



**Institute for State Control of Veterinary Biologicals and Medicines  
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(Reference Member State)**

**PUBLICLY AVAILABLE ASSESSMENT REPORT FOR AN  
IMMUNOLOGICAL VETERINARY MEDICINAL PRODUCT**

**Lenzelta suspension for injection for cattle**

Product name Lenzelta suspension for injection for cattle	Application number CZ/V/0204/001/DC
Applicant Boehringer-Ingelheim	DCP
Publicly available assessment report	

## PRODUCT SUMMARY

EU procedure number	CZ/V/0204/001/DC
Name and pharmaceutical form	Lenzelta suspension for injection for cattle
Applicant	Boehringer Ingelheim Vetmedica GmbH Binger Strasse 173 55216 Ingelheim am Rhein Germany
Active substance(s)	<i>Escherichia coli</i> , inactivated RP ≥ 1 <i>Staphylococcus aureus</i> , inactivated RP ≥ 1
ATC vetcode	QI02AB17
Target species	Cattle (cows and heifers)
Indication for use	For active immunisation of healthy cows and heifers, in herds of dairy cattle with repeated occurrence of mastitis, to reduce the incidence and severity of clinical mastitis caused by <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> .

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## PRODUCT INFORMATION

The Summary of Product Characteristics (SPC), the labelling and package leaflet for this immunological veterinary medicinal product (IVMP) are available in the Union Product Database (UPD).

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## SUMMARY OF ASSESSMENT

Legal basis of original application	Full application in accordance with Article 8 (full dossier) of Regulation (EU) 2019/6 as amended.
Date of completion of the original decentralised procedure	29/10/2025
Date immunological veterinary medicinal product first authorised in the Reference Member State (MRP only)	Not applicable
Concerned Member States (CMS) for original procedure	AT, BE, DE, DK, EL, ES, FI, FR, IE, IT, LU, NL, NO, PT, SE, UK(NI)
CMS for subsequent use procedure	-
Withdrawn CMS during original decentralised procedure	-

### 1. SCIENTIFIC OVERVIEW

The IVMP is manufactured and controlled using validated methods and tests that ensure the consistency of the IVMP released on the market.

The IVMP can be safely used in the target species; the slight reactions observed are indicated in the SPC.

The IVMP is also safe for the user, the consumer of foodstuffs from treated animals and for the environment, when used as recommended. Suitable warnings and precautions are indicated in the SPC.

The efficacy of the IVMP was demonstrated according to the claims made in the SPC.

The overall risk/benefit analysis is in favour of granting a marketing authorisation for this IVMP.

### 2. QUALITY DOCUMENTATION (physicochemical, biological or microbiological information)

#### 2.A. Product description

Each 2 ml dose contains:

**Active substances:**

*Escherichia coli*, serotype O111, strain J5, inactivated: RP ≥ 1\*

*Staphylococcus aureus*, strain DSM 4910, inactivated: RP ≥ 1\*

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- \* Relative potency (RP) is determined by comparing the antibody level with the antibody level in serum of mice prepared with a reference batch of vaccine compliant with the challenge test in target animals.

**Adjuvant:**

Aluminium hydroxide gel 2%                      0.4 ml

**Excipients:**

Thiomersal    0.2 mg

Formaldehyde                                        ≤ 1 mg

Sodium chloride

Water for injections

The IVMP is an established pharmaceutical form and its development is adequately described in accordance with the relevant European guidelines.

The container/closure system:

Type I glass vials of 10 ml with chlorobutyl elastomer closure and aluminium or flip off caps.

Type II glass vials of 50 or 100 ml with chlorobutyl elastomer closure and aluminium or flip off caps.

Translucent plastic (HDPE) vials of 15, 60 or 120 ml with chlorobutyl elastomer closure and aluminium or flip off caps.

Package size

Plastic box of 10 glass or plastic vials of 5 doses (10 ml)

Cardboard box of 1 glass or plastic vial of 5 doses (10 ml), 25 doses (50 ml), 50 doses (100 ml)

The choice of the active substances, cultivation media, formulation, inactivating agent and adjuvant are justified.

The selection of the manufacturing process of the active substance and the finished product is explained.

**2.B. Description of the manufacturing method**

The IVMP is manufactured fully in accordance with the principles of good manufacturing practice at a licensed manufacturing site.

Process validation data on the IVMP are provided in accordance with the relevant European guidelines.

The inactivation process and the detection limit of the control of inactivation are correctly validated.

**2.C. Production and control of starting materials**

The active substances (*Escherichia coli*, *Staphylococcus aureus*), are the established active substances.

The active substance specifications are considered adequate to control the quality of the material. Batch analytical data demonstrating compliance with this specification are provided.

Starting materials of non-biological origin used in production comply with indicate pharmacopoeia monographs or in-house specifications.

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Biological starting materials used are in compliance with the relevant Ph. Eur. monographs and guidelines and are appropriately screened for the absence of extraneous agents according to the Ph. Eur.

The master and working seeds were produced according to the seed lot system as described in the relevant guideline and satisfactorily tested according to current European requirements.

## 2.D. Control tests during the manufacturing process

The tests performed during production are described and the results of three consecutive runs, conforming to the specifications, are provided.

In-process control tests are carried out on intermediate stages of manufacture in order to verify the consistency of the manufacturing process and the final IVMP.

A specification was set for each intermediate and the analytical methods are described and validated, if applicable.

A shelf life and storage conditions for the intermediate IVMP are defined based on data resulting from stability studies.

## 2.E. Control tests on the finished product

For all tests, a short description of the techniques for analysing the finished product is provided. The tests and their specifications and limits are justified and are considered appropriate to adequately control the quality of the IVMP.

Satisfactory validation data for each analytical method are provided, if appropriate.

The tests performed on the final product conform to the relevant requirements and monographs, if applicable; any deviation from these requirements is justified.

Information regarding the establishment and replacement of reference material is provided.

Batch analytical data from the proposed production site are provided demonstrating compliance with the determined specification.

The tests include in particular:

Appearance

Sterility

Airtightness

Extractable volume

pH

Potency\*

Potency test of *E. coli* component

Potency test of *S. aureus* component

Identity\*

Aluminium Content\*

Thiomersal content\*

Endotoxins assay \*\*

Formaldehyde content\*

\*performed as an in-process control on bulk vaccine.

\*\*performed on bacterin just before vaccine bulk formulation

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The demonstration of the batch-to-batch consistency is based on the results of 3 batches produced according to the method described in the dossier. Other supportive data provided confirm the consistency of the production process.

## 2.F. Batch-to-batch consistency

Full protocols of three consecutive batches of the product, representative of the routine production and giving the results for all tests performed during production and on the finished product, are provided in order to ensure that quality is consistent from batch to batch and to demonstrate conformity with the predefined specifications.

## 2.G. Stability tests

Stability data on the active substances are provided in accordance with applicable European guidelines, demonstrating the stability of the active substance when stored under the approved conditions.

The storing of the live antigens (*E. coli* and *S. aureus*) during the manufacturing process can be storage at the temperature 2-8 °C up to 72 hours. The inactivated and concentrated antigens (*E. coli* and *S. aureus*) can be storage at the temperature 2-8 °C up to 6 months.

The stability results of the tested three batches of bulk vaccine confirmed storage for up to 1 month at 2 - 8 °C after filling into 10L bottles and before filling into the final primary container.

Stability data on the finished product have been provided in accordance with applicable European guidelines, demonstrating the stability of the product throughout its shelf life (2 years) when stored under the approved conditions at (2 °C – 8 °C).

The in-use shelf-life of the broached vaccine (10 hours) is supported by the data provided. The recommendations in the product leaflet should be followed.

## 2.H. Other information

Not applicable.

## 3. SAFETY DOCUMENTATION (safety and residues tests)

### 3.A. General requirements

The safety of the IVMP when administered to the target species, the potential harmful effects (residues in IVMP, substance in foodstuff), the potential serious risk for human beings during product administration and to the environment are adequately described.

The vaccine is administered intramuscularly at dose 2 ml to pregnant cows and heifers.

- First injection is 45 days before expected delivery date.

- Second injection is 3 weeks after the first application.

This complete immunization program must be repeated with each pregnancy.

Safety studies have been performed with a vaccine batch with maximum antigen content produced according to the described production process.

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Field studies have been performed with a representative vaccine batch produced according to the described production process.

### 3.B. Pre-clinical studies

The safety of the administration of one dose, the repeated administration of one dose and safety related to reproductive performance was performed as one controlled laboratory study on the basis of Ph. Eur. 5.2.6. which in total included 16 animals (8 vaccinated animals and 8 control animals with placebo administration).

Rectal temperatures, general health status and local reactions were observed.

The safety studies demonstrate that the administration of one dose and the repeated administration of a dose can be considered to be safe, when used in accordance with the recommended vaccination schedule. The average temperature did not exceed a difference of 1.5 °C for all heifers and none of the heifer raised the body temperature by more than 2 °C. No systemic reactions were observed. Local reactions at the vaccine site were obtained for one individual, there was a swelling with a diameter of 0-2 cm after second dose application. The swelling then grew up to average about 2-5 cm but then decreased again. Before the third dose, the swelling gradually decreased.

The observed reactions are reflected in the relevant SPC and package leaflet sections:

Common (1 to 10 animals / 100 animals treated):	Injection site swelling <sup>1</sup> Elevated temperature <sup>2</sup>
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<sup>1</sup> swelling (up to 5 cm<sup>2</sup>) for up to 2 weeks.

<sup>2</sup> a slight and transient increase in body temperature up to 1.5 °C may occur and disappear spontaneously within the first 24 hours after the injection.

Effects on reproductive performance were examined. No effect on the reproductive performance in vaccinated pregnant heifers and on health status of new born calves was observed thus the following is stated in the SPC and package leaflet:

*“Can be used during the last trimester of pregnancy.”*

There are no data suggesting that this product might adversely affect the immune system of the vaccinated animal or its progeny therefore a specific study was not carried out.

The vaccine is inactivated and thus the specific tests to be performed for live vaccines are not applicable.

The adjuvant and excipients used are aluminium hydroxide, formaldehyde and thiomersal. The excipients and adjuvant are included in the Appendix of the Commission Regulation (EU) No 37/2010 – the substances that are not subject to determination of residues. For this reason, the presence of the residues was not tested. Based on this information, no withdrawal period is proposed.

No specific assessment of the interaction of this product with other medicinal product was made. Therefore, an appropriate warning in the SPC is included.

### 3.C. Clinical trials

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Two farms were selected for the clinical trial that do not vaccinate animals against any of the mastitis agents for which the vaccine is intended.

A total number of 32 pregnant heifers were selected on two farms for clinical evaluation of the safety and efficacy of the vaccine, with 16 animals available in each of these farms, which were divided into 2 groups (8 vaccinated and 8 controls).

Body temperature measurements (rectal) were performed. The temperature was measured daily for 3 days before the application of the test vaccine, 4 hours after the application and then daily for 4 days in both groups of animals in both breeding facilities. The vaccine met the criteria for safety testing in the clinical trial. The average body temperature in all heifers did not exceed a difference of 1.5 °C and none of the heifers had an increase in body temperature of 2 °C or more (from the average pre vaccination temperature). The standard physiological temperature for cattle is in the range 37,5-39,5 °C.

The evaluation of local and systemic reactions was monitored only in vaccinated groups of animals for 14 days after each injection. No animals enrolled in the clinical trial experienced local injection site reactions following vaccine administration during the study period. No systemic reactions following vaccine administration were observed in any animal enrolled in the clinical trial during the study period.

The last indicator monitored in the safety evaluation of the vaccine was the effect of vaccination on the course of pregnancy and parturition, but also on the viability of newborn calves. No animals were aborted or affected during pregnancy and birth as a result of vaccination. The births of all vaccinated animals in both farms were uneventful, the newborn calves were viable and in good physical condition with no signs of defects or deficiencies that could be attributed to the vaccine tested.

The safety of the administration of vaccine was demonstrated in field conditions in pregnant cows/heifers, confirming results of laboratory studies.

### **3.D. Environmental Risk Assessment**

The applicant provided a first phase environmental risk assessment in compliance with the relevant guideline, which showed that no further assessment is required. The assessment concluded that there is a negligible risk to the environment associated with use of the vaccine. No warnings are therefore required.

### **3.E. Assessment required for veterinary medicinal products containing or consisting of genetically modified organisms**

Not applicable.

### **3.F. Residue tests to be included in the pre-clinical studies**

The adjuvant and excipients used are aluminium hydroxide, formaldehyde and thiomersal. The excipients and adjuvant are included in the Appendix of the Commission Regulation (EU) No 37/2010 – the substances that are not subject to determination of residues. For this reason, the presence of the residues was not tested. Based on this information, no withdrawal period is proposed.

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## 4. EFFICACY DOCUMENTATION

### 4.A. General requirements

The vaccine is intended for active immunisation of herds of healthy cows and heifers, in herds of dairy cattle with repeated occurrence of mastitis, to reduce the incidence and severity of clinical mastitis caused by *Staphylococcus aureus* and *Escherichia coli*.

The vaccine is presented as a liquid suspension (2 ml/dose). It should be administered intramuscularly. This vaccine is intended for pregnant heifers and cows. It is applied in 2 doses – first dose is applied 45 days before expected parturition date, second dose is applied 3 weeks after first administration.

The challenge strains used for the vaccine efficacy testing were selected as different from the vaccinal strains:

The *E. coli* (MSLB 8204) strain was prepared.

The *S. aureus* CCM 4223 (MSLB 3109) strain was obtained from Veterinary Research Institute in Brno. The challenge strain belongs to the “CCM 4223” type whereas the vaccinal strain belongs to the “DSM 4910” type. The challenge doses were calibrated before initiation of the efficacy testing to ensure the appropriate severity of the challenges.

For the efficacy testing the batches of the vaccine containing the minimum amount of active substances were used.

The field testing used the vaccine with standard antigens content.

### 4.B. Pre-Clinical Studies

The efficacy of the product was demonstrated in laboratory studies under well-controlled conditions in accordance with the relevant requirements, which show that the vaccine reduces the incidence and severity of clinical mastitis caused by *Staphylococcus aureus* and *Escherichia coli*.

Onset of immunity: 4 weeks after completion of the primary vaccination course.

Duration of immunity: up to 6 months after completion of the primary vaccination course.

#### Onset of immunity:

The vaccinated group of animals received a vaccine with minimal antigen content, leaving the control group without administration. Six days after parturition, all animals were subjected to a challenge experiment. For bacterial challenge with *E. coli* strain WSLB 8204/1, 16 animals (8 vaccinated + 8 control) were used and for bacterial challenge with the *S. aureus* strain WSLB 3109/1, also 16 animals (8 vaccinated + 8 control) were used. The animals were observed for the next 7 days after the administration.

The rectal temperature was measured. The blood samples were taken from the vaccinated group of animals on the day of vaccination before the vaccine administration and 10 days before expected parturition. The blood samples were taken as well on the day of challenge before the administration of challenge inoculum to the heifers from both groups of animals.

Comparison of vaccinated and control groups of animals:

a) rectal temperature

- temperature score

- number of days with higher physiological temperature and fever

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- AUC (ROC) curve of measured rectal temperatures
- b) number of clinical symptoms
- c) somatic cell numbers (logarithms of AUC)
- d) numbers of unsatisfactory milk samples (SB content  $\geq$  200.000/mL)
- e) serological evaluation
- vaccinated group 45 days before parturition x 6 days after parturition
- non-vaccinated group 45 days before parturition x 6 days after parturition
- animals before vaccination
- animals before the challenge experiment

### Rectal temperature

*E. coli*: Statistical comparison of indicators related to rectal temperature revealed that for all parameters evaluated, statistically significant differences were achieved between the vaccinated and non-vaccinated groups at the level of probability  $P < 0.05$ .

*S. aureus*: Compared to the *E. coli* challenge experiment, no significant febrile statuses were observed.

Statistical comparison of indicators related to rectal temperature revealed that for all parameters evaluated, statistically significant differences were achieved between the vaccinated and control groups at a level of probability  $P < 0.05$ .

**The typical signs** of acute mastitis such as hardening, swelling, redness and other such as milk changes (flakes, clots or watery milk) were evaluated. Each sign was included in the statistical evaluation as one point.

*E. coli*: All animals in the vaccinated group suffered from symptoms of acute mastitis during the challenge experiment, with the most common symptoms being swelling and hardening of the quarter. Changes in the consistency of the milk also occurred to a large extent in addition to hardening of the quarters and swelling in the non-vaccinated group.

A statistical comparison of the vaccinated and non-vaccinated groups based on the incidence of acute mastitis symptoms, as well as a statistical comparison of the groups based on rectal temperature indicators, resulted in a statistically significant difference at the  $P < 0.05$  probability level.

*S. aureus*: Only 2 animals in the vaccinated group suffered from symptoms of acute mastitis during the challenge experiment, with only changes in milk consistency. No animal suffered from swelling or hardening of the infected quarters. Changes in milk consistency in both animals persisted for 2 days. In the non-vaccinated group, hardening of the quarters, swelling and also changes in the consistency of the milk occurred to a large extent in the animals.

By statistical comparison of the vaccinated and non-vaccinated groups based on the incidence of symptoms of acute mastitis, a statistical difference was achieved for the *S. aureus* challenge strain between the vaccinated and non-vaccinated groups, where the incidence of symptoms was higher in the non-vaccinated group than in the vaccinated group.

**The milk samples for the somatic cell count** evaluation were taken twice a day at each milking from each quarter separately into 50 ml plastic bottles in an amount of 25 ml with the addition of a preservative compound and the analysis of bottled samples were carried out at accredited laboratories on the Bentley FCM fluoro-optoelectronic analyser by flow cytometry according to the ČSN EN ISO 13366-2. Compliant milk samples contained a maximum of 200.000 somatic cells per 1 mL of milk.

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The numbers of non-compliant milk samples for each strain were compared separately for the vaccinated and non-vaccinated groups, but also for the whole vaccine in total, regardless of the used challenge strain. Milk with a somatic cell count higher than 200.000 / mL was considered non-compliant. It was found that the number of non-compliant samples in vaccinated animals decreased.

A significant increase in somatic cell count was observed in all animals after challenge with both bacterial strains. The higher incidence of somatic cells count as well as clinical symptoms, was not localized only in the infected quarters, but in many cases affected the entire mammary gland. Even in the observation of somatic cell count, it was found that infection caused by the *E. coli* strain was more aggressive than *S. aureus* infection.

Statistical testing showed a decrease in the mean of somatic cells count in milk. Due to the very high variance of values and the small number of animals, the difference in the number of somatic cells count between the vaccinated and the non-vaccinated group was not statistically significant for any of the used bacterial strains.

### **Serological evaluation**

The obtained sera were used for serological evaluation. The aim was to determine that the test item has the potential to stimulate the immune system to produce antibodies against the contained bacterial strains of *E. coli* and *S. aureus*. Evaluation of the antibody response in blood serum was performed by ELISA.

It was confirmed that the amount of antibodies against *E. coli* was several times higher in 10 days before parturition than in the collection before vaccination. It follows that the vaccine stimulates the immune system to make antibodies.

The statistically significant difference was confirmed in the comparison of the groups at the time of blood sampling 6 days after parturition, i.e. on the day of challenge, when the antibody content in the vaccinated group was statistically higher than in the non-vaccinated group. The statistical difference was confirmed for antibodies formation in the vaccinated animals in the comparison of the sampling 45 days before parturition and 6 days after parturition.

It was confirmed that the amount of antibodies against *S. aureus* was several times higher in 10 days before parturition than in the collection before vaccination. It follows that the vaccine stimulates the immune system to make antibodies.

The statistically significant difference was confirmed in the comparison of the groups at the time of blood sampling 6 days after parturition, i.e. on the day of challenge, when the antibody content in the vaccinated group was statistically higher than in the non-vaccinated group. The statistical difference was confirmed for antibodies formation in the vaccinated animals in the comparison of the sampling 45 days before parturition and 6 days after parturition.

### **Conclusion**

Statistical analysis showed a significant decrease in **clinical signs** of acute mastitis after application of both challenge strains in the vaccinated group, which was also shown in the reduction of rectal temperature rise of fever and the number of days when the physiological temperature of animals is above 39.5 °C.

No statistically significant differences were obtained in the evaluation of **somatic cell count**.

It was confirmed that the amount of **antibodies** against both *E. coli* and *S. aureus* was several times higher in 10 days before parturition than in the collection before vaccination. It follows that the vaccine stimulates the immune system to make antibodies, which was confirmed with the serological evaluation where the significant statistical difference between vaccinated and non-vaccinated animals were obtained in the comparison of the sampling 45 days before parturition and 6 days after parturition, i.e. on the day of challenge.

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### Duration of immunity:

In the efficacy study duration of immunity, the vaccinated group of animals received a vaccine with minimal antigens content, leaving the control group without administration. Five months (150 days) after parturition, all animals were subjected to a challenge experiment according to their classification with either *Escherichia coli* strain WSLB 8204/1 or *Staphylococcus aureus* strain WSLB 3109/1 and the animals were observed for the next 7 days after the administration.

Statistical analysis showed a significant decrease **in clinical signs** of acute mastitis after application of both challenge strains in the vaccinated group, which was also shown in the reduction of rectal temperature the number of days when the physiological temperature of animals is above 39.5 °C.

It was confirmed that the number of **antibodies** against *E. coli* and *S. aureus* was several times higher 6 days after parturition than in the sampling before vaccination. It follows that the vaccine stimulates the immune system to produce antibodies, which was confirmed by serological evaluation, where there is a significant statistical difference, which coincides with the results found in a previous study of the onset of immunity.

Despite the decrease in the level of antibodies, a significantly higher level of antibodies was demonstrated 150 days after parturition than before vaccination of the animals, which indicates the ability of the vaccine to protect animals from mastitis caused by bacterial strains of *E. coli* and *S. aureus* up to 5 months after parturition.

### 4.C. Clinical trials

Efficacy of vaccination was also demonstrated under field conditions in a controlled field trial.

The clinical evaluation was conducted at two independent test sites.

The clinical evaluation of the safety and efficacy, a total of 32 animals from 2 farms, with 16 animals available in each of these farms, which were divided into 2 groups (8 vaccinated and 8 controls).

The efficacy of the tested vaccine was evaluated on the basis of the amount of somatic cells and myeloperoxidase enzyme activity in milk samples, but also on the basis of the antibody response of the immune system of vaccinated animals against individual antigens contained in the vaccine.

Blood samples were collected from vaccinated and control animals, at 7 days before vaccination, on the day of vaccination, on the day of revaccination, 6 days after parturition and every 14 days thereafter for 5 months.

Milk samples were collected from both vaccinated and control animals 6 days after parturition and every 14 days thereafter for 5 months.

During the course of the clinical trial, exceptional milk samples were taken outside of the established sampling. These were taken mainly from those mammary gland quarters where signs of acute mastitis were detected. The analysis of the abnormal samples was intended to identify the causative agent of mastitis and thus contribute to the evaluation of the efficacy of the investigational vaccine.

During the clinical trial, mastitis occurred in both breeds. The symptoms of acute mastitis were the same in both breeds. The most frequent symptoms were hardening, swelling, tenderness and soreness of the mammary gland, as well as a change in the milk secretion through small elements (flakes) to a secretion completely unlike milk.

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During the clinical evaluation, a total of 100 abnormal samples were taken in the A1 breed to detect the pathogen causing mastitis. The analyses revealed that mastitis caused by both strains against which the vaccine is intended to be used, i.e., *E. coli* (4 confirmed samples out of 100 samples taken = 4 %) and *S. aureus* (77 confirmed samples out of 100 samples taken = 77 %), occurred in the farm. In addition to these bacterial strains, mastitis caused by *S. uberis* (19 confirmed samples out of 100 samples = 19 %).

A total of 36 abnormal samples were taken during the clinical trial in A2 to detect the pathogen causing mastitis. During the analyses it was found that mastitis caused by both strains against which the vaccine is intended to be used, i.e., *E. coli* (28 confirmed samples out of 37 samples taken = 75,68 %) and *S. aureus* (8 confirmed samples out of 36 samples taken = 22,22 %), occurred in the farm. Mastitis caused by other bacterial strains did not occur in the farm during the clinical evaluation.

During the clinical trial, a total of 1220 milk samples were collected for somatic cell evaluation and 136 emergency samples for detection of the pathogen causing mastitis. Samples confirming *E. coli* (31 confirmed samples out of 1220 samples collected) accounted for 2.54% and *S. aureus* (85 confirmed samples out of 1220 samples collected) accounted for 6.97% of the total. The bacterial strain *S. uberis* (19 confirmed samples out of 1220 samples taken) was represented by 1.56 %. Thus, mastitis samples accounted for 11.23 % of the total number of samples collected. The remaining 88.77 % of the samples did not have significantly elevated somatic cell counts due to mastitis.

The results show that while the dominant mastitis strain in the A1 farm was caused by *S. aureus*, the dominant mastitis strain in the A2 farm was caused by *E. coli*. The results further indicate that the appropriate animal breeds were chosen for the clinical evaluation.

The increase in somatic cell count was observed in both farms mainly in control animals. The high somatic cell counts in the control animals persisted for a long period of time (up to 7 consecutive sampling points), while in the control animals, higher counts were detected at a maximum of 1 sampling point. Somatic cell counts in the vaccinated group reached maximum values of lower millions of somatic cells, whereas in the control group they were higher millions to tens of millions of somatic cells. There was also sampling of non-milk-like secretions that could not be analyzed and therefore these samples were labelled as NA. However, these were only 2 samples obtained from control animal from breed A1. Mastitis occurred very sporadically in vaccinated animals in breed A1, in breed A2 no vaccinated animal suffered from mastitis during the observation period.

During the observation period, a reduction of the Somatic Cell Count (SCC) in vaccinated cows was observed.

Statistical processing of the results of myeloperoxidase activity confirmed that the activity of the enzyme increases in mastitis. In the evaluation of the samples, it was also found that there were statistically significant differences in myeloperoxidase activity between the vaccinated and control groups in both breeds. Thus, myeloperoxidase enzyme activity can be considered as an indicator of ongoing mastitis disease, while the efficacy of the vaccine was demonstrated, with vaccinated animals having lower levels of enzyme activity than control animals, whose increased activity was due to the occurrence of mastitis during the clinical trial, while vaccinated animals had almost no mastitis.

Prior to the start of the clinical trial, antibody levels against individual strains were at low levels. A large increase in antibodies was observed between revaccination and the onset of immunity, i.e., 6 days after delivery, when there was a sharp increase in levels against *E. coli* up to 80% and *S. aureus* up to 110%. During follow-up, levels gradually decreased and the downward trend continued until the end of observation.

Statistical analyses were also performed to compare the levels of antibodies against both strains in vaccinated animals before vaccination, 6 days after birth (onset of immunity) and at

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the last sampling (6+126 days after birth) in both breeds. In all cases of comparison, statistically significant differences in antibody levels for both strains were confirmed, i.e. a statistically significant increase in antibodies at the time of onset of immunity compared to pre-vaccination levels was found, but also a statistically significant decrease in antibodies at the last collection compared to the levels at the time of onset of immunity was found during the clinical evaluation, but despite this, antibody levels in vaccinated animals after the last collection were statistically significantly higher than before vaccination. These results confirm that the vaccine used is effective and stimulates the body to produce antibodies against bacterial strains of *E. coli* and *S. aureus* that have a protective potential for at least 5 months.

The results obtained in this clinical study support the findings obtained in the laboratory trials.

## 5. OVERALL CONCLUSION AND BENEFIT-RISK ASSESSMENT

The data submitted in the dossier demonstrate that when the product is used in accordance with the Summary of Product Characteristics, the risk benefit profile for the target species is favourable and the quality and safety of the product for humans and the environment are acceptable.

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## POST-AUTHORISATION PROCEDURES

The SPC and package leaflet may be updated to include new information on the quality, safety and efficacy of the veterinary medicinal product. The current SPC/labelling/package leaflet is/are available in the Union Product Database (UPD).

## COMMITMENTS

The list of agreed commitments				
No.	Commitment identification	Action required to fulfil the commitment *	Deadline	Requested by Member State
1.	Replacement of the <i>in-vivo</i> potency test by an <i>in-vitro</i> test.	Variation submission	31/10/2028	ES, UK(NI), GB