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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)	*Escherichia coli,* inactivated RP ≥ 1 *Staphylococcus aureus,* 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 *Staphylococcus aureus* and *Escherichia coli*.

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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).

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Withdrawn CMS during original decentralised procedure	-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*

***** 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.

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

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

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 of the active substances and of the IVMP are described and the results of three consecutive runs, conforming to the specifications, are provided.

2.E. Control tests on the finished product

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

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

2.F. Batch-to-batch consistency

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.

2.G. Stability tests

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

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.

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: 45 days before expected parturition date.

- Second injection: 3 weeks after the first administration.

This full vaccination schedule 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.

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[1] Elevated temperature[2]

[1] swelling (up to 5 cm^2) for up to 2 weeks.

[2] 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

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.

Animals were selected on the two farms for clinical evaluation of the safety and efficacy of the vaccine.

Rectal temperature measurements were performed. The average body temperature in all heifers after vaccination 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 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.

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 applicated in 2 doses – a first dose is applicated 45 days before expected parturition date, a second dose is applicated 3 weeks after the first administration.

Relevant challenge strains different from the vaccinal strains and administered at an appropriate dose to ensure the severity of the challenges were used to demonstrate the vaccine efficacy in preclinical efficacy studies.

For the pre-clinical efficacy studies, 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, animals were subjected to a challenge experiment either using a relevant *E. coli* challenge strain or a relevant *S. aureus* challenge strain. The animals were observed for the next 7 days after the administration.

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 animals from both groups.

A comparison of vaccinated and control groups of animals was performed considering the rectal temperature, the clinical symptoms, the somatic cells numbers and serological data.

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.

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.

The typical signs of acute mastitis such as hardening, swelling, redness and other such as milk changes (flakes, clots or watery milk) were evaluated.

E. coli: 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 between the vaccinated and non-vaccinated groups, where the incidence of symptoms was higher in the non-vaccinated group than in the vaccinated group.

S. aureus: 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. Compliant milk samples contained a maximum of 200.000 somatic cells per 1 mL of milk.

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. It was found that the number of non-compliant samples in vaccinated animals was lower.

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 enrolled in the study, 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

A seroconversion in vaccinated animals was observed with the production of antibodies against *E. coli* and *S.aureus*. The antibodies titers were several times higher in the samples collected 10 days before expected parturition (2 weeks after completion of the primary vaccination course) compared to the samples collected before vaccination.

For both *E.coli* and *S.aureus* antigens, a statistical significant difference between the vaccinated and the control groups was confirmed 6 days after parturition (i.e. on the day of

challenge, 4 weeks after completion of the primary vaccination course), with significantly higher serological titers in the vaccinated group compared to non-vaccinated group.

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 and the number of days when the physiological temperature of animals is above 39.5 °C compared to the control group.

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

Antibody levels against E. coli and S. aureus were several times higher 10 days before parturition (2 weeks after completion of the primary vaccination course) compared to before vaccination. This shows the vaccine effectively stimulates antibody production. This was further confirmed by a significant difference between vaccinated and non-vaccinated animals 4 weeks after completion of the primary vaccination course (day of challenge).

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 using relevant challenges (either *Escherichia coli* or *Staphylococcus aureus*) 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 and the number of days with a rectal temperature above 39.5 °C.

The **antibodies titers** against *E. coli* and *S. aureus* were several times higher 6 days after parturition (4 weeks after completion of the primary vaccination course) compared to the titers measured in the samplings collected before vaccination. Despite the decrease in the level of antibodies over time, a significantly higher level of antibodies was demonstrated 150 days after parturition compared to the antibody titer observed 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

The results from vaccination-challenge trials conducted under laboratory conditions have been supplemented with data from field studies.
The clinical evaluation was conducted at two independent test sites.
The clinical evaluation of the safety and efficacy was performed on animals from 2 farms (A1 and A2), in which animals were divided into 2 groups.

The efficacy of the tested vaccine was evaluated on the basis of the somatic cells counts and myeloperoxidase enzyme activity in milk samples, but also on the basis of the antibody responses 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 additionally collected in cows showing signs of acute mastitis. The analysis of the abnormal samples was intended to identify the causative agent of mastitis.

During the clinical trial, mastitis occurred in animals from both farms. The symptoms of acute mastitis were the same in both farms. 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.

During the clinical trial, milk samples were collected for somatic cell evaluation in addition to the samples used for detection of the pathogen causing mastitis. Samples confirming *E. coli* accounted for 2.54% and samples confirming *S. aureus* accounted for 6.97% of the total. The bacterial strain *S. uberis* was also represented in 1.56 % of the samples. 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 selected farms and animal breeds were appropriately chosen for the clinical evaluation.
A significant difference was observed between the vaccinated and control groups in regards to the prevalence of mastitis related to *E. coli* and *S. aureus* in both farms.
During the observation period, there was a statistical difference in the number of somatic cells between the vaccinated and the control group in both farms A1 and A2. This difference was due to the higher frequency, severity and duration of mastitis in the control animals compared to the vaccinated animals.

Myeloperoxidase activity was statistically higher in samples collected from cows showing mastitis. There were also statistically significant differences in myeloperoxidase activity between the vaccinated and control groups in both breeds 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 the last sampling (6+126 days after birth) in both farms. 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.

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

No.	Commitment identification
1.	Replacement of the *in-vivo* potency test by an *in-vitro* test