8 December 2022
EMA/949569/2022
Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Brucellin Aquilon (EMEA/V/C/005577/0000)

Vaccine common name: brucella abortus, strain AQ1302, protein extract

Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted.

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Introduction

The applicant Aquilon Cyl S.L. submitted on 20 July 2021 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Brucellin Aquilon, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 18 March 2020 as Brucellin Aquilon has been developed by means of a biotechnological process.

The applicant applied for the following indication: 'For in vivo diagnostic of *Brucella*-infected pigs through a positive skin reaction'.

The approved indication is: "For in vivo diagnosis of *Brucella*-infected pigs through a positive skin reaction after a positive serological *Brucella* test".

The active substance of Brucellin Aquilon is a concentrated purified protein extract of *Brucella abortus*, strain AQ1302, which does not contain O-polysaccharide (O-PS) component present in other bacteria, which is considered to be the cause of false positive serological reactions (FPSR) in serological tests for *Brucella*. This allows for the unequivocal differentiation of *Brucella*-infected animals from *Brucella*-free animals amongst those showing a positive O-PS serological test. The target species is pigs. The product is intended for administration by intradermal injection.

Brucellin Aquilon is presented as a solution for injection for pigs containing ≥ 1 RP (relative potency) of the active substance per dose (0.1 ml) in multi-dose vials containing 2.5 ml of the solution.

The applicant is registered as an SME pursuant to the definition set out in Commission Recommendation 2003/361/EC.

The rapporteur appointed is Frédéric Klein and the co-rapporteur is Cristina Muñoz Madero.

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC – full application.

On 8 December 2022, the CVMP adopted an opinion and CVMP assessment report.

On 26 January 2023, the European Commission adopted a Commission Decision granting the marketing authorisation for Brucellin Aquilon.

Marketing authorisation under exceptional circumstances

Not applicable.

Scientific advice

The applicant received scientific advice from the CVMP on 17 March 2016 (initial request), 8 September 2016 (clarification), 12 April 2017 (follow-up request), 20 June 2019 (follow-up request) and 17 June 2021 (initial request). The scientific advice pertained to the quality, safety and clinical development part of the dossier.

The applicant followed the scientific advice with regard to the studies to be undertaken but clarifications and justifications about the results of the sensitivity and the specificity studies of the test were required to be included in the final PI.

MUMS/limited market status

The applicant requested eligibility of this application for MUMS/limited market by the CVMP, and the Committee confirmed that, where appropriate, the data requirements in the relevant CVMP guideline(s) on minor use minor species (MUMS) data requirements would be applied when assessing the application. MUMS/limited market status was granted as in vivo diagnosis of *Brucella*-infected pigs is considered a minor use.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the system of pharmacovigilance (Version 5, dated February 2013) of CZ Vaccines S.A.U. A statement signed by the applicant and the qualified person for pharmacovigilance, indicating that CZ Vaccines S.A.U. has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country has been provided in the DDPS.

The DDPS of CZ Vaccines S.A.U. has been submitted for several mutual recognition and decentralised procedures and it had been concluded in these procedures that this version fulfils the requirements of Directive 2001/82/EC.

The marketing authorisation holder (MAH) for the product is Aquilon Cyl S.L. Information on the responsibilities and contractual arrangements between Aquilon Cyl S.L and CZ Vaccines S.A.U. has been provided. In addition, a signed pharmacovigilance statement between Aquilon Cyl S.L. and the QPPV has been given.

It can be noted that from 28/01/2022 a pharmacovigilance system master file (PSMF) should be in place.

Manufacturing authorisations and inspection status

Manufacturing and QC testing of Brucellin Aquilon active substance and intermediates as well as manufacturing, QC testing and batch release of Brucellin Aquilon finished product are performed by CZ Vaccines S.A.U. (A Relva s/n - Torneiros, 36410 O Porriño, Pontevedra, Spain). This site has a valid GMP certificate. The manufacturing authorisation was issued by the Spanish competent authority, Agencia Española de Medicamentos y Productos Sanitarios (AEMPS).

Overall conclusions on administrative particulars

The GMP status of the active substance and finished product manufacturing site has been satisfactorily established and is in line with legal requirements.

Part 2 – Quality

Chemical, pharmaceutical and biological/microbiological information (quality)

Qualitative and quantitative particulars of the constituents

Qualitative and quantitative particulars

The finished product consists of concentrated purified protein extract of *Brucella abortus* strain AQ1302 with saline solution as excipient. Details of the composition of the immunological veterinary product per 0.1 ml dose were provided.

Container and closure

The product is filled into 3 ml type I glass vial containing 25 doses (2.5 ml). The vials are closed with a perforable butyl rubber stopper and a flip-off aluminium seal. The container components comply with the European Pharmacopoeia chapters 3.2.1 (Glass containers for pharmaceutical use) and 3.2.9 (Rubber closures for containers for aqueous parenteral preparations).

Glass vials are washed and dried and then sterilised. The stoppers are washed and sterilised.

Product development

Classic serological diagnostic tests for swine brucellosis often yield false positive results (FPSR) due to cross-reactivity with other bacteria that share common epitopes in the polysaccharides in the cell wall. Brucellin Aquilon is an O-PS-free protein-rich cytosolic extract of a rough (R) mutant (*B. abortus* Tn5::*per*) of the smooth (S) *B. abortus* 2308 reference strain carrying a disrupted *per* (perosamine synthase) gene, which is unable to synthesise perosamine, and hence totally lacks O-PS. Accordingly, the *B. abortus* Tn5::per mutant enables to obtain *Brucella* O-PS free protein extracts. Therefore, as the bacteria causing FPSR do not share protein antigens with *Brucellae* and these protein extracts cause characteristic cutaneous responses in *Brucella*-infected animals, Brucellin Aquilon allows the unequivocal differentiation of *Brucella*-infected pigs (skin test positive) amongst those with a FPSR (skin test negative).

Justification of the use of Brucella abortus

B. abortus, B. melitensis and *B. suis* are antigenically highly homogeneous so that the analysis of cytosolic antigens reveals no significant differences. Analysis of the corresponding genomes shows the striking similarities that exist among the three species as they only reveal a low number of features that may be important in host specificity. Accordingly, strains of these three species are equally suitable for the preparation of allergens.

Justification of the use of Brucella abortus Tn5::per strain

An obvious condition of cytosolic extract (CP) extracts intended to screen out FPSR is that they strictly need to be free of S-LPS or O-PS. The *per* gen codes for the perosamine synthase which is strictly required for the synthesis of this sugar. In the O-PS biosynthesis pathway, perosamine is then formylated and polymerised to generate the N-formyl-perosamine homopolymer that constitutes *Brucella* O-PS. Therefore, perosamine synthase dysfunction totally blocks its synthesis and results in a stable R mutant.

Brucellin Aquilon is based on the cytosolic extract of a mutant of *Brucella abortus* 2308: the strain *B.*

abortus Tn5::*per*. Since this mutant presents a disruption of the *per* gen (ORF BAB1_0544; perosamine synthase), it lacks the O-polysaccharide. Strain *B. abortus* 2308_DMB13 was identified by Southern blot: it was shown that the mini-Tn5 was inserted in the *per* gene.

The use of a cytosolic extract was justified.

Justification of the excipient

Pyrogen-free saline solution will be used in Brucellin Aquilon. This saline solution does not induce any skin reaction. No preservative is added to the composition due to shelf life for this product once opened: it should be used immediately and a maximum of 25 animals will be treated with each vial.

Composition of batches used in the clinical trials

The preclinical studies were performed with an experimental batch while the clinical trials were performed with GMP batches. Comparability between the experimental batch and the GMP batches was demonstrated. Furthermore, the first GMP industrial batch has been considered as the standard batch.

Description of the manufacturing method

The strain AQ1302 of *Brucella abortus* used for the production is cultured in an appropriate medium and then inactivated with phenol. The inactivated culture is concentrated by microfiltration and purified by cell lysis. The obtained antigen is centrifuged, and the supernatant is collected.

The soluble antigen obtained is formulated to the target protein concentration; sterile saline solution is added, if needed.

The validation of the inactivation step has been provided and is considered adequate.

Production and control of starting materials

Starting materials listed in pharmacopoeias

Starting materials listed in a Pharmacopoeia that are used for routine production processes of Brucellin Aquilon including media components are included in the dossier. Copies of the certificates of analysis from the suppliers are provided.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

Active substance

The production of the master seed bank (MSB) and working seed bank (WSB) are described. The following control tests were performed on the MSB and WSB: vacuum, residual moisture, purity, viable germ count, dissociation index and identification. A risk assessment of the transmission of animal spongiform encephalopathy agents through the strain AQ1302 of *B. abortus* is provided. It is concluded that AQ1302 strain of *B. abortus* does not present a potential risk of transmitting animal spongiform encephalopathy and complies with the established in the Note for guidance EMA/410/01 rev. 3.

Substances of biological origin

Raw materials of biological origin used during the manufacturing process are included in the dossier. These are components of the agar and culture medium. Certificates of analyses are provided, and these raw materials are accepted for use for production of Brucellin Aquilon.

Starting materials of non-biological origin

Substances of non-biological origin

Raw materials of non-biological origin used during the manufacturing process are described in the dossier. A certificate of analysis was provided.

In-house preparation of media and solutions consisting of several components

Information regarding the qualitative and quantitative composition of all culture media, their production and treatment processes and their storage conditions is provided in the dossier.

Control tests during the manufacturing process

The applicant has provided an overview and description of the control tests performed during manufacturing: Gram staining (purity); colony morphology (purity); growth test (purity); dissociation index; viable germ count; optical density; inactivation/sterility; sterility; dry weight; protein concentration. Test descriptions and the limits of acceptance were presented.

The tests for viable germ count, dry weight and inactivation/sterility have been adequately validated.

The in-process tests are deemed sufficient to control all the critical steps during the manufacturing.

Control tests on the finished product

The description of the methods used for the control of the finished product and the specifications were provided. Method validations were provided for the protein concentration test, the phenol content test, sterility test and the potency test. The control tests on the final bulk and the finished product are: extractable volume; appearance; protein concentration; phenol content; pH; sterility; potency; protein profile/identification (SDS-PAGE); presentation.

The in vivo potency test measures the skin reaction in sensitised guinea pigs. A detailed description was provided. The in vivo potency test has been validated and is deemed acceptable. The applicant has proposed the SDS-PAGE protein profile assay as identity assay. This assay was described in detail and acceptance criteria were defined which seem sufficiently specific.

Batch-to-batch consistency

The applicant provided batch data from three antigen batches and from three batches of finished product. The test results of the antigen batches and the finished product batches were compliant with the specifications. These batch data are sufficient to demonstrate batch consistency and to confirm the validated status of the manufacturing process.

Stability

The applicant has provided stability data for the antigen (up to 24 months for concentrate antigen and soluble antigen) and the finished product (up to 27 months).

For the concentrate antigen (after concentration) and soluble antigen (after purification), a shelf life of 24 months is proposed when stored at -30 ± 10 °C.

For the concentrate, stability data up to 24 months are provided for 1 batch and a stability study was initiated for a second independent batch. For the soluble antigen, stability data are available for 2

batches up to 24 months. An additional stability study was initiated for a third independent batch. Importantly, to confirm that long term storage of the antigen does not impact the final product quality and potency (which is not assessed at antigen level), the applicant also started a stability study of a finished product batch that was formulated using antigen that had been stored for 31 months. At time point zero (0 months), all results comply with the specifications, including potency and protein profile. Taken together, given the MUMS status of the product, the currently available data are deemed sufficient to approve the proposed shelf life of 24 months for the antigen concentrate and the soluble antigen when stored between -20 °C and -40 °C.

For the finished product, the applicant proposed a shelf life of 24 months when stored at 2-8 °C, based on the currently available stability data. Stability data were provided for two batches of the finished product up to 27 months and 15 months respectively, including potency and protein profile. All results complied with the specifications. No trends are observed. Furthermore, an additional stability study was initiated with another finished product batch. At time point zero (0 months), all results comply with the specifications, including potency and protein profile. Taken together, given the MUMS status of the product, the currently available data are deemed sufficient to approve the proposed shelf life of 24 months for the finished product when stored between 2 °C and 8 °C.

No in-use stability is defined since the product is to be used immediately.

New active substance (NAS) status

Brucellin Aquilon is an in vivo diagnostic skin test for pigs whose active substance is based on a protein-rich cytosolic extract of a rough mutant (*Brucella abortus* AQ1302 = *Brucella abortus* Tn::5 *per*) of the smooth *B. abortus* 2308 reference strain carrying a disrupted *per* (perosamine synthase) gene, which is unable to synthesise perosamine, the most important component of the O-PS, which enables to obtain *Brucella* O-PS free protein extracts.

The O-PS-free cytosolic protein extract of the *Brucella abortus* AQ1302 strain has not been used previously in a veterinary diagnostic kit or any other veterinary medicinal product registered in the EU. Therefore, the active substance in Brucellin Aquilon is considered a new active substance.

Overall conclusions on quality

The applicant has described the composition and development of the product and its active ingredient, the manufacturing process, the tests performed during manufacture and on the finished product, the batch data and the stability data.

Based on the review of the data presented, the quality of Brucellin Aquilon is considered acceptable.

The applicant is given the following recommendation.

<u>*Recommendation*</u>: The applicant is requested to provide the results of the ongoing stability studies for the two independent antigen batches, including test results on soluble antigen, as well as the results from the stability study of the finished product batch formulated with aged antigen when these data are available. The applicant is also requested to provide the results of the ongoing stability studies for the finished product when these data are available.

Part 3 – Safety

Introduction and general requirements

Brucellin Aquilon is a MUMS product intended to be used as a diagnostic tool (skin test) to differentiate *Brucella*-infected from *Brucella*-free pigs amongst those tested positive for *Brucella* with serological tests relying on O-PS antigens.

The active substance of Brucellin Aquilon, i.e. *Brucella abortus* strain AQ1302 protein extract, is a new active substance not authorised for a veterinary medicinal product in the EU before. A full safety file in accordance with Article 12(3)(j) has been provided.

The active substance is a concentrated purified protein cytosolic extract of the *B. abortus* strain AQ1302 (originally *Brucella abortus* Tn5::*per*), which has been genetically modified to carry a disrupted perosamine synthase (*per*) gene. This genetic modification renders the bacteria unable to synthesise perosamine, the most important component of the O-PS (which is shared by some Gram-negative bacteria causing FPSR). The strain AQ1302 was used to manufacture a laboratory batch which was used in pre-clinical studies as well as two industrial batches intended for laboratory and clinical studies.

Studies included in the dossier have been conducted in compliance with general requirements of Directive 2001/82/EC and Ph. Eur. 0062. There are no specific legal requirements concerning *Brucella* antigens for *in vivo* diagnostic testing (allergic skin test). Regarding animal welfare, the pre-clinical studies complied with Directive 2010/63/EU and applicable national provisions (Spanish Royal Decree 1369/2000) but were not conducted in accordance with GLP standards. However, the studies were conducted within a quality system in accordance with appropriate quality standards. The results of the pre-clinical studies have been published in 2 peer-reviewed articles.

According to the proposed SPC, one dose (0.1 ml) of Brucellin Aquilon should be injected intradermally with an adequate medical device in a non-pigmented area of the skin in the perianal region close to the tail. Forty-eight hours after inoculation, a reading is performed to detect any inflammatory reaction and/or haemorrhage at the injection site (positive reaction).

Three scientific advices were given concerning the different batches of Brucellin Aquilon used in pre-clinical and clinical studies, concerning the safety in pregnant females as well as adverse immunological effects. The applicant followed the scientific advice with regard to the safety studies.

Safety documentation

Both the pre-clinical studies and the clinical trials were carried out in *Brucella*-free commercial farms in Spain. In the two pre-clinical laboratory studies, a *Brucella*-free farm and a *Brucella*-infected farm were included, but the primiparous sowsof the *Brucella*-infected farm were not monitored for safety parameters after administration of the product.

Study reference	***Study title***	***Batch used***
CITA1302-02	Determination of optimal dose and reading interval. Safety of one dose and overdose in negative animals. Efficacy in positive and negative animals: diagnostic sensitivity and specificity.	Experimental batch BA-169 (manufactured by UNAV)

CITA 1302-04	Macroscopical and microscopical evaluation of the injection site. Safety of one dose in negative animals. Efficacy in positive and negative animals: diagnostic sensitivity and specificity.	
AQ 1302-07	Clinical study to determine the safety and diagnostic specificity of the Brucellin BM skin test in *Brucella*-free pigs (intradermal administration).	2013429 (manufactured by CZV; standard batch)
AQ1302-08	Clinical study to determine the safety of the Brucellin BM skin test in *Brucella*-free reproductive sows (intradermal administration)	
AQ1302-10	Clinical study to determine the safety of an overdose and repeated dose as well as the absence of sensitization of the Brucellin BM skin test in *Brucella*-free pigs (intradermal administration)	

The safety of the administration of one dose and of an overdose of Brucellin Aquilon was investigated in studies CITA 1302-02 and CITA 1302-04, with an experimental batch administered to Landrace x Large-White crossbred primiparous sows aged from 8 months.

The industrial batch (2013429) used in some of the safety studies was also administered to 50% Large White/25% Landrace/25% Tai Zumu x Pietrain crossbred pigs in the 3 clinical trials, which complied with GCP standards.

Laboratory tests

Safety of the administration of one dose and of an overdose

In study CITA1302-02, 4 doses (200 + 50 + 12 + 6 µg) of the experimental batch BA-169 of Brucellin Aquilon were administered to each of 5 *Brucella*-free primiparous sows, and local and general reactions were monitored. No local reactions were detected over the 14 days of the monitoring at any dose, including the proposed dose (50 µg) as well as the 4-fold overdose (200 µg). No general reactions to the highest global dose of 268 µg/gilt (200 + 50 + 12 + 6 µg) as well as any increase in body temperature beyond physiological variations over the 4 first days were observed. The maximal increase of rectal temperature was 0.85 °C.

Study CITA1302-04 was devised to detect histological changes at the injection site following the administration of the proposed dose (50 µg) in addition to conventional safety parameters. Following injection to 7 pregnant primiparous sows from a *Brucella*-free herd, local and general signs (rectal temperature and clinical signs) were monitored over 14 days (except for the temperature, which was monitored over 4 days).

The injection did not trigger any local or general reaction. The maximal increase of rectal temperature was 0.53 °C one day after the injection.

The safety at the proposed dose (50 µg) was also investigated with an industrial batch in 5–5.5-month

old fattening pigs (study no AQ1302-07). Brucellin Aquilon was compared to a placebo (saline solution) (90 pigs in each group). Neither local (macroscopic or microscopic lesions) nor general clinical reactions (scored with a standardised clinical scale) or an increase in body temperature were noticed in any of the groups.

A double dose of an industrial batch of Brucellin Aquilon was also administered in study AQ1302-10, and neither local nor general reactions were reported.

Safety of the repeated administration of one dose

The safety of a 2-fold overdose followed by with three repeated standard doses was investigated with an industrial batch in 5-month old fattening pigs (GCP-compliant study AQ1302-10). Pigs treated with antibiotics or anti-inflammatory products in the last month or week, respectively, were not included in the study. A group of 15 cross-bred pigs from a commercial *Brucella*-free farm were injected with Brucellin Aquilon and then compared to a placebo group of 15 other pigs injected with saline. Pigs were injected twice the recommended dose at day 0 and the normal dose at day 14, 21 and 28, and were monitored for local reactions (macroscopic and microscopic) and general reactions over 35 days. The temperature was measured over the 4 days after the 1st injection.

Within the 4 days after the initial administration of the overdose, no differences between groups with regard to body temperature, local and general reactions, or histological findings were observed., During the study period, animals from both groups suffered from respiratory infections and lethargy, and while increases of temperature or general signs were reported, no statistically significant differences between the testing groups at the end of the observation period were found. After three repeated injections, body temperature, clinical signs and local reactions, either macroscopic or microscopic, did not differ between the two groups.

In brief, neither local nor systemic reactions were reported after an overdose or repeated injections of Brucellin Aquilon in fattening pigs. The lack of local and general reactions after the 4 injections together with the lack of seroconversion are indicative of a lack of sensitisation to *Brucella abortus* strain AQ1302 antigens.

Examination of reproductive performance

A dedicated GCP-compliant study (study no AQ1302-08) was carried out in a *Brucella*-free farm to detect any impact of an industrial batch of Brucellin Aquilon on the fertility and the fecundity of sows. One hundred commercial cross-bred sows (Large White/Landrace/Tai Zumu/Pietrain) were included at 5 different stages of their reproduction cycle (1st, 2nd or 3rd third of pregnancy, 2nd week of lactation, after weaning and before insemination) after stratification according to the number of pregnancies and were injected with either saline or with 50 µg of Brucellin Aquilon (10 sows in each group) according to the aforementioned procedure. In each group, parity was evenly distributed (6 nulliparous sows, 6 sows with more than 5 farrowings and 8 sows in between these stages), except in the group intended to investigate fertility only, in which no nulliparous sows were included. Sows treated with antibiotics or anti-inflammatory products in the last month and week, respectively, were not included in the study.

The immediate reactions, either local or general, were monitored in each of the 10 groups for 14 days after administration of 50 µg. Over the first 4 days, rectal temperature was measured and local reactions as well as general clinical signs were recorded. Moreover, the pregnancy was monitored to record any abortions or adverse reactions attributable to Brucellin Aquilon. Parameters recorded included the number of piglets born alive, dead or malformed, the litter weight at birth and placenta expulsion. The piglets of the groups of sows injected either in the last third of gestation or in lactation

were also monitored until weaning to measure their mortality rate, the number of weaned piglets per sow and their mean bodyweight at weaning. Any interruption of lactation was also recorded. Finally, the impact of Brucellin Aquilon on fertility was followed in the groups of lactating sows and sows injected during their dry period, in which both the weaning to service interval and the weaning to fertile service interval were determined.

Immediate local reactions were equally reported in both groups (score of 1 out of 4) and were attributed to the injection procedure itself rather than to an allergic reaction. Clinical signs did not differ between the 2 groups and only one control sow injected with saline (in the dry period) showed an increase of temperature above 2 °C.

Regarding fecundity parameters, only the mean bodyweight of piglets at weaning slightly differed between the 2 groups. However, this difference was inconsistent across experimental groups and therefore it is not considered relevant. No difference was reported with regards to fertility parameters.

In conclusion, no effects of Brucellin Aquilon on reproductive performance of the sows at any stage of the reproductive cycle and age were found in this study.

In addition, in safety study CITA 1302-02 and CITA 1302-04, no clinical signs associated with reproduction parameters were reported during a two-week observation period in any of the 17 pregnant primiparous sows which were injected with an experimental batch of the product at different stages of gestation.

Overall, it is concluded that Brucellin Aquilon can be used during pregnancy and lactation, as laid down in section 4.7 of the SPC.

Examination of immunological functions

Brucellin Aquilon is an allergenic extract from *Brucella abortus* strain AQ1302. It is therefore anticipated to trigger an allergic reaction in *Brucella*-infected pigs and no such reaction in *Brucella*-free animals.

This allergic reaction has been characterised in the efficacy part of the dossier as a local mixed type III and IV hypersensitivity reaction without general manifestations (study CITA1302-04). This allergic reaction remained unchanged following the administration of a quadruple dose (CITA1302-02) or repeated injections (CITA 1302-06).

No such allergic reactions were reported in *Brucella*-free animals in the safety studies regardless of the dose or following repeated administration of the product (AQ1302-10).

No other immunological investigation has been undertaken and no other concern of immunological nature is expected with the data provided.

User safety

The applicant has presented a user safety risk assessment which has been conducted in accordance with CVMP guidelines on user safety for immunological and pharmaceutical veterinary medicinal products (EMEA/CVMP/IWP/54533/2006 and EMEA/CVMP/543/03-Rev.1, respectively). Brucellin Aquilon is a multi-dose ready-to-use product intended to be administered by healthcare professionals. The main potential route of accidental contact with Brucellin Aquilon is considered accidental self-injection. The administration of the product is intended to be performed by use of a medical device which is considered to lower the risk of accidental self-injection. Adequate information to that effect has been included in section 4.5 of the SPC. The excipients are commonly used in other immunological veterinary medicinal products and do not pose a risk for the user. In conclusion, the CVMP concludes

that the product does not pose an unacceptable risk to the user when used in accordance with the SPC.

Study of residues

MRLs

The active substance being a principle of biological origin intended to diagnose the state of immunity towards *Brucella suis* is not within the scope of Regulation (EC) No 470/2009.

The excipients listed in section 6.1 of the SPC are either allowed substances for which Table 1 of the Annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required (sodium chloride) or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product (water for injection).

Withdrawal period

The withdrawal period is set at zero days.

Interactions

The applicant has not provided data investigating interactions of Brucellin Aquilon with other veterinary medicinal products and therefore proposes to include a statement in section 4.8 of the SPC that "No information is available on the safety and efficacy of this immunological veterinary medicinal product when used with any other veterinary medicinal product. A decision to use this immunological veterinary medicinal product before or after any other veterinary medicinal product therefore needs to be made on a case-by-case basis".

Field studies

The safety of the Brucellin Aquilon has been investigated in the GCP-compliant study AQ 1302-07 where 90 male and female cross-bred fattening pigs (Large White/Landrace/Tai Zumu/Pietrain) from 3 *Brucella*-free farms where skin-tested according to the aforementioned procedure. Pigs treated with antibiotics or anti-inflammatory products in the last month and week, respectively, were not included in the study. The 5–5.5-month-old pigs were randomly assigned to the Brucellin Aquilon group or control group (saline). Both local and general reactions (temperature and clinical signs) were monitored over 2 weeks according to predefined forms and a biopsy of the injection site was carried out in 5 piglets randomly selected from each group 2, 7 and 14 days after injection. The biopsy was graded according to the vascular reaction and the cellular infiltration to appraise whether an allergic reaction was present. Brucellin Aquilon-injected pigs did not differ from controls in terms of local or general reactions. In both groups, the mean temperature increased less than 1.5 °C and no animal showed a rectal temperature of over 2 °C. Coughing was reported in 1 control and 4 treated pigs, and apathy was reported in 3 controls. Mild macroscopical alterations (score of 1 in about 20% of controls and about 30% of treated animals) were reported 4 hours after inoculation and the following day in relation with the intradermal inoculation. These macroscopical lesions correspond with mild microscopical inflammation (score 1) of the perivascular superficial dermis which were observed in all but one animal in the control group and in all treated pigs over the 2-week monitoring period. They were associated with the trauma of the puncture itself and none of them could be classified as allergy-like. These results corroborate that Brucellin Aquilon is not allergenic in *Brucella*-free pigs.

A second GCP-compliant study (AQ1302-08) carried out with an industrial batch was devised to address the safety of Brucellin Aquilon on the reproduction of females with no concerns observed (see

the section "Examination of the reproductive performance" for details).

A third GCP-compliant safety study (AQ1302-10) carried out with an industrial batch was devised to investigate the safety of an overdose and of the repeated administration of Brucellin Aquilon in *Brucella-free* pigs with no concerns observed (see section "Safety of the repeated administration of one dose" for details).

Environmental risk assessment

An environmental risk assessment in accordance with the "Note for guidance: environmental risk assessment for immunological veterinary medicinal products" (EMEA/CVMP/074/95) has been provided by the applicant. Based on the data provided, the ERA can stop at phase I. Brucellin Aquilon is not expected to pose a risk for the environment when used according to the SPC.

Overall conclusions on the safety documentation

Brucellin Aquilon is an allergenic extract of the rough strain of *B. abortus* strain AQ1302. Its safety was tested with both an experimental batch and an industrial batch in studies carried out in farms free of brucellosis. The 2 studies performed with the experimental batch were designated as "laboratory" studies, while those carried out with the industrial batch were qualified as "field" studies. No local or general reactions were reported in any of these studies. The lack of local reaction was corroborated by microscopical investigation of the injection site (CITA1302-04). The safety of the product in target animals is thus considered acceptable when administered according to the recommended schedule and via the recommended route.

The safety of the skin test in *Brucella*-infected animals is additionally supported by results obtained from efficacy studies described in detail in section 4, with no adverse effects reported apart from a local inflammation at the injection site consistent with the anticipated local allergic reaction. Additionally, no sensitisation was reported in *Brucella*-free 5-month-old fattening piglets.

No adverse effects emanating from Brucellin Aquilon on the fecundity and fertility of sows were detected. The product is thus considered to be safe when used in pregnant animals at all stages of gestation as well as in lactating animals. The SPC has been updated accordingly.

Regarding user safety, Brucellin Aquilon does not pose an unacceptable risk to the user (veterinarians) when used in accordance with the SPC. Appropriate warnings have been included in the product literature.

No Brucellin Aquilon residues in porcine edible tissues are expected and a withdrawal period of zero days is considered adequate.

An appropriate environmental risk assessment was provided. The product is not expected to pose a risk for the environment when used according to the SPC.

Part 4 – Efficacy

Introduction and general requirements

Brucellin Aquilon is a MUMS product intended to be used as a diagnostic tool (skin test) to differentiate *Brucella*-infected from *Brucella*-free pigs amongst pigs with a positive reaction in the classical *Brucella* serological tests which detect the O-polysaccharide (O-PS) moiety of *Brucella* smooth lipopolysaccharide (S-LPS). Tests based on this S-LPS are not specific of *Brucella* and cross-antigenicity

have been reported with other bacteria such as *Yersinia enterocolitica* O:9, *Francisella tularensis*, *Vibrio cholerae*, *Escherichia coli* O:157, few *Salmonella* serotypes*, Afipia clevelandensis*, leading to FPSR (false positive serologic reaction).

The active substance is based on a concentrated purified protein extract from the cytosol of the *Brucella abortus* strain AQ1302 (originally *Brucella abortus* Tn5::*per*), which has been genetically modified to carry a disrupted *per* (perosamine synthase) gene. This genetic modification makes the bacteria unable to synthesise perosamine, the most important component of the O-PS (which is shared by some gram-negative bacteria causing FPSR). The AQ1302 strain was used to manufacture an experimental laboratory batch which was used in the pre-clinical studies as well as two industrial batches that have been used in pre-clinical and clinical studies.

Studies included in the dossier have been conducted in compliance with general requirements of Dir. 2001/82/EC and Ph. Eur. 0062. There are no specific legal requirements concerning *Brucella* antigens for in vivo diagnostic testing (allergic skin test). Regarding animal welfare, the pre-clinical studies complied with Directive 2010/63/EU and applicable national provisions (Spanish Royal Decree 1369/2000).

Five scientific advices were given concerning the different batches of Brucellin Aquilon used in pre-clinical and clinical studies, concerning the sensitivity and the specificity of the test. The applicant followed the scientific advice with regard to the study to be undertaken but clarifications and justifications about the results of the sensitivity and the specificity studies of the test were required to be included in the final PI.

Challenge model:

A *Yersinia enterocolitica* O:9 sensitising model was established in sows (study AQ1302-11) to more accurately address the main cause of FPSR in pigs.

Efficacy parameters and tests:

According to the recommendations of use described in the proposed SPC, Brucellin Aquilon should be injected intradermally with an adequate medical device in a non-pigmented area of the skin in the perianal region close to the tail. Forty-eight hours after inoculation, a reading is performed to detect any inflammatory reaction and/or haemorrhage at the injection site (positive reaction). The applicant states that a regular dose of Brucellin Aquilon must trigger a readable skin reaction in *Brucella* infected animals. The efficacy parameters relevant for Brucellin Aquilon are:

- Diagnostic specificity (Sp): proportion of *Brucella*-free animals that are correctly identified as negative by Brucellin Aquilon (true negative rate).
- Diagnostic specificity (Sp) in *Brucella*-free animals affected by FPSR: proportion of brucellosis free animals positive in the serological tests that are correctly identified as negative by Brucellin Aquilon.
- Diagnostic sensitivity (Se): proportion of *Brucella*-infected animals (microbiological culture as gold standard test for *Brucella suis* infection confirmation) that are identified as positive by Brucellin Aquilon (true positive rate).
- Relative diagnostic Sensitivity (RSe): proportion of Rose Bengal Test positive animals in *Brucella*-infected population that are identified as positive by Brucellin Aquilon.

Also, the possible development of anergy (i.e. absence of immune response to a particular antigen after the administration of repeated doses) has been investigated. It has been confirmed that *Brucella suis*- infected pigs would react positive in the Brucellin Aquilon skin test even after repeated doses of

the product.

Efficacy documentation

Firstly, the procedure to be followed to conduct the allergic skin test and some factors of variations were established in 3 preclinical studies (recommended dose, reading time/onset of reaction, anergy).

The specificity and the sensitivity of the skin test have been determined in the different pre-clinical and clinical studies. All these studies were performed in Spanish commercial farms but one (AQ1302-11) which was carried out in a GLP-accredited animal facility.

An experimental batch of the product manufactured in the Universidad de Navarra (Spain) was used in all "pre-clinical" studies while 2 batches (an industrial and a standard) were used in "clinical" studies. Both batches have been concluded to be equivalent in further experiments determining their biological activity in an established guinea pig model (more information in section 2).

Study reference	**Study title**	**Batch used**
CITA1302-01	Efficacy in positive animals: diagnostic sensitivity of the test - I	BA-169 (Experimental Manufactured by UNAV)
CITA1302-02	Determination of optimal dose and reading interval. Safety of one dose and overdose in negative animals. Efficacy in positive and negative animals: diagnostic sensitivity and specificity	
CITA 1302-03	Efficacy in positive animals: diagnostic sensitivity of the test - II	
CITA 1302-04	Macroscopical and microscopical evaluation of the injection site. Safety of one dose in negative animals. Efficacy in positive and negative animals: diagnostic sensitivity and specificity	
CITA 1302-05	Efficacy in positive animals: Relative diagnostic sensitivity of the test	
CITA 1302-06	Efficacy in positive animals: absence of anergy	
AQ 1302-11	Study to determine the diagnostic specificity of Brucellin skin test in *Yersinia enterocolitica* O:9 sensitised pigs	2016980 (industrial)
AQ 1302-07	Clinical study to determine the safety and diagnostic specificity of Brucellin BM skin test in *Brucella* free pigs (Intradermal administration)	2013429 (industrial)
AQ1302-09	Clinical study to determine the diagnostic specificity of Brucellin BM skin test in False Positive Serological Reactions (pigs) (Intradermal administration)	
AQ1302-12	Clinical study to determine the specificity of Brucellin Skin test in False Positive Serological Reactions cases of *Brucella suis* (Intradermal administration)	

Laboratory trials

Six pre-clinical studies were carried out by CITA (Agrifood research and Technology Centre of Aragón). They were claimed to be performed according to the principles of quality assurance.

These studies combined efficacy and safety evaluation of the experimental batch BA-169, in pregnant Landrace x Large-White crossbred sows naturally exposed to *Brucella suis* which were either serologically diagnosed as *Brucella suis*-positive or -negative or have had an abortion caused by *B. suis,* later confirmed by bacteriological culture.

Another pre-clinical study (AQ1302-11) which was also claimed to be conducted according to the principles of quality assurance, was performed in an animal facility where pigs were experimentally sensitised to *Y. enterocolitica* O:9 and skin tested with the industrial batch 2016980.

The efficacy of Brucellin Aquilon was determined by a qualitative assessment of the injection sites carried out independently by at least 3 different trained technicians who reported the presence or lack of "evident inflammatory response" and when present, qualified as: erythema mild (ME) or not (E), colour from reddish to an almost black (DN), papule (P) and nodule (N).

The applicant has provided a summary of the studies, including information which has been considered relevant for this application.

Test parameter determination

In a first approach to determine the optimal dose and the onset of reaction, 50 and 100 µg/0.1 ml of Brucellin Aquilon were tested in 18 primiparous sows older than 8 months that aborted due to *B. suis* (CITA1302-01). The two sites of administration were checked 24 and 48 h after the injection and, while no reaction was noticed in 2/18 primiparous sows at 24 h, they were all positive at 48 h without qualitative differences observed between the 2 doses.

The determination of the optimal dose and the onset of reaction was consolidated in the study CITA1302-02. In this study, two different ranges of Brucellin Aquilon were investigated. Primiparous sows aged more than 8 months were enrolled after abortion caused by *B. suis* in a naturally-infected farm (NIF) and were compared to pregnant sows from a Spanish officially accredited *Brucella*-free farm (OFF).

Firstly, eight NIF primiparous sows were administered with 50 and 100 µg doses and monitored over 14 days. The inter-individual differences were higher than those between the 2 doses. While five primiparous sows were considered positive 24 h after injection, they were all positive at 48 h or 72 h with an inflammation turning from the vasoactive step into the cellular one.

Secondly, 4 doses ranging from 6 to 200 µg were administered both to 5 NIF and 5 OFF sows. Investigations were run according to the same procedures. While no skin reaction was noticed in OFF sows, all the NIF sows showed a positive reaction. The reactions were more severe from 48 h after inoculation and when a dose of 50 µg or higher was used.

The dose of 50 µg/0.1 ml and the 48 h reading time point were thus chosen by the applicant.

In a similar confirmatory study (CITA 1302-03), 16 *B. suis*-aborted primiparous sows were intradermally injected 50 µg/ml of Brucellin Aquilon in 4 perianal sites according to the aforementioned procedure. The overall result was that 13 out of 16 aborted primiparous sows were positive in the skin test, i.e. a sensitivity of 81%.

The histology of the skin reaction after injection of Brucellin Aquilon (50 µg) was investigated in study CITA1302-04 (in 2 different farms, 7 animals each. While in the Spanish officially accredited *Brucella*-

free farm, none of the pregnant sows exhibited macro- or microscopical lesion at 48 h, lesions associated with both mild type III and type IV allergic reactions were reported at the injection site of all the *B. suis*-aborted primiparous sows in the other farm. As anticipated, the type III-associated antibody reaction tends to happen within 24 h, whilst the type IV- associated mononuclear cell infiltration of the injection site by 48 h. In this study, Brucellin Aquilon specificity was also considered in comparison with the same amount of an allergen from *Ochrobactrum intermedium*. The *B. suis*-aborted primiparous sows did react alike to both allergens while the microscopic scoring highlighted a slight lower mean intensity with this heterologous antigen. This result highlights the lack of genuine specificity of Brucellin Aquilon. The conditions under which the specificity of Brucellin Aquilon has been validated are clearly explained in the SPC.

Ochrobactrum spp. have been isolated from the digestive flora of 2% of cattle (2/100), 4% of broiler chickens (8/200) and 14% of pigs (14/100) at the slaughterhouse (Alonso 2017). Moreover, it has been reported to cause opportunistic infections in humans, goat and sheep but only one report has been published in a pig, causing neurological clinical signs (Gu 2020). The LPS of *Ochrobactrum anthropic* has been shown to react with sera of *B. melitensis* infected sheep, goat or rabbit (Velasco 1997).

Therefore, insofar as *Ochrobactrum spp.* infected pigs may give FPSR, Brucellin Aquilon would not help to differentiate them from true *B. suis*-infected-pigs and the actual frequency of these opportunistic infections is unknown.

The sensitivity of Brucellin Aquilon has been monitored in a farm where *B. suis* circulated (study CITA1302-05). Primiparous sows older than 8 months (n=80) were tested with Brucellin Aquilon according to aforementioned procedure after being diagnosed *B. suis* antibody positive, associated with brucellosis typical clinical signs. A total of 64 out of 80 gilts were positive to the skin test at 48 h.

The repeated use (two administrations) of Brucellin Aquilon has been studied in study CITA1302-06 where 79 primiparous sows older than 8 months from a naturally infected farm were included in the study after being diagnosed *B. suis* antibody positive with brucellosis typical clinical signs. A second Brucellin Aquilon injection was administered to groups of 15 primiparous sows within a variable time frame (7, 14, 21, 42 days) in accordance with the proposed SPC. Regardless the time frame, all the primiparous sows were positive. Conversely to what was reported by Blasco (1994) in sheep, no such one-week anergy period was observed in pigs after a second injection.

In study AQ1302-11, the specificity of Brucellin Aquilon has been challenged in a *Yersinia enterocolitica* O:9 infection experimental model. Thirty five male and female cross-bred pigs (18-19 weeks-old) from a *Brucella*-free farm were sensitised twice (at day 0 and day 7) with a fresh culture of a field strain of *Yersinia enterocolitica* O:9 administered orally (5 ml) and subcutaneously (2 ml, in the neck area). Those (33) which became *Brucella* antibody positive (cross-reactive antibodies detected by Rose Bengal test) were included in the study and were injected with Brucellin Aquilon (50 µg) 2 days later according to the aforementioned procedure. After 48 hours, 100% of the *Yersinia enterocolitica* O:9 sensitised pigs were negative.

Field trials

Three clinical studies were designed for the evaluation of the efficacy of Brucellin Aquilon (=diagnostic specificity), among them, only study AQ1302-07 has been completed. Study AQ1302-09 is still running.

The safety and the specificity of Brucellin Aquilon was investigated in study AQ 1302-07 where 420 animals from 3 *Brucella*-free farms were tested according to the procedure proposed by the applicant. All pigs (5 – 5.5 months old) tested showed negative results in the skin test.

An on-going trial has been undertaken in boar quarantine centres of Catalonia (Spain) since March 2021 (AQ1302-09). However, no *B. suis*-serologically-positive pig has up to now been detected and enrolled in the study which is still running.

The specificity was also being investigated in 3 farms free from *B. suis* reproductive clinical signs (AQ 1302-12). Sows older than 5 months (157) were tested by the Rose Bengal test. Nine sows were found Rose Bengal positive and thus skin tested with Brucellin Aquilon. These sows were found negative 48 hours later and no *Brucella* were isolated in their vaginal sample by culture. Pictures of the skin reaction of each of the animals tested were provided.

Finally, the repeated injections of Brucellin Aquilon (4 administrations 1 week apart) triggered no sensitisation (AQ-1302-10; safety field study) which would have decreased the specificity. In addition, the sensitivity observed after a second administration did not decrease (no anergy reported - CITA1302-06).

Overall conclusion on efficacy

The dose of 50 µg was established based on two dose finding studies range: 6 – 200 µg/dose, and the time to read the skin test reaction was set in one study, 48h after the intradermal injection.

The results from 3 other studies with a laboratory batch of the product show that Brucellin Aquilon is effective for bearing out *Brucella* infection in *Brucella*-aborted primiparous sows with a good sensitivity and one study with an industrial batch showed that the product is effective for detecting FPSR animals with a good specificity in an experimental model of *Y. enterocolitica* O9 infection at the proposed dose.

The sensitivity of the reaction put forward by the applicant (94%) has been calculated in the population of primiparous sows which were undoubtedly infected by *B. suis* (i.e. those which experienced a *B. suis* abortion) (studies CITA1302-01, 2, 3, 4). This subpopulation is very particular because primiparous sows are very susceptible to the infection and the immune reaction triggered in case of abortion is considered to be of a higher magnitude; extrapolation to other groups of pigs (e.g. *B. suis* infected primiparous sows which farrowed live piglets) is not straightforward; for instance, a sensitivity of 80% has been found in *B. suis* seropositive primiparous sows in a *B. suis*-naturally-infected farm (CITA1302-05).

The specificity (100%) of Brucellin Aquilon has been successfully demonstrated against *Y. enterocolitica* O:9 in an experimental setting including 33 sensitised pigs (males and females). This bacterium is a major serologically cross-reacting organism in pigs. Besides, no skin reactions were reported from 12 Brucella-free (control) primiparous sows (studies CITA1302-02 and CITA1302-04).

However, as experimentally determined (Dieste-Perez 2015), allergic skin reactions triggered by Brucellin Aquilon or a cytosolic extract of *Ochrobactrum intermedium* are indistinguishable. And the LPS of *Ochrobactrum anthropic* do react with sera of *B. melitensis* infected sheep, goat or rabbit (Velasco 1997). Therefore, insofar as *Ochrobactrum* spp. infected pigs may give FPSR, Brucellin Aquilon would not help to differentiate them from true *B. suis* infected pigs, and the actual frequency of these opportunistic infections is unknown. Besides, no information about the other bacteria which have a cross-antigenicity with *Brucella* were provided in the dossier with the exception of *Yersinia enterocolitica*.

In conclusion, the specificity of Brucellin Aquilon is quite high because for the time being *Y. enterocolitica* is the main bacteria responsible of FPSR and, while they are abundant in the environment of pigs and even in their flora, infections with immunologically cross-reactive α-proteobacteria bacteria seems to happen very infrequently. These circumstances may change with *Brucella* and *Yersinia* control measures. Nevertheless, relevant information including the conditions where Brucellin Aquilon has been validated are in the SPC.

Brucellin Aquilon has been investigated mainly in primiparous sows, but efficacy and safety studies have also been performed in fattening pigs, males and females. However, information is missing about the performance of Brucellin Aquilon in pigs at different stages of the disease. This is acceptable for a MUMS product.

A warning for the product not to be used in pigs treated with anti-inflammatory drugs has been included in the SPC.

In summary, Brucellin Aquilon, when used as recommended, was shown to bring about an optimal both type III and IV allergic reactions 48 hours after intradermal injection, enabling the detection of *Brucella* false positive reactions with a high sensitivity and specificity within the current epidemiological situation, which is characterised by *Y. enterocolitica* O:9 infections. Appropriate information has been included in the SPC.

Part 5 – Benefit-risk assessment

Introduction

Brucellin Aquilon is an injectable solution containing ≥ 1 RP (relative potency) of concentrated purified protein extract of *Brucella abortus* strain AQ1302 (originally *Brucella abortus* Tn5::*per*) per 0.1 ml dose. Relative potency is expressed as compared to the reference standard which has been qualified in terms of potency (in the in vivo potency test) against a clinical batch used in the safety and efficacy studies (potency ratio of reference standard over pre-clinical batch was 1.37). The active substance is innovative. It does not contain O-polysaccharide, present on other bacteria causing the false positive serological reaction (FPSR) in brucellosis serological tests. The indication of the product is: "For *in vivo* diagnosis of *Brucella*-infected pigs through a positive skin reaction after a positive serological *Brucella* test". The proposed dose of 0.1 ml is to be injected intradermally in the perianal area. The product is presented in multi-dose vials containing 2.5 ml of the solution.

The applicant has provided evidence that *Brucella* suis-aborted-primiparous sows did react with Brucellin Aquilon while pregnant sows and fattening piglets from *Brucella*-free farms didn't. And the allergen was safe for both *Brucella*-free and -infected pigs.

Brucellin Aquilon is innovative because is the first diagnostic test product intended to discriminate pigs giving false positive serological reactions to *Brucella* serological tests from real *Brucella*-infected animals, by reading the skin reaction of the pig. Brucellin Aquilon uses the immune system of the animals to reveal the presence of *Brucella*.

The eligibility to the centralised procedure was agreed upon by the CVMP on 18 March 2020 as Brucellin Aquilon has been developed by means of a biotechnological process. The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC (full application). The product has been classified as MUMS/limited market and therefore reduced data requirements apply, which have been considered in the assessment.

Benefit assessment

Direct therapeutic benefit

The potential benefit of Brucellin Aquilon is that it provides a reliable in vivo diagnostic tool (skin test) to differentiate *Brucella*-infected from *Brucella*-free pigs amongst those with a positive reaction by *Brucella* LPS related serological tests. Brucellin Aquilon is able to discriminate serological false positive reactions caused by cross-reacting bacteria, particularly *Y. enterocolitica* O:9. The specificity,

sensitivity and safety of Brucellin Aquilon has been established in well devised field studies conducted to an acceptable standard. The conduct of the skin test using Brucellin Aquilon has been sufficiently validated. It provides a diagnostic result 48 hours after administration.

Brucellin Aquilon triggered no sensitisation which would have decreased the specificity when used repeatedly nor was reported an anergy after a 2nd administration.

Additional benefits

Brucellin Aquilon is easy and safe to apply by the veterinarian because it is injected with a medical device.

Risk assessment

Quality:

The available data confirm the adequate quality of Brucellin Aquilon.

Based on the available stability data and taking into account the MUMS status of the product, the proposed shelf lives for antigen and finished product are deemed acceptable. Nevertheless, the applicant is recommended to provide updated stability data of the ongoing studies to confirm the proposed shelf lives of the antigen and the final product.

Safety:

Risks for the target animal:

Administration of Brucellin Aquilon in accordance with SPC recommendations is well tolerated both in *Brucella*-free and -infected pigs. No adverse reactions were observed in the studies presented.

Risk for the user:

The user safety for this product is acceptable when used according to the SPC recommendations. Standard safety advice is included in the SPC.

Risk for the environment:

Brucellin Aquilon is not expected to pose a risk for the environment when used according to the SPC recommendations.

Risk for the consumer:

Brucellin Aquilon is not expected to pose a risk for the consumer when used according to the SPC recommendations.

Special risks:

None have been identified except the misdiagnosis of pigs already suspected to be infected by *Brucella suis*.

Risk management or mitigation measures

Appropriate information has been included in the SPC and other product information to inform on the potential risks of this product relevant to the target animal, and the user, and to provide advice on how to prevent or reduce these risks.

Evaluation of the benefit-risk balance

At the time of submission, the applicant applied for the following indication:
"For in-vivo diagnostic of Brucella infected pigs through a positive skin reaction.
Brucellin Aquilon has been specifically designed to identify *Brucella*-infected pigs and differentiating these from the brucellosis free pigs affected by False Positive Serological Reactions (FPSR) in the current brucellosis serological tests."

Further the assessment of the dossier the CVMP agreed to the following indication(s):

"For in vivo diagnosis of Brucella-infected pigs through a positive skin reaction after a positive serological *Brucella* test.

Brucellin Aquilon has been specifically designed as a second line diagnostic test to differentiate *Brucella*-infected pigs, from the age of 5 months, from the *Brucella*-free pigs having given false positive serological reactions (FPSR) in brucellosis serological tests based on anti-O-PS antibodies (e.g. Rose Bengal)."

Information on development, manufacture and control of the active substance and finished product has been presented and lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. It is well tolerated by the target animals and presents an acceptable risk for users and the environment when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Veterinary Medicinal Products (CVMP) concluded that the application for Brucellin Aquilon is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned medicinal product.

In addition, the CVMP has recommended that the applicant provides the results of the stability studies which were ongoing at the time of authorisation.