# THE CLINICAL TESTING OF THE POLYVALENT INACTIVATED VACCINE USED FOR THE IMMUNOPROPHYLAXIS IN OVIN CLOSTRIDIOSIS

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Summary

The goal of this paper's researches is the clinical testing and immunological effectiveness of the ULTRACHOICE 8 vaccine destined for the immunization against the clostridiosis that developed in sheep.

The researches has been made respecting the testing protocol of the ULTRACHOICE 8 product of the PFIZER ANIMAL HEALTH – USA company, under the coordination of the I.C.P.B.M.V. Bucuresti, for the assimilation, registering and marketing of the vaccine in Romania.

## Materials and methods

The clinical testing of the ULTRACHOICE 8 vaccine (inactivated vaccine, polyvalent, against anaerobiosis in bovine and ovine) was realized in the didactic farm of the U.S.A.M.V.B. Timisoara, on a series of 35 adult sheep made and an equal number of adult sheep represented the proof series.

*Clostridium chauvoei* strain F..... 2,28 U.O.*
*Clostridium haemolyticum* strain IRP-135..... 216 L+U.T.** and 3,6 U.O.
*Clostridium novyi* strain 8296..... 6000 DLM***
*Clostridium perfringens* type C strain PC8 ..... 300 L+U.T.
*Clostridium perfringens* type D strain 317 ..... 100 L+U.T.
*Clostridium septicum* strain A (IRP-111) ..... 32 L+U.T.
*Clostridium sordellii* strain 5918 ..... 20 L+U.T.
Formaldehidă (prezervant) ..... ≤0,2% w/v
Stimugen-adjuvant water soluble
PH 6,2-6,4

*Legend*:
* -culture opacity units;
** -toxin units before inactivation;
*** -toxin lethal limits before inactivation.

The vaccine was administered in doses of 1 ml sc at the sheep in the axillary region. The vaccination consisted of two administrations (vaccination I and vaccination II) in an interval of 4 weeks.

After the vaccination the local and general reactions were observed to discover any side effects at the inoculating place or as a general reaction.

For testing the immune response of each animal belonging to the vaccinated group and the ones that have not been vaccinated, there have been taken blood samples to obtaining the serum as it follow:

- $T_0$ - before the first vaccination;
- $T_1$- before the second vaccination and at 4 weeks from the first vaccination;
- $T_2$- at 4 weeks from the second vaccination

The evaluation of the immune response after the vaccination was realized by mouse neutralization test at I.C.P.B.M.V. Bucharest. For some technical reasons the immune response was tested for 3 antigenic components of the vaccine: for *Clostridium perfingens* type C, *Clostridium perfingens* type D and *Clostridium novyi*.

The mouse neutralization test was made respecting the classic methodology (5). The samples were ordered in:

- 4 samples average ($O_1$, $O_2$, $O_3$, $O_4$) for $T_0$;
- 4 samples average ($O_1$, $O_2$, $O_3$, $O_4$) for $T_1$;
- 4 samples average ($O_1$, $O_2$, $O_3$, $O_4$) for $T_2$;

## Results and discussions

After the vaccination of the sheep with the ULTRACHOICE 8 the obtained results were, as a whole, demonstrating that the vaccine induces an immune response that protects the vaccinated animals against the genus *Clorstridium* for which it is recommended. These aspects are confirmed by the serological test results.

The ULTRACHOICE 8 vaccine administered to the sheep in two inoculations did not generate fever, modifications of the general status or other symptoms which would denote the affect of the general status.

After the first and second vaccination there have been observed local reactions, at the place of the inoculation: nodule, doughy edema and lameness. These local reactions overcame between 3-4 days after the vaccine administration and have totally disappeared after 21 days at the animals from the first and second vaccination.

The serological results concerning the clinical testing of the vaccine ULTRACHOICE 8 and its post-vaccinational immune response have been made at the I.C.P.B.M.V. Bucuresti by Dr. Necsulescu Marius within the clinical testing protocol of the vaccine.

The post-vaccinal response immune has been tested by the seroneutralisation reaction on mice, with 3 existing antigenic components in the composition of the vaccine. The high costs of the diagnostic kits have enforced the testing only on those 3 components and another motive was that the technical file elaborated by the producer permits the establishment of the post-vaccinal response immune with minimum 3 antigen components.

The obtained results of the serological test are presented in the 1-3 tables.

The immune response against *Clostridium perfingens* type C was very intense, after the second vaccination, highly than the admissible limits. The geomean average of their titers was 14.56 I.U./ml. With the *Clostridium perfingens* type D the immune response surpassed, after the II vaccination the minimum admisibility standard, the geometrical average o the titer was 3.36 I.U./ ml.

In the case of the *Clostridium novyi* the immune postvaccinal response was equaly intense surpassing the minimum admissible limit, and the geometrical average of the obtained titer after the II vaccination was 5.42 I.U./ml. The results proved a protective post-vaccinal immune response obtained only after vaccination II. After vaccination I the immune response was low at the minimum admissible limits.

At the witness group, not vaccinated, the results aren't presented in the tables because the obtained titers were under the admissibility limits.

We mention that the admissibility margin of the antibody antitoxic post-vaccinal titers expressed by International Units/ milliliter was established by the producer.

Analyzing the present results, it is clear that the ULTRACHOICE 8 vaccine can be used in immunoprofilaxy of diseases produced by the genus *Clostridium* in ovine.

**Table 1**

**The serological tests results at the immunized sheep with the ULTRACHOICE 8 vaccine (*Clostridium perfrigens* type C)**

| **Minimum admissibility limit** | **≤0,1 I.U./ml** | **≥5 I.U./ml** | **≥10 I.U./ml** |
|---|---|---|---|
| Merged proof | Obtained value $T_0$ | Obtained value $T_1$ | **Obtained value $T_2$** |
| O1 | <0.1 I.U./ ml | 8 I.U./ ml | **15 I.U./ ml** |
| O2 | <0.1 I.U./ ml | 8 I.U./ ml | **15 I.U./ ml** |
| O3 | <0.1 I.U./ ml | 8 I.U./ ml | **15 I.U./ ml** |
| O4 | <0.1 I.U./ ml | 8 I.U./ ml | **15 I.U./ ml** |
| | | | **MG $T_2$ = 14.56 I.U./ml** |

**Table 2**

**The serological tests results at the immunised sheep with the ULTRACHOICE 8 vaccine (*Clostridium perfrigens* type D)**

| **Minimum admissibility limit** | **≤0,1 I.U./ml** | **≥1 I.U./ml** | **≥2 I.U./ml** |
|---|---|---|---|
| Merged proof | Obtained value $T_0$ | Obtained value $T_1$ | **Obtained value $T_2$** |
| O1 | <0.1 I.U./ ml | 1.5 I.U./ ml | **4 I.U./ ml** |
| O2 | <0.1 I.U./ ml | 1.5 I.U./ ml | **4 I.U./ ml** |
| O3 | <0.1 I.U./ ml | 1.5 I.U./ ml | **4 I.U./ ml** |
| O4 | <0.1 I.U./ ml | 1.5 I.U./ ml | **4 I.U./ ml** |
| | | | **MG $T_2$ = 3.36 I.U./ml** |

**Table 3**

**The serological tests results at the immunised sheep with the ULTRACHOICE 8 vaccine (*Clostridium novyi*)**

| **Minimum admissibility limit** | **≤0,1 I.U./ml** | **≥1 I.U./ml** | **≥2 I.U./ml** |
|---|---|---|---|
| Merged proof | Obtained value $T_0$ | Obtained value $T_1$ | **Obtained value $T_2$** |
| O1 | <0.1 I.U./ ml | 2 I.U./ ml | **6 I.U./ ml** |
| O2 | <0.1 I.U./ ml | 2 I.U./ ml | **4 I.U./ ml** |
| O3 | <0.1 I.U./ ml | 2 I.U./ ml | **6 I.U./ ml** |
| O4 | <0.1 I.U./ ml | 2I.U./ ml | **4 I.U./ ml** |
| | | | **MG $T_2$ = 5.42 I.U./ml** |

## Conclusions

- ULTRACHOICE 8 vaccine administered at adult sheep, in two inoculations, has not produced general reactions and the local reactions were transient.
- ULTRACHOICE 8 vaccine induced a protective immune response only after vaccination II.
- The geomean average of the post-vaccinal antibody titer, represented in I.U./ ml. for three antigens components have overvalue the minimum admissibility limits.
- The obtained results after the clinical and laboratory testing recommend the usage of the ULTRACHOICE 8 vaccine in the immunoprofilaxy of the clostridiosis in sheep.

## References

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