ASSURING THE SAFETY, QUALITY AND EFFICACY OF VETERINARY MEDICINES

United Kingdom
Veterinary Medicines Directorate
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NATIONAL PROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

Zylexis for Horses

MODULE 1

PRODUCT SUMMARY

Name, strength and pharmaceutical form	Zylexis for Horses, Lyophilisate and Solvent for Suspension for Injection
Applicant	Zoetis UK Limited 5th Floor, 6 St. Andrew Street London EC4A 3AE
Active substance(s)	Inactivated Parapoxvirus ovis, strain D1701 minimum 460 Units* IFN per ml. *interferon units as determined in an *in vitro* stimulation assay in cattle lymphocytes.
ATC Vetcode	QL03AX
Target species	Horses, from 10 months of age.
Indication for use	Zylexis for horses acts by stimulation of the non-specific immune mechanisms and is of potential clinical value in the reduction of clinical signs of stress/crowding associated equine respiratory disease. In a field study, reduction of clinical signs (defined as the first time-point at which significant differences were evidenced between groups) was shown on day 5 after administration of the full treatment schedule and lasted less than a week. This is a Limited Marketing Authorisation. A full set of supporting efficacy data is not available for this product.

MODULE 2

The Summary of Product Characteristics (SPC) for this product is available on the Veterinary Medicines Directorate website (www.vmd.defra.gov.uk)

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	Limited Marketing Authorisation application in accordance with Schedule 1, paragraph 26 of the Veterinary Medicines Regulations.

I. SCIENTIFIC OVERVIEW

Zylexis for Horses is a Limited Marketing Authorisation, under European Medicines Agency (EMA) guidelines for MUMS (Minor Use Minor Species)/Limited Markets. This type of Marketing Authorisation is intended for use where limited numbers of a product are expected to be sold, and where a need for a specific drug is required. Due to the nature of the use of such drugs, a full dossier meeting all requirements for the marketing of the drug is not required, provided that the safety, quality and efficacy of the product can be supported by available data, and a favourable benefit-risk profile is determined. There is a requirement for Periodic Safety Update Reports (PSURs) to be submitted for annual reassessment of this product. Moreover, results of any additional studies must be submitted to the Veterinary Medicines Directorate (VMD).

Zylexis for Horses is an immunomodulator containing inactivated Parapoxvirus ovis (PPVO), and is presented as a freeze dried lyophilisate to be reconstituted with solvent Water for Injections. It acts by stimulation of the non-specific immune mechanisms and is of potential clinical value in the reduction of clinical signs of stress/crowding associated equine respiratory disease. In a field study, reduction of clinical signs (defined as the first time-point at which significant differences were evidenced between groups) was shown on day 5 after administration of the full treatment schedule and lasted less than a week. The product is administered by intramuscular injection. Three injections of a single 2ml dose for each animal are recommended. The first two injections are administered with a 48-hour interval (day 0 and day 2) and the third injection should be administered on day 9.

The product is produced and controlled using validated methods and tests which ensure the consistency of the product released on the market. It has been shown that the product can be safely used in the target species. The product is safe for the user, and for the environment, when used as recommended. Suitable warnings and precautions are indicated in the SPC. The efficacy of the product was demonstrated according to the claims made in the SPC. The overall risk/benefit analysis is in favour of granting a marketing authorisation.

II. QUALITY ASPECTS

A. Composition

The product contains inactivated PPVO strain D1701 and excipients polygeline, MEM with Earle's salts (EMEM) and water for injection.

The PPVO strain used in this product originates from Germany. It was isolated in 1972 from a diseased lamb. More than 170 *in vitro* cell passages were performed for virus attenuation. This strain was chosen by its ability to reduce clinical symptoms of disease following an infection.

The lyophilisate component of the product is freeze dried and supplied in a Type 1 glass vial (1 dose for horses). The solvent component of the product is supplied in separate Type 1 glass vials containing 2ml of solvent. The Type 1 glass vials comply with the European Pharmacopoeia. The vials are closed with bromobutyl rubber stoppers closed with an aluminium cap. The product is presented in a cardboard box containing 1, 3, 5 or 6 vials of lyophilisate together with 1, 3, 5 or 6 vials of solvent. Not all pack sizes may be marketed. The particulars of the containers and controls performed are provided and conform to the regulation.

The choice of the inactivating agent and absence of preservative are justified. The inactivation process and the detection limit of the control of inactivation are correctly validated.

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

B. Method of Preparation of the Product

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

The antigen is produced by culturing the PPOV in a cell line. After growth, the antigen fluids are harvested and inactivated. The inactivated antigen fluids may be filtered and virus concentration by ultrafiltration may be performed. The antigen fluids are then stabilised before being aseptically transferred to sterile containers where they may be stored at -40°C or below for up to 30 months until require for preparation of the final product.

To produce final product the inactivated stabilised PPVO antigen fluids are thawed, aseptically blended and freeze-dried. At the end of the freeze-drying process, rubber stoppers are inserted into the vials and the vials are sealed with the aluminium caps. After freeze-drying samples are taken for final product quality control testing. The freeze-dried lyohpillisate is then stored at +2 - +8°C until required for packaging.

To produce the solvent component of the product, the diluent (Water for Injection) is filled into sterilized glass vials, stoppered using sterilized rubber stoppers and sealed with aluminium caps. The filled vials are then steam-sterilized. Samples are taken for final product quality control testing, and the

vials of solvent are stored at room temperature until required for the assembly process. Process validation data on three batches of the product have been presented in accordance with the relevant European guidelines.

B. Control of Starting Materials

Starting materials of non-biological origin, including the solvent Water for Injections, used in production comply with European Pharmacopoeia requirements.

Biological starting materials used are in compliance with the applicants in-house specifications. The active substance PPOV is manufactured in accordance with the principles of good manufacturing practice.The Master Seed Virus (MSV) was established in 1995. The MSV was tested in compliance with European Pharmacopoeia requirements between 1995 and 1997. Tests included those for identity, sterility, mycoplasma, and extraneous agents. The Working Seed Virus (WSV) are prepared by a maxium of 4 passages of the MSV in the cells line. Before use in the antigen production, each batch of WSV is tested for sterility, mycoplasma, extraneous agents and virus titre.The master and working seeds have been produced according to the Seed Lot System as described in the relevant guideline.

D. Specific Measures concerning the Prevention of the Transmission of Animal Spongiform Encephalopathies

Scientific data and certificates of suitability issued by the EDQM have been provided and compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products has been satisfactorily demonstrated.

E. Control tests during production

The tests performed during production are described and the results of 3 consecutive runs, conforming to the specifications, are provided. Tests performed before virus inactivation include absence of mycoplasma, virus titre, sterility and detection of bovine viral diarrhoea (BVD). Tests performed after inactivation include tests for residual live virus, residual sodium thiosualfate and sterility.

F. Control Tests on the Finished Product

The tests performed on the final product conform to the relevant requirements; any deviation from these requirements is justified. The tests include in particular those for appearance, identification, sterility, detection of bovine viral diarrhoea virus, potency and residual humidity for the freeze-dried component.
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.

The solvent component of the product (Water for Injections) is tested in accordance with European Pharmacopoeia. The results obtained from three batches of the diluents demonstrate compliance with the requirements.

G. Stability

Stability data testing the potency of 3 batches of the active substance have been provided in accordance with applicable European guidelines, demonstrating the stability of the active substance when stored under the approved conditions.

Stability data on 3 batches of the finished product have been provided in accordance with applicable European guidelines, demonstrating the stability of the finished product when stored under the approved conditions. Tests on stability of the finished product include potency, residual humidity and sterility.

J. Other Information

The application was supported with regard to quality. The following precautions are included on the SPC and product literature:

Store in a refrigerator (2°C - 8°C).
Protect from light.
Do not freeze.

The finished product has a shelf-life of 24 months and should be used immediately after reconstitution.

III. SAFETY ASSESSMENT

The batches employed in the safety tests were produced according to the manufacturing process of the product. The virus and the cell cultures employed in these batches were at the normal passage level as used for production. To achieve target potency for the trials, the immunomodulator was adjusted by resuspension of the vials containing the freeze-dried pellet in respective volumes of the diluent. The safety studies were conducted under Good Clinical Practice – veterinary (GCPv). They were not conducted in accordance with Good Laboratory Practice (GLP), however, this is considered acceptable under the "*Guildeline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets*" which states that GLP requirement can be waived.

Laboratory trials

The safety of the administration of one dose was not studied. This was justified on the basis of requirements of the Minor Use Minor Species (MUMS) guidelines. The overdose studies presented do not show any adverse reactions, therefore the absence of a single does study is justified.

A laboratory study was conducted on the safety of the administration of an overdose and repeated overdose. A suitable number of Shetland ponies between 2-5 years of age received 6 injections intramuscularly with Zyelxis for Horses, or a placebo on trial days 0, 2, 9, 16, 23 and 30. In one group, the

ponies were injected with a pre-licensing batch containing 2x the antigen content of a routine dose in double the volume of diluents (2560 IFN units per injection). The remaining ponies received a placebo preparation. The ponies were observed for clinical signs starting 2 days before the first injection through to 4 days after the last injection (37 days total observation period). The rectal temperature was measured daily, and the injection sites were observed daily for any local reactions. Two Zylexis treated ponies and one placebo pony were treated with antibiotics from trial day +25 through to +29 due to clinical respiratory symptoms. No other clinical signs of disease were observed. General condition and attitude of ponies in both treatment groups did not change. Rectal temperatures in both treatment groups were considered not to be clinically different. Neither local swellings (visable or palpable) nor systemic reactions were detected in any of the trial animals.

No laboratory investigation of effect on reproductive performance was conducted due to the unavailability of pregnant animals. No inherent risk was expected which necessitated specific laboratory trials examining reproductive performance, the influence of Zylexis for Horses on the reproductive performance of the rest of the target species was examined in field trials.

No specific studies have been conducted for the examination of immunological functions. Based on presented trial results, the experience with precursor products and the nature of the product, no negative effects on immunological functions are to be expected. In addition the product is an inactivated product without an adjuvant.

Zylexis for Horses contains inactivated virus and thus the specific tests to be performed for live vaccines are not applicable.

Horses are not considered a food producing species in the UK. Therefore studies of residues have not been carried out and a withdrawal period of zero days in the SPC has been set.

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

A user risk assessment was presented for Zylexis for Horses. The assessment concluded that due to the nature of the ingredients of the product, it is considered that no specific risk management is required. The measures to be taken are those already in place for the use of all injectable products administered to horses, as stated in the SPC. No specific warning is provided to the person administering the product or to the person in direct contact with the animal.

Field studies

Four field safety studies were carried out. The first field study was carried out investigating the safety of one Intramuscular (IM) injection containing 10 times the antigen dose of the routine product in one day old foals. A total of 30 healthy foals at the age of one day after birth received one IM injection with either Zylexis for Horses or a placebo. In one group, 20 foals were injected with one batch of Zylexis for Horses containing 10x the antigen content of a routine dose in double the volume of diluent (920 IFN units/animal). The remaining 10 foals

received a placebo preparation. The foals were examined for clinical signs for 14 days after injection. The rectal temperature was measured daily starting immediately before the injection through to the fourth day after injection. The injection sites of all foals were observed for local reactions for 14 days after injection. During the observation period no clinical signs of disease were observed and general condition and attitude of the foals in both treatment groups were not disturbed. There were no clinically relevant differences between the groups mean rectal temperatures, and no local swellings (visible or palpable) or systemic reactions were detected in any trial animals.

A further field study looked at the effects of repeated IM injections of one dose (460 IFN units per/dose) in 4 to 10 month old horses. Two groups of 26 and 27 young horses were used. One group was injected via IM injection with the Zylexis preparation three times on day 0, 2 and 9. For the second group a placebo preparation was used. The animals were observed up to day 28. The injection site was inspected and palpated at each examination. No adverse local or systemic effects could be seen in any of the horses.

An additional field study was carried out to look at the safety of one IM injection of an overdose of 10x the antigen content of a routine dose in double the volume of diluent in pregnant horses between 4 to 268 days before parturition. 30 pregnant mares were included in the study in total. 20 foals were injected with one batch of Zylexis for Horses containing 10x the antigen content of a routine dose in double the volume of diluent (230 IFN units/animal). The remaining 10 foals received a placebo preparation. The mares were observed for clinical signs for 14 days after injection. The rectal temperature was measure daily beginning 2 days before injection until 4 days after injection, and the injection sites of all mares were observed for local reactions for 14 days after injection. During the observation period clinical signs of disease were observed only in one placebo treated mare. General condition and attitude of the mares in both treatment groups were not changed. Group mean rectal temperature differences were not considered to be clinically relevant. There were no local swellings (visible or palpable) or systemic reactions detected in any trial animals. The course of pregnancy was not disturbed through injection of Zylexis. Twenty-nine mare gave birth to a clinically healthy foal. Only one foal (from the placebo group) died 35 minutes after birth.

The final field study also investigated the safety of one IM injection of an overdose of 10x the antigen content of a routine dose in double the dose of diluent in pregnant horses, but this study looked at mares between 1 and 254 days before parturition. 30 pregnant mares were included in the study. 20 animals received one IM injection with pre-licensing batch of the test product containing 10x the antigen content of a routine dose (920 IFN units/ animal) in double the volume of diluent and the remaining 10 animals received a placebo preparation. The mares were observed for clinical signs for 14 days after injection. The rectal temperature was measure daily beginning 2 days before injection until 4 days after injection, and the injection sites of all mares were observed for local reactions for 14 days after injection. During the observation period no mare showed clinical signs of disease and the general condition and attitude of the mares in both treatment groups was not disturbed. Group mean rectal temperature differences were not considered to be clinically relevant. There were no local swellings (visible or palpable) or systemic reactions detected in any trial animals. The course of pregnancy was not disturbed

through injection of Zylexis. Each of the 30 mares gave birth to a clinically healthy foal.

The field studies presented on pregnant mares were not conducted with the recommended dosage schedule of three doses and therefore the studies do not confirm the safety of the product in pregnant animals with the full vaccination schedule. Additionally, no data was presented with regards to safety in stallions. Therefore, the following warnings have been listed on the SPC:

- No information is available to support the use of the authorised schedule in pregnant mares. One overdose (4ml) was investigated in pregnant mares in the second half of pregnancy up to a few days before parturition. No systemic or local reactions or negative effects were observed in these mares. There is no information concerning safety in stallions.

Ecotoxicity

The applicant carried out an assessment with regards to ecotoxicity hazard. It was concluded that the constituents contained in Zylexis for Horses are common in animal and human health products. With regards to the constituents and to the results of the safety trials there are no indications for any negative impact on the environment or human health. The explanation and justifications presented for the negative ecotoxicity hazard are acceptable, and no warnings regarding ecotoxicity are therefore required.
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IV CLINICAL ASSESSMENT (EFFICACY)

Clinical Studies

Laboratory Trials

The efficacy of the product has been demonstrated in four laboratory studies in accordance with the relevant requirements.

The first study was looking at the in vivo effect of Zylexis on humoral and cellular immunity in horses challenged with equine infectious anaemia virus (EIAV). It was conducted to determine whether or not the non-specific immunization of horses with Zylexis could enhance and restore their immune responsiveness to the challenge infection with the EIAV. Additionally, the product efficacy was tested in the case of simultaneous IM administration of 2 mL Pb-acetate preparation that is known to interfere with the replication of the virus. A suitable number of horses between 2-15 years old free from antibodies against EIAV were randomly allocated to three groups. A placebo group was administered saline twice, 12 days pre-challenge and on the day of challenge. A second group received 2 ml of Zylexis (640 IFN units/ml) twice 12 days pre-challenge and on the day of challenge. A third group received Zylexis and Pb-acetate simultaneously 12 and 10 days pre-challenge. Following treatment, all study animals were challenged by intravenous (IV) and Intramuscular (IM)

administration of lyophilised blood containing EIAV. Blood samples were collected for detailed analysis on day 15 post infection. Results showed that Zylexis was effective in stimulating cell-mediated immunity (helper and cytolyitc T cells), and particularly in inducing the elements of humoral immunity (B cells and immunoglobulins) and therefore was responsible for the inhibition of viral infectivity and reduction of immunosuppression caused by EIAV. This study was not conducted with the recommended dosage schedule of three doses and was not conducted with a batch of product at the minimum potency and therefore the study does not confirm the efficacy of the product for an indication concerning EIAV. However, the EIA UK code of practice describes that EIA is notifiable by law under the Infectious Diseases of Horses Order 1987. As there is currently no cure for EIAV, any horse testing positive will be subject to compulsory slaughter under the control of the Defra Divisional Veterinary Manager (DVM). The applicant therefore has decided not to claim any indication derived from the EIAV study.

A second study was carried out for an evaluation of Zylexis to induce proliferations of the immune cells from different species. Selected donors were of both sexes and various ages from species including goats, cows, rabbits, horses, swine, dogs, cats and humans and were clinically healthy. Randomly selected peripheral blood collected from the donors, and mesenteric lymph node and spleen cells collected from swine, were used in this study in order to assess the in vivo cellular immune response induced by Zylexis administration in the different species, based on the stimulation of lymphocyte cell cultures seen in vitro by three different concentrations (5, 10, 20 µm/ml of 640 IFN units/ml). Results showed that all three concentrations of Zylexis induced proliferative responses of peripheral blood lymphocytes (PBL) from all species tested in a similar fashion to those induced by common mitogens (PHA, ConA, PWM) or specific antigens (PLS, KLH). Zylexis exhibited highest stimulatory effect for porcine, caprine, and human immune cells, high effect for horse, cat, cow and rabbit PBL, and low effect for dog PBL. This study relies on in vitro data and results are not correlated to an in vivo benefit. Therefore results can only be considered as information and can’t be taken into account to document the efficacy of Zylexis. However, it is considered that the study provides important information on the potential mechanism of action of the product which is relevant to the user.

A third study was carried out to investigate the immune method of action of Zylexis in horses. A suitable number of horses were injected IM on days 0, 2 and 9. Cytokine were assayed by RT-PCR at 6, 24 and 48 hours after each injection. One group of horses also received Tetanus toxoid for comparison and another group were normal controls. It was found that there were no significant effects of time on IFN-gamma levels but there was 2.5 fold increase within 24 hours after the first injection and decline gradually thereafter. TNF-alpha expression was significantly higher at 54 hours (approx. 2 days), 222 and 264 hours (11 days) than in the controls following Zylexis injection.

The applicant also provided bibliographic data on two published studies on the immune method of action. One was carried out to determine the effect of immunostimulant Zylexis on neutrophil, macrophage, and lymphocyte function following *ex vivo* exposure to *Rhodococcus equi.* Foals were randomly assigned

to one of 3 treatment groups and bronchoalveolar lavage and blood were collected and on days 0, 1, 24 and 36. The study concluded that neutrophils from the foals treated with Zylexis had significantly greater ability to phagocytose *R.equi*, undergo oxidative burst, TNF induction in monocyte derived macrophages and IL-12 in BAL macrophages. The study is considered to be supportive in showing that Zylexis evokes immune response in horses following in vitro exposure of neutrophils, monocytes, and macrophages to *Rhodococcus equi.*

Details of a second published study looking at the effect of a commercial preparation of inactivated PPVO on cytokine gene expression by equine peripheral blood mononuclear cells both in vitro and in vivo were provided. Peripheral blood mononuclear cells (PBMC) from one group of yearling horses were tested in vitro and a second group of yearling horses were treated in vivo. The results indicate that PPVO stimulated IFN-gamma production both in vitro and in vivo. Increased cytokine expression could account for its immunomodulatory activity. The study is considered to be supportive in showing that Zylexis stimulates IFN-gamma production in horses both in vitro and in vivo.

A final laboratory study was carried out looking at the ability of Zylexis to induce interferon (IFN) in PBMC of horses and dogs to prove the principle of protective immune stimulation established in bovine, also in these species. EDTA blood from healthy adult cross breed horses and health 1 year-old dogs was collected, prepared and used for the tests. ED cells (horses and cattle lymphocyte system) or A72 cells (dog lymphocyte system) were infected with VS virus. The infected culture was then examined for the occurrence of cytopathogenic effects. The results showed that Zylexis is able to induce interferon in lymphocytes of different species and that the degree of IFN production varies between species.

Field Trials

In a field study, reduction of clinical signs (defined as the first time-point at which significant differences were evidenced between groups) was shown on day 5 after administration of the full treatment schedule and lasted less than a week. This is a Limited Marketing Authorisation. A full set of supporting efficacy data is not available for this product.

V OVERALL CONCLUSION AND BENEFIT– RISK ASSESSMENT

Zylexis for Horses is an immunomodulator containing inactivated PPVO, strain DI 701. It is a Limited Marketing Authorisation, under European Medicines Agency (EMA) guidelines for MUMS (Minor Use Minor Species)/Limited Markets. Due to the nature of the application, a full dossier meeting all requirements for the marketing of the drug is not required, provided that the safety, quality and efficacy of the product can be supported by available data, and a favourable benefit-risk profile is determined.

The raw materials used for the preparation of the active ingredient comply with the relevant European Pharmacopoeia requirements. The product is free from any adjuvants, antibiotics and preservatives. The manufacturing process takes place in a Good Manufacturing Process (GMP) compliant facility and other production materials assure that no manufacturing risks are associated with the product.

Zylexis has been tested for safety in five studies, one under laboratory conditions and four under field conditions. None of the studies demonstrated any adverse effects. With regards to the constituents in the product and to the results of the trials conducted, there are no indications for any negative impact on the environment, animal or human health.

Several laboratory studies and one field study have been provided in support of the efficacy of the product. Several studies demonstrate that the product can evoke immunological response and stimulate lymphocytes in vitro which points to a possible mode of action of the product. However, they do not give assurance about how the product will perform in vivo. Therefore the approved claim for indications of the product is a non-specific one for stimulation of immune mechanisms in the target species and is of potential clinical value in the reduction of clinical signs of stress/crowding associated equine respiratory disease.

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

MODULE 4

POST-AUTHORISATION ASSESSMENTS

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 is available on the Product Information Database of the Veterinary Medicines Directorate website.

(www.gov.uk/check-animal-medicine-licensed)

The post-authorisation assessment (PAA) contains information on significant changes which have been made after the original procedure which are important for the quality, safety or efficacy of the product.

The PAA for this product is available on the Product Information Database of the Veterinary Medicines Directorate website.

(www.gov.uk/check-animal-medicine-licensed)