TE can determine the secure intravenous administration of the vaccine suspension.

The excess of the hybrid material, used for inactivation in concentration (30 mg/L), was removed due washing partically in advance.
Two rabbits that were put into immunization scheme were elected from one litter in order to provide closely related signs and immunity. They passed the full course of 4 immunizations with increasing antigenic load of “ghost” *E. coli* O 104.

- One of rabbits was immunized with the antigen inactivated by the hybrid material (rabbit No1).
- The other rabbit was immunized with antigen prepared in a conventional manner – treated with heat (rabbit No2).

It was established that the rabbit immunized with “ghost” bacterial cells forms more rapidly titre of specific antibodies from those that has been immunized with the antigen treated by the classical method (Table 2).

The presence of a specific titer after the second immunization was observed only by rabbit No1.
Table 2: Determination of specified antibodies against *E. coli* O104 during and after the end of immunization scheme.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Titer 3 days after the first immunization</th>
<th>Titer 3 days after the second immunization</th>
<th>Titer 3 days after the third immunization</th>
<th>Titer 3 days after the fourth immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td>No 1</td>
<td>Without titer</td>
<td>At 1:50 dilution, good positive slide agglutination</td>
<td>At 1:400 dilution, good positive slide agglutination</td>
<td>At 1:400 dilution, very good positive slide agglutination</td>
</tr>
<tr>
<td>No 2</td>
<td>Without titer</td>
<td>Without titer</td>
<td>At 1:50 dilution, very good positive slide agglutination</td>
<td>At 1:400 dilution, very good positive slide agglutination</td>
</tr>
</tbody>
</table>

**Legend:** Rabbit No 1 – immunized with antigen treated with hybrid material; Rabbit No 2 – immunized with antigen treated with heat.
TEM image (Figure 5) demonstrates the changes in the cell structure of the received at this manner E. coli O104 “ghosts”.

The picture shows the advantage in the introduction of washing step after the inactivation of the antigen with the polymer in a method of treatment.

Figure 5: TEM image of the E. coli O 104 – “ghost” cells with discarded cellular content and visible presence in a cell of PVA/AgNps.

Infection per os of the immunized rabbits was provided with 1 ml of a billionth suspension of alive bacterial cells E. coli O 104.

The rabbits showed a mild discomfort during the next day with transient loss of appetite. After this period they recovered without clinical signs of disease.
2. ENTEROTOXIGENIC E.coli (ETEC)

ETEC produce one or more fimbrial adhesins that mediate their attachment to specific receptors on mucosal epithelial cells, producing of enterotoxines. This change the water and electrolyte efflux of the small intestine and lead to neonatal diarrhea and post-weaning diarrhea in farm animals. For protection against ETEC diarrhea are commonly used commercially available vaccines, that are given parentally. They content inactivated whole-cells, purified fimbrial subunit or heat labile enterotoxin (LT).

Fimbrial adhesins of neonatal porcine ETEC are F4 (K88), F5 (K99), F6 (987P) and F41. The most responsible for diarrhea in young pigs are the F4 ETEC strains and for post weaning diarrhea – F4 or F18. Some F18 ETEC strains also produce Shiga Like Toxin Ile (SLT Ile) and can cause oedema disease and not diarrhea.
Verotoxigenic *E. coli* (VTEC) on animals and food were monitored and the report covers primarily VTEC O157 H7 on the skin of young cattle and sheep fleeces. The monitoring is extended to the *E. coli* serogroups O26, O103, O111 and O145, which also cause human infection.

In the last provided (still unpublished) experiment immunization was conducted according to the established schedule of two rabbits.

For the test was chosen the strain *Escherichia coli* O 157 H7.

The MBC of PVA/AgNps was established in this case at silver concentration 0,03 mg/L and show sensitivity to silver.
Both antigens of *Escherichia coli* O 157 H7 were prepared as inactivated in **two different ways**:  

- the first - *with the hybrid material*;  
- the second - *with formalin*.

The **formalin**, added for the inactivation of the second immunization antigen *E.coli* O157H7, was **in quantities equal to the volume of the added polymer** to the first antigen.

The suspensions are **washed aseptic twice with injection water after centrifugation** of 5000-6000 rpm for 15 minutes to remove the added inactivators.
TEM image of the treated with PVA/AgNps antigens for immunization shows the existence of ghosts - cells in the first antigen (Figure 6) with removed cell content and preserved cell wall.

The TEM image of cell of the second antigen, treated with formalin (Figure 7) shows presence of a strong thinning of the cell shell, in some places as eaten away.

Figure 6: E.coli O157H7 treated with PVA/AgNps.

Figure 7: E.coli O157H7 treated with formaldehyde.
To establish the influence of the processing of the antigen before each subsequent immunization have been blood samples taken to determine in stage agglutination reaction the reached specific titer (Table 4).

**Table 4:** The specific titers of sera from the three immunized rabbits after second, third and fourth immunization.

<table>
<thead>
<tr>
<th>Serum obtained after an immunization with</th>
<th>Specific titers after the second immunization</th>
<th>Specific titers after the third immunization</th>
<th>Specific titers after the fourth immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O-titer</td>
<td>K-titer</td>
<td>O-titer</td>
</tr>
<tr>
<td><em>E. coli</em> O157H7, treated with PVA/AgNps</td>
<td>800</td>
<td>0</td>
<td>1600</td>
</tr>
<tr>
<td><em>E. coli</em> O157H7, treated with formaldehyde</td>
<td>200</td>
<td>0</td>
<td>1600</td>
</tr>
</tbody>
</table>
1. The study showed that both strains *S. Enteritidis*, *S. Newport-Puerto Rico* and *S. Typhimurium* are appropriate to be chosen as candidates for their incorporation in order to create “ghost” vaccine for veterinary use.

2. “Ghost” *E.coli* O104 vaccine creates protective immunity.

3. The TE was established as very good, which allows the intravenous use of the hybrid material without expecting pathological changes in cells.

4. It was proven advantage when using antigen, inactivated by the PVA/AgNps hybrid material (*E.coli* O104, O157H7) to such treated by classical methodology (by heat inactivation or by formaldehyde), expressed in faster development of specific titer.
THANK YOU FOR YOUR ATTENTION!

3. Bertschinger and Fairbrother, 1999
6. Haesebrouck F et al., 2004
17. Pencheva D., Velichkova E., Mileva M., Briaskova R., Genova-Kalou P., Kantardjiev T., PVA/AgNps as inactivator for "ghosts"-vaccines for veterinary use, 25-28 April, 2015, 25-th ECCMID, Copenhagen, Denmark.