Veterinary Microbiology 173 (2014) 232–240

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Efficacy of a *Parapoxvirus ovis*-based immunomodulator against equine herpesvirus type 1 and *Streptococcus equi equi* infections in horses

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ARTICLE INFO

Article history:
Received 11 April 2014
Received in revised form 14 July 2014
Accepted 15 July 2014

Keywords:
Zylexis
Immunomodulator
Parapoxvirus ovis D1701
Equine herpesvirus type 1
Streptococcus equi equi
Strangles

ABSTRACT

The efficacy of Zylexis®, an immunomodulator in horses based on inactivated *Parapoxvirus ovis* (iPPVO), was assessed using an equine herpesvirus type 1 (EHV-1) challenge model in the presence of a natural infection with *Streptococcus equi equi* (*S. equi*). Eleven horses were treated with iPPVO and twelve were kept as controls. Six horses were challenged with EHV-1 and commingled with the horses on study. Animals were dosed on Days −2, 0 (just before commingling) and Day 7. On Day 11 significantly less nasal discharge, enlarged lymph nodes, EHV-1 shedding and lower rectal temperatures were observed in the iPPVO-treated group. In addition, iPPVO-treated horses showed significantly fewer enlarged lymph nodes on Days 17 and 19, significantly less lower jaw swelling on Day 3 and significantly lower rectal temperatures on Days 12 and 13. Dyspnoea, depression and anorexia were only recorded for the control group. Following challenge seven out of 11 horses in the iPPVO treated group shed EHV-1 but on Days 11, 12, 13, 14, 15 and 16 quantitative virus detection in this group was significantly lower as compared to the controls. All animals shed *S. equi* but the percentage of animals with positive bacterial detection was lower in the iPPVO group than in the control group from Day 14 through Day 28. This difference was significant on Day 24. No injection site reactions or adverse events were observed. In conclusion, Zylexis administration is safe and reduced clinical signs and shedding related to both EHV-1 and *S. equi* infections.

1. Introduction

From birth, horses are at risk of exposure to a variety of pathogens which cause respiratory disease with high incidence worldwide. These include viruses such as equine herpesvirus type 1 (EHV-1) and 4 (EHV-4) and equine influenza virus (EIV), and bacteria such as *Streptococcus equi equi* (*S. equi*) and *Rhodococcus equi* (Mumford et al., 1998; Morley et al., 2000; Waller and Jolley, 2007; Ataseven et al., 2009; Parkinson et al., 2011; Pusterla et al., 2011; Tel et al., 2011; Muscatello, 2012; Legrand et al., 2013). In addition, opportunistic pathogens such as the bacterium *Streptococcus equi zooepidemicus* are part of the normal upper airway mucosa in horses but can cause

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http://dx.doi.org/10.1016/j.vetmic.2014.07.015
0378-1135/

disease when the immune system is weakened by predisposing factors like stress or other infections (Newton et al., 2008; Lindahl et al., 2013).

Although vaccination is the most important tool for prevention of infection, commercial vaccines are not available in all cases or they might not be updated with the most recent field isolates which may predispose to vaccine breakdowns. Some horses might still have high levels of maternal derived antibodies at the time of vaccination which may cause interference with the vaccine. Other horses demonstrate a poor immune response for various other reasons like incorrect vaccine administration, reduced vaccine quality due to incorrect storage, or reduced immune response due to their genetic background (van Maanen et al., 1992; Mumford et al., 2003; Gildea et al., 2011; Brinkmeyer-Langford et al., 2013). When no vaccination is performed or when response to vaccination is poor, the horse will rely on the non-specific innate immune system, the first line of defense in case of infection. Immunomodulators such as Zylexis®, containing inactivated *Parapoxvirus ovis* (iPPVO), are administered to optimize innate immune responses and to assist the horse in coping with pathogens for which they were not or are no longer primed. In horses, iPPVO administration has been shown to increase interferon (IFN) gamma/beta and tumour necrosis factor (TNF) alpha mRNA expression, both *in vivo* and *in vitro*. Increased expression of IFN alpha was only described *in vitro* and an elevated Interleukin (IL)-15 and IL-18 expression was only noted *in vivo* (Horohov et al., 2008). All of these cytokines are associated with a Th1, cell mediated response. Also increased phagocytosis was reported following stimulation with this immunomodulator *in vitro* (Buttner, 1993; Ryan et al., 2010). The mode of action for Zylexis® and its effect on respiratory infection under field conditions has recently been reviewed extensively (Paillot, 2013).

In this study, iPPVO-treated and untreated foals were brought in contact with EHV-1 infected foals. This scenario is representative of what could happen in the field when foals are weaned and collected together. EHV-1 was selected as a model because of the importance of this pathogen and encouraging results obtained previously (Ziebell et al., 1997) and the availability of sero-negative horses. During the first week of the study foals were diagnosed with a *S. equi* infection. This provided the opportunity to monitor the efficacy of Zylexis® as an immunomodulator in horses in the presence of a viral (EHV-1) and bacterial (*S. equi*) dual infection.

2. Materials and methods

2.1. Animal details and husbandry

Twenty-nine male Gypsy Cob horses between 6 and 8 months of age were sourced from a single breeder and were enrolled on the study. Each horse was identified by its chip number. Prior to transport to the study site they were wormed using a combination of ivermectin and praziquantel. After arrival at the site animals were treated with fenbendazole. Horses were kept in quarantine for two weeks prior to study start.

Throughout the study horses were housed in one barn which contained two pens. Each pen contained three directly challenged foals, five or six foals belonging to T01 and six foals belonging to T02. Straw was used as bedding material. Water and good quality hay were provided *ad libitum* and small amounts of pelleted feed were additionally given.

The study protocol was reviewed and approved by the Zoetis Zaventem Ethics Review Assessment Team. The study was carried out under the ADAS Home Office License.

2.2. Investigational and control product

A batch of Zylexis® produced under commercial conditions was used for this study. This immunomodulator consists of the freeze-dried iPPVO strain D1701 containing L2 as stabilizer. The freeze-dried pellet was resuspended in 2 mL of water for injection (WFI) just prior to administration by IM injection.

The control product was WFI from the same batch as the one used for re-suspending the investigational product.

2.3. Study design

Results reported by Ziebell et al. (1997) suggested that reduction in nasal discharge is an important efficacy variable to assess the effect of iPPVO after EHV-1 challenge. Treatment groups of 12 horses would allow a difference of 58% to be detected between treatment groups with 80% power testing at the 5% level of significance (two-tailed). However, only 11 horses were available for inclusion in treatment group T01 which reduced the potential power to 78%.

Twenty-three horses were randomly distributed over two treatment groups, group T01 (iPPVO-treated; $n = 11$) and group T02 (WFI-treated; $n = 12$) using a randomized complete block design. Blocks were based on age in days. Six blocks were randomly assigned to each of two pens. On Days −2, 0 and 7 these animals received an IM injection in the left neck (Days −2 and 7) or right neck (Day 0) with 2 mL Zylexis or WFI. On Day 0, post treatment administration, horses from both treatment groups were challenged by commingling with EHV-1 infected horses. They were brought into contact with horses ($n = 6$) which did not receive any treatment and were challenged approximately 3 h earlier by intranasal aerosol with $10^{5.9}$ $TCID_{50}$ per animal of the European EHV-1 strain 121412 (Heldens et al., 2001). The challenge strain was provided by the Irish Equine Centre (IEC).

On Day 3 horses were diagnosed with a concurrent *S. equi* infection. A decision was made to continue with the study and to evaluate the efficacy against both EHV-1 and *S. equi* infection.

2.4. Injection site reactions and clinical observations

During the study the general health of all animals was observed and recorded on a daily basis. All other variables described in this paragraph were monitored for treatment groups T01 and T02 only.