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Review paper

A systematic review of the immune-modulators *Parapoxvirus ovis* and *Propionibacterium acnes* for the prevention of respiratory disease and other infections in the horse

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ABSTRACT

Inactivated *Parapoxvirus ovis* (iPPVO) and *Propionibacterium acnes* (*P. acnes*) are currently used in equine medicine as immune-modulators for prophylactic treatment or adjunct to conventional therapy in order to improve immune defences, to prevent or treat infectious diseases. Their mode of action relies on a non-antigen specific interaction with the innate and/or adaptive immune responses. iPPVO stimulates and regulates cytokine secretion by leucocytes, while *P. acnes* acts primarily through the activation of macrophages. This report aims to describe their activity as immune-modulators and to summarise the scientific literature and reports available about their use in horses, particularly in the prevention or treatment of equine respiratory diseases. This systematic review regroups articles published in peer-review journals, clinical trials reports, conference proceedings and other information made available in the last 2 decades.

Contents

1. Innate immunity to respiratory infection and principle of immune-modulation

The equine respiratory disease complex (ERDC) is a term used to regroup a set of common equine viruses such as equine herpesvirus type 1 and 4 (EHV-1/4), equine influenza virus (EIV), equine arteritis virus (EAV) or equine adenovirus (Landolt et al., 2007; Slater, 2007a,b).[1] These respiratory viruses all primarily induce an indistinguishable respiratory disease in horses, characterised by pyrexia, cough and nasal discharge. Depending on the causative

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[1] Respiratory Diseases of Horses: Introduction – The Merck Veterinary Manual. 2011. http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/121300.htm.

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pathogen, specific secondary pathology may occur (*e.g.* EHV-1 abortion or myeloencephalopathy). Occasionally, ERDC is complicated by secondary bacterial infection mostly caused by *Streptococcus equi* spp *zooepidemicus*, an opportunistic pathogen of the respiratory tract. *Streptococcus equi* spp *equi* (*S. equi*) and *Rhodococcus equi* (*R. equi*) are other important primary respiratory bacterial pathogens, causative agents of Strangles and pneumonia in young foals, respectively (Giguere et al., 2011; Waller et al., 2011).

Vaccination is essential to prevent or limit the development of ERDC associated respiratory diseases and/or bacterial infections. Regrettably, protection induced by vaccination is not always optimal. In absence of pre-existing immunity induced by vaccination, maternal derived antibody or natural exposure to the pathogen, the mucosal innate immunity represents an essential line of defence of the horse. Its role is to contain the infection until the pathogen-specific adaptive immune response has been stimulated. Innate immunity starts with an immediate response composed of preformed factors (*e.g.* complement proteins) and resident mononuclear cells (*e.g.* macrophages, dendritic cells (DC) (Kohlmeier and Woodland, 2009; Shishido et al., 2012)). Alveolar macrophages are predominant in the lumen of the respiratory tract and in broncho-alveolar lavage (BAL) fluids of horses (Flaminio et al., 1998; Hoffman, 2008). The primary activity of macrophages is to destroy pathogens. Alongside DC, macrophages also act as professional antigen presenting cells (APC) by recognising invading-pathogens through their pattern-recognition receptors (PRRs), which is essential for the development of both innate and adaptive immune responses (Iwasaki and Medzhitov, 2004; Kohlmeier and Woodland, 2009). Their last important function is synthesis of pro-inflammatory cytokines including interleukins (IL-1, IL-6, IL-8, IL-12) and tumour necrosis factor alpha (TNF alpha) along with mediators that recruit new phagocytic cells to local sites of infection. The combination of IL-1, IL-6 and TNF alpha (also called endogenous pyrogens) will cause a rise in body temperature. Other cytokines (*e.g.* Type I Interferon; IFN alpha and beta) are also rapidly synthesised by both virus-infected cells and cells of innate immunity (*e.g.* macrophages, plasmacytoid cells and DC), during the early phase of infection (Kohlmeier and Woodland, 2009). Interferon and IL-6 synthesis were measured in horses after EHV-1 or EIV infection (Edington et al., 1989; Wattrang et al., 2003). These molecules present a wide range of antiviral activities, inducing up-regulation of major histocompatibility complex (MHC) class I molecules and antiviral resistance in uninfected cells. These cytokines compose the inducible phase of the innate response and play an essential role in stimulation of natural killer (NK) cells that are an early defence against intracellular infections. NK cells are activated by IFN alpha and IL-12 synthesised by macrophages, and are cytotoxic for virus-infected cells with absent or abnormally low levels of MHC class I expression (a means of immune evasion to avoid recognition by cytotoxic T-lymphocytes). Cytokines and chemokines locally produced during the infection are also essential for the maturation and the trafficking of DCs to draining lymph nodes where the adaptive immune response will be initiated (Kohlmeier and Woodland, 2009). IFN gamma synthesised by NK cells (He et al., 2004) will drive the development of a T-helper 1 (Th1) adaptive immune response, most effective against intracellular pathogens (Boehm et al., 1997). Modification of the cytokine balance initiated during the innate response may have a deep impact on subsequent development of T helper cells, T regulatory cells and cytotoxic lymphocytes responses, all major actors of the adaptive immunity (Fig. 1).

Nonspecific immune-modulators are molecules that interact with the innate and/or adaptive immune response but are not specific to an antigen. Their activity is based on the activation of innate cells and subsequent cytokine production (Rush and Flaminio, 2000). Through their ability to increase immune competence (activation, suppression and/or regulation), immune-modulators have a role to play to achieve or improve protection against infection, and to support animals whose immune function and response are sub-optimal, impaired/suppressed or dysregulated. Immune-modulator products have been used for years in veterinary medicine. *Parapoxvirus ovis* (PPVO) and *Propionibacterium acnes* (*P. acnes*) are 2 immune-modulators commercially available for the treatment of dogs, cats, horses and cattles. This review aims to summarise the scientific literature currently available on their use in horses.

2. Methods

This systematic review regroups articles published in peer-review journals and clinical trials reports, which characterise PPVO and/or *P. acnes* activity *in vitro* and their use *in vivo* in horses. Due to the limited amount of scientific literature available on this subject, conference proceedings, manufacturing reports, round-table discussions, reviews and abstracts were also considered. The language of publication is mostly English, but not exclusive. Publication date ranged from 1980s to the present time. Studies and reports were identified by searching electronic databases (Pubmed/Medline, Scopus and GoogleScholar) and scanning reference lists of articles/reports (Fig. 2). The PRISMA (Preferred Reporting Items for Systematic reviews) guidelines were consulted for the preparation of this review (Moher et al., 2009). The last search was run in September 2012. Information was extracted from each included study and report on context of the study (*e.g.* pathogen), type of intervention (dose, duration and frequency), number of participants in each group and type of outcome (specific to the pathogen targeted). Details of the search strategy are presented in supplementary data 1 (Table 1).

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetimm.2013.01.010.

3. Immune-modulators used in horses

3.1. *Parapoxvirus ovis*

PPVO (Orf virus) belongs to the poxvirus family. PPVO is a host restricted DNA virus that primarily induces skin lesions in sheep and goats and is transmissible to humans (Haig and Mercer, 1998). One strategy used by poxviruses

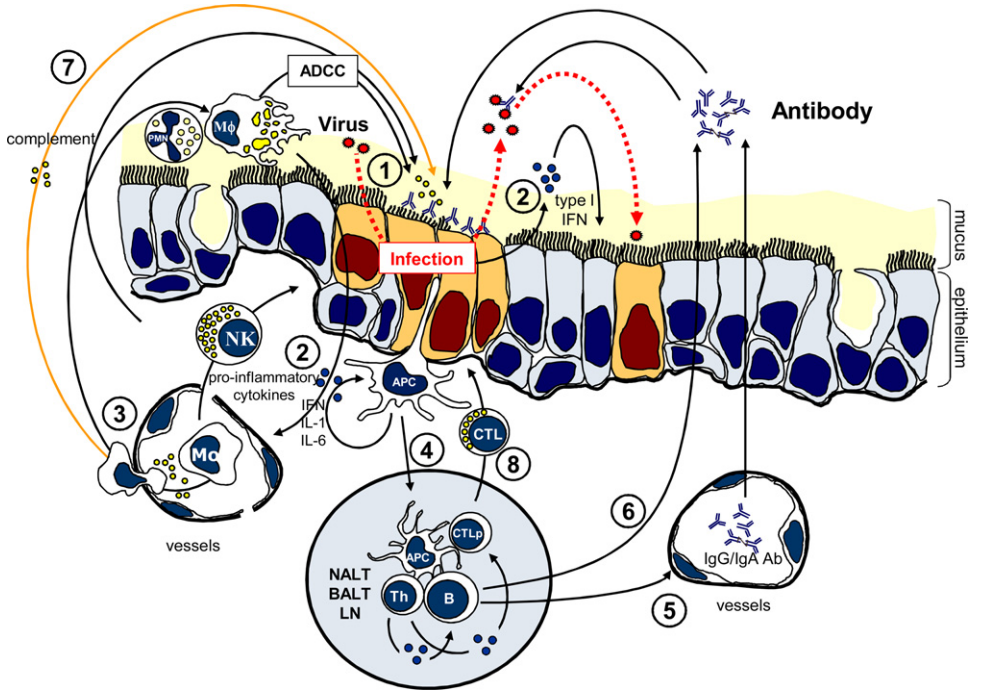

Fig. 1. Immune response to respiratory virus infection (adapted from (Paillot et al., 2008, 2009). Epithelial cells become infected (1) inducing the local synthesis of type I IFN, IL-1 and IL-6 that present a wide range of antiviral activities, inducing up-regulation of MHC class I molecules and antiviral resistance in uninfected cells. (2). Alveolar macrophages (Mϕ) and neutrophils (PMN) respond to the infection by releasing pro-inflammatory cytokines (2), leading to the containment of the infection, rise of body temperature and to the recruitment of further phagocytic and natural killer (NK) cells (3). NK cells are activated by IFN alpha and IL-12 synthesised by macrophages, and are cytotoxic for virus-infected cells. Their activity is not MHC restricted. NK cells synthesise IFN gamma that drives the development of the adaptive immune response. DC migration to lymphoid tissues and viral antigen presentation (4); induce the synthesis of serum (5); or mucosal antibodies (6); able to neutralise excreted virus and to stimulate antibody dependent cell cytotoxicity (ADCC) (7). Virus-specific CTL that lyse infected cells are also stimulated (8). APC: antigen presenting cell. BALT & NALT: Bronchus- & Nasal-associated lymphoid Tissue. CTL: cytotoxic T lymphocytes (CTLp: CTL precursor). LN: lymph node. Th: T-helper lymphocyte. B: B lymphocyte.

to evade immunity is to produce a variety of immune-modulatory proteins that support viral replication in spite of an active immune response. PPVO is known to express modulatory factors such as inhibitors of GM-CSF and IL-2 (GIF: GM-CSF inhibitor factor)(Deane et al., 2009; Seet et al., 2003) and a viral IL-10 homologue (Haig and Fleming, 1999; Haig and Mercer, 1998). Some of these immune evasion/modulatory mechanisms are maintained after virus inactivation, which could be used to the host's advantage (Friebe et al., 2004; Weber et al., 2003). Inactivated PPVO (iPPVO) has been shown to elicit immune-modulatory and anti-herpetic activity in several disease models (*e.g.* genital herpes disease in guinea pigs, human hepatitis B virus infection in transgenic mice, Pseudorabies virus in mouse models, or pig transmission model of Aujesky's disease, infectious bovine rhinotracheitis in the natural host)(Biuk-Rudan et al., 2004; Castrucci et al., 2000; Friebe et al., 2004; Weber et al., 2003).

3.1.1. *iPPVO activities in vitro*

iPPVO-mediated immune-modulation has been investigated *in vitro* in several species and was primarily associated with synthesis of early pro-inflammatory cytokines (IL-6, IL-8, TNF alpha) by monocytes or APC, IL-2, IFN alpha/beta and Th1 cytokines (IL-12, IL-18 and IFN gamma) by T-helper lymphocytes (Fachinger et al., 2000b; Friebe et al., 2004; Weber et al., 2003).[2] IFN gamma synthesis by T lymphocytes and/or NK cells is believed to be a keystone component of iPPVO activity. Interestingly, iPPVO-mediated cytokine response seems to be auto-regulated by an IL-4 (Th2 cytokine, Th1-cytokine antagonist), IL-10 (regulatory cytokine) and IL-1R antagonist (natural inhibitor of IL-1 beta) response in peripheral blood mononucleated cells (PBMC), which could explain the absence of notable side effects or tissue damage after iPPVO administration (Friebe et al., 2004; Weber et al., 2003). This complex cytokine response is also associated with activation of several cell populations. In combination with a co-stimulatory signal (*e.g.* Concanavalin A, ConA; a polyclonal T-cell mitogen), iPPVO had the ability to stimulate monocytes and Th1-like cells, involving soluble factors such as complement proteins (Friebe et al., 2004). Phagocytosis and oxidative burst were also increased in human neutrophils (Forster et al., 1994) and in canine monocytes (Schutze et al., 2009, 2010),[3] but this activity was not observed in swine leukocytes (Fachinger et al., 2000b). iPPVO induced activation of murine bone-marrow derived DC, which was characterised by IFNalpha/beta, IL-12 and TNF alpha expression and MHC class I and II

[2] Studies sponsored by Bayer AG Animal Health.
[3] Studies sponsored by Pfizer Animal Health.

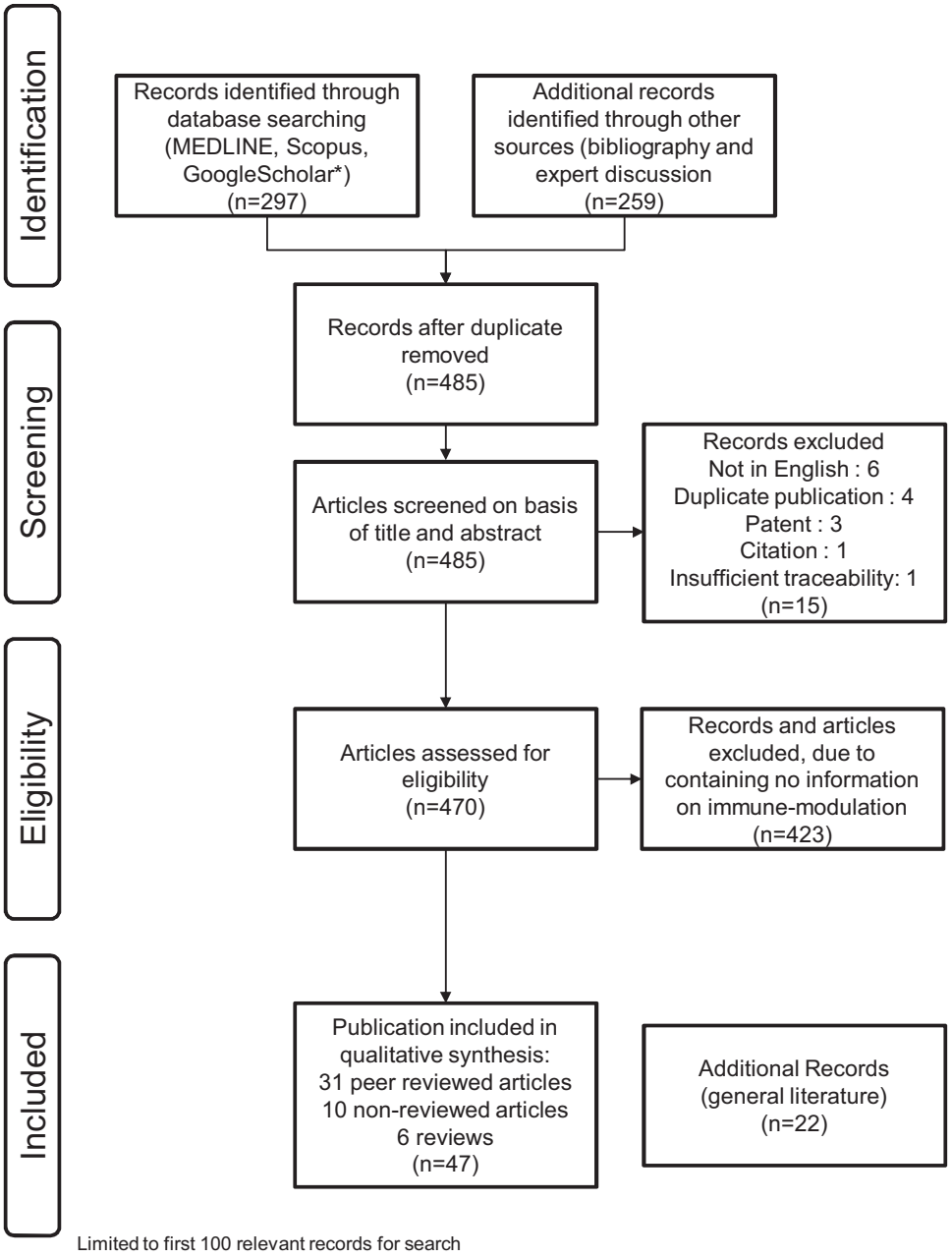

Fig. 2. Flow chart of the search strategy used to identify and select articles with information on PPVO and *P. acnes* used in horses (adapted from Moher et al., 2009).

and CD86 up-regulation *in vitro* (Siegemund et al., 2009). These responses were PRR Toll-like receptor (TLR) independent (TLR2 and 4). Finally, oligoclonal superantigen-like stimulation of swine and canine T CD4+ lymphocytes has also been demonstrated (Fachinger et al., 2000a; Schutze et al., 2009). This T-cell activation is antigen independent and may require increased MHC class II expression, as demonstrated on canine monocytes (Schutze et al., 2009). To date, the range of iPPVO activities reported *in vitro* with equine leukocytes is less extensive. *In vitro* stimulation of equine PBMC with iPPVO induced a statistically significant increase of IFN alpha/beta mRNA expression. In combination with ConA, iPPVO induced increased expression of IFN gamma and TNF alpha mRNA (Horohov et al., 2008) and intracellular IFN gamma production (Breathnach and Horohov, 2009). Increased phagocytosis was also reported after incubation of equine granulocytes with whole parapoxvirus or purified envelope proteins (Buttner, 1993).

3.1.2. *In vivo iPPVO/Zylexis activities in the horse*

The inactivated PPVO strain D1701 was commercialised as Baypamun® (Bayer, Inc) and more recently as Zylexis® (Pfizer AH, Inc). The D1701 strain was attenuated through

Table 1
Summary of iPPVO and *P. acnes* immune-modulation. i.m., intramuscular administration; i.v., intravenous administration; Inj., injection.

Immune-modulator	Type of study	Context	Outcomes	References
iPPVO i.m. 3 inj. Within 9-day period	*In vitro*	Cell stimulation.	Pro-inflammatory cytokine synthesis (IL-6, IL-8, TNFalpha), cytokine synthesis (IL-2, IL-4, IL-10 IFNalpha/beta, IL-12, IL-18, IFNgamma).	Fachinger et al. (2000a,b) Friebe et al. (2004) Weber et al. (2003) Horohov et al. (2008) Breathnach and Horohov (2009) Ryan et al. (2010)
			Increased phagocytosis/oxidative burst, DC and T cell activation.	Buttner (1993) Schutze et al. (2009, 2010) Fachinger et al. (2000b)
	In vivo	Cell stimulation.	Conflicting results on cytokine stimulation (IFNgamma, TNFalpha, IL-15 and IL-18).	Horohov et al. (2008) Breathnach & Horohov (2009) Sturgill et al. (2011a)
		Respiratory diseases associated with stress factors and co-mingling.	Conflicting results on efficacy. Reduced disease severity associated with EHV-1/4 infection. No impact on EIV infection.	Lindner et al. (1993) Ziebell et al. (1997) Donecker and Holland, (2005) ([5]) Lunn & Rush (2004)
		Immune-depression associated with transport.	Reduced cortisol levels and improved immune response.	Lunn & Rush (2004) Mayr ([4]) Valpotic et al. (1998)
		Application in foals and *R. equi* infection.	Conflicting results on disease incidence. No effect on *R. equi* infection.	Rush (2001) Sturgill et al. (2011a)
P. acnes i.v. 3 inj, 2–3 days apart	*In vitro*	Cell stimulation.	Macrophages, CD4 T lymphocytes, NK cell activation, IFNgamma, IL-1, IL-2, IL-6, TNFalpha, TLR and NK-lysin stimulation.	Davis et al. (2003) Flaminio et al. (1998) Tizard et al. (1992) Jugeau et al. (2005)
	In vivo	Stress related respiratory diseases.	Improved recovery. Decreased frequency of illness.	Evans et al. (1988) Vail et al. (1990) Nestved, 1996
		Cell stimulation and *R. equi* infection. Endometritis.	IFNgamma stimulation. Increased phagocytosis and oxidative burst. Improved diagnosis and pregnancy rate.	Sturgill et al. (2011b) Ryan et al. (2010) Zingher (1996) Rohrbach et al. (2007)

cell-culture passage (135 passages in embryonic lamb-kidney cultures, 137 passages in embryonic bovine lung, 49 passages in MA-104 cells and 62 passages in Vero cells).[4] iPPVO is recommended for prophylactic and metaphylactic treatment of infectious diseases in the horse. Three intramuscular (i.m) injections within a 9-day period is the usual advised administration. *In vivo*, yearlings treated with iPPVO (3 i.m injections, days 0, 2 and 9) showed transient IFN gamma and TNF alpha gene expression in blood when measured 24 and 48 h after the first immunisation, respectively. A single intradermal inoculation of iPPVO induced a statistically significant local tissue increase of IFN beta, IFN gamma, IL-15 and IL-18 mRNA expression in the 24–48 h following administration (Horohov et al., 2008)[3]. In the last 15 years, iPPVO has been tested against several infectious respiratory diseases of the horse.

Stress factors such as weaning, transport and co-mingling predispose horses to infection and increase susceptibility to respiratory pathogens such as *Streptococcus zooepidemicus* and EHV-1/4. The incidence of respiratory infection may reach 60% after transportation and could involves reduced macrophages activity (Foreman et al., 1992; Laegreid et al., 1988; Nestved, 1996; Stull et al., 2004). iPPVO was used as prophylactic treatment in a group of 63 Thoroughbreds subjected to stressful events. Foals received 3 i.m iPPVO injections immediately prior to and after weaning (days −6, −4 and +5). The prevalence of respiratory diseases was non-significantly reduced (p-value = 0.14; Fisher exact test) in the treated group (7.9%) when compared with the control group (24%) (Lindner et al., 1993). Another study sponsored by Bayer AG (Animal Health) demonstrated that the use of iPPVO (i.m injection, days 0, 2 and 9) to treat foals (4–10 month old, n = 26) in commingling and crowded conditions induced a statistically significant reduction of the overall clinical signs of respiratory infection, with noteworthy decrease in mucopurulent nasal discharge (severity and duration) when compared to a placebo treated group (n = 27). The clinical signs of disease started 2 days after co-mingling and peaked at the end of the second week of contact. They were associated with EHV-1/4 transmission and infection, as 43.1% and 54.6% animals had seroconverted against EHV-1 and EHV-4 at the end of the trial, respectively (Ziebell et al., 1997). This study highlights the beneficial use of iPPVO to limit the severity of respiratory infectious diseases in young horses (Ziebell et al., 1997). A more recent study sponsored by Pfizer AH evaluated iPPVO used for protection in yearlings exposed by contact-challenge to EHV-1 or EHV-4.[5] iPPVO treated yearlings (n = 9; 4 doses, i.m injection) and controls (n = 9) were commingled with 12

[4] Mayr, A. Paramunization by pox virus inducers (non-immunizing vaccines) as a new concept in prophylaxis and therapy in equine medicine. http://www.harness.org.au/hra/papers/Pmayr2.pdf.

[5] Donecker, J.M. & Holland R.E. Efficacy of the immunomodulator Zylexis® in horses challenged with equine herpesvirus. 2005. Pfizer Zylexis Technical Bulletin V3.

horses experimentally infected with the EHV-1 strain ERP A183. This study design was repeated using 10 yearlings in both iPPVO and control groups and co-mingling with 12 horses infected with the EHV-4 strain T446. All controls were successfully infected with either EHV-1 or EHV-4. iPPVO treated horses showed a 40% (EHV-1 study) and 61% (EHV-4 study) reduction in disease severity (measured by nasal discharge scoring) when compared to controls (p-value = 0.0206 and p-value = 0.0001, respectively. The statistical method used is not defined in the report). The neutralising antibody (SN) titre was also increased in the iPPVO treated groups, which support a potential immune-enhancing activity. The significance of the antibody response was not reported in the study. Virus shedding after infection was not affected by the iPPVO treatment.

The use of iPPVO was reported in horses subjected to stress transport and exposed to equine influenza infection. This work was presented by Lunn & Rush, at the 50th Annual Convention of the American Association of Equine Practitioners (AAEP). Twenty yearling horses received 3 injections of iPPVO (route of administration not specified) on days −2, 0 (transport) and +2 of transport and commingling with 10 horses experimentally infected with EIV. Twenty untreated naive horses going through similar experimental conditions were used as controls. All treated and controls horses were infected. The iPPVO treatment had little effect on the clinical signs of disease (only dyspnoea was significantly reduced when compared with controls, p-value ≤ 0.05, Wilcoxian rank-sum test) and no effect on virus shedding measured by titration in embryonated eggs (Lunn and Rush, 2004). This method of titration lacks sensitivity and subtle differences may have been missed (Paillot et al., 2010). However, the level of SRH antibody in response to infection was described as significantly lower in the controls horses (p-value not reported), when compared with iPPVO treated or experimentally infected horses. The lower SRH antibody response in control animals was associated with transport stress and it was concluded that iPPVO had contributed to re-establish normal immune response in potentially immune-suppressed individuals (Lunn and Rush, 2004). Such an activity on the transportation-associated immuno-suppression had been previously reported (Mayr and Mayr, 1999). Ten horses received intramuscular injection of iPPVO on days −4 and −2 prior to transport. The stress associated with loading and movement was measured by the elevation of cortisol serum concentration (a glucorticoid released in response to stress). Cortisol levels remained stable in iPPVO treated animals while it increased in controls (reviewed in (Mayr and Mayr, 1999) and [4].

iPPVO has also been evaluated in neonatal foals. Rush reported that iPPVO administration (route of administration not specified) immediately after birth and 24 or 48 h *post-partum* reduced the incidence of disease (reviewed in (Rush, 2001). *R. equi* is a facultative zoonotic intracellular bacterium that infects and replicates in alveolar macophages, inducing pneumonia and enteritis in young foals. Th1-induced IFN gamma response has been shown to be associated with protection against *R. equi* infection, through macrophage activation. However, young foals have difficulty producing Th1 cytokines after mitogenic stimulation during the first 3 months of age (Breathnach et al., 2006). A first study investigated the activity of cells purified from iPPVO treated foals (3 i.m injections on days 0, 2 and 8) when confronted to pathogen stimulation. An increased IL-12p40 and TNF alpha mRNA expression was measured in monocyte-derived macrophages purified 4 days after the second iPPVO *in vivo* injection and stimulated *in vitro* with *R. equi* for 4 h when compared with cells purified from control animals (injected with saline placebo) (Ryan et al., 2010).[6] An increase of *R. equi* phagocytosis by macrophages and neutrophils oxidative burst (essential for *R. equi* clearance) was also measured when compared with pre-treatment baseline in iPPVO animals. Hovewer, these differences were not significantly different from activity measured in controls at the same time point and therefore could reflect natural variation of macrophages and neutrophils activity with time. iPPVO activity against *R. equi* infection was further investigated at an American Quarter horse breeding farm with a history of foal pneumonia associated with *R. equi* infection. Twenty eight neonatal foals were treated with iPPVO from 24 to 48 h of age (3 i.m injection, day 0, +24 h and +9 days). Frequency of cytokine synthesising cells and the prevalence of pneumonia were compared with an untreated control group ($n = 31$ foals of similar age). PBMC were purified and stimulated *in vitro* with PMA/Ca Ionomycin (a strong T-cell mitogen combination) prior to analysis by IFN gamma or IL-4 ELISPOT assays. A statistically significant increase ($p = 0.027$) of IFN gamma synthesising cells were measured in blood samples from 7–14 days old foals treated with iPPVO when compared with control group animals. The frequency of IL-4 synthesising cells was equivalent between groups. The incidence of pneumonia was not affected by either iPPVO treatment or the frequency of IFN gamma synthesising cells (Sturgill et al., 2011a). However, a similar study (7 neonatal foals, i.m injected on days 0, 2 and 9) failed to detect an increase of intracellular IFN gamma synthesis after iPPVO treatment when compared to controls untreated foals ($n = 6$) (Breathnach and Horohov, 2009).

The review of *in vivo* studies tends to indicate that iPPVO reduces the severity of respiratory disease, which may improve overall recovery. Its use did not induce noticeable side effects. It is postulated that systemic IFN gamma stimulation induced by iPPVO may contribute to reduce the incidence of infectious diseases in horses. It appears that the protective activity of iPPVO may be variable depending of the nature of the pathogen but the number of documented studies remains limited. The recent study by Sturgill et al. (2011a) indicated that iPPVO had no effect on *R. equi* infection in young foals. However, the greater impact of iPPVO administration on EHV infection and subsequent secondary bacterial infection may result from the combination of iPPVO anti-herpetic activity and stimulation of IFN gamma synthesis. Its ability to directly prevent disease occurrence is arguable. The impact of pre-existing immunity induced by repeated injection of iPPVO is unknown in the horse. Previous results from permissive

[6] Study partially funded by Pfizer Animal Health.

species and dogs indicated that iPPVO-specific antibodies are non-neutralising. Furthermore, iPPVO-specific antibody titres seemed correlated with increased phagocytosis in dogs, through formation of immune-complexes and increased stimulating activity of iPPVO (Schutze et al., 2010). Anecdotally, iPPVO was also evaluated as potential treatment of equine sarcoids with no effect measured (Studer et al., 1997).

All these *in vitro* and *in vivo* studies are consistent with the new immune-modulator B2 (product of the B2L gene, a homolog of vaccinia F13L gene), recently identified by a functional screen of PPVO D1701 genome. The B2 protein is non-immunogenic but induced DC accumulation at the site of administration. B2 also enhanced antibody response and protective immunity when co-administered with an antigen *in vivo* in mice, which highlights its potential as adjuvant (McGuire et al., 2012). This adjuvant activity was previously reported in Tetanus toxoid/toxin hypermimmune horses that received iPPVO parenteral administration during tetanus immunisation. Both cellular and tetanus antibody responses were increased in iPPVO treated horses when compared with control horses (Valpotic et al., 1998).

3.2. Propionibacterium acnes

Propionibacterium acnes (*P. acnes*, formerly known as *Corynebacterium parvum*) is a commensal bacteria part of the skin flora that is reported to have immune-stimulatory and regulatory properties. When administered intravenously, *P. acnes* is likely to be taken up by macrophages in the liver and spleen (Cox, 1988; Tizard et al., 1992). It is believed that *P. acnes* immune-modulation is linked to a delayed degradation once phagocytised by macrophages (Kalis et al., 2005; Tchaptchet et al., 2012). This characteristic is the result of *P. acnes* peptidoglycan structure (Kamisango et al., 1982). It has also been suggested that repetitive CpG sequences in *P. acnes* genome could be at the origin of its immunostimulatory activity (Rush and Lunn, 2004). Unmethylated CpG oligodeoxynucleotide (CpG ODN) are repetitive CpG patterns that are recognised by the PRR TLR9. TLR9 is expressed by numerous innate cells such as DC, NK cells, monocytes and macrophages. Stimulation of macrophages, NK cells, CD4+ T lymphocytes, lymphokine activated killing (LAK) activity and an increased IFN gamma, IL-2 and NK-lysin (antimicrobial peptide) mRNA expression has been detected in healthy horses treated with inactivated *P. acnes* (Davis et al., 2003; Flaminio et al., 1998), with IFN, IL-1, IL-6 and TNF upregulation within 3 h of administration (Tizard et al., 1992). Bacterial fraction of *P. acnes* has been shown to stimulate TLR-2 and TLR4 by human keratinocytes *in vitro* (Jugeau et al., 2005). Recent work in mice reported that *P. acnes* immune-modulatory activities are dependent of TLR9 and/or IFN gamma expression (Kalis et al., 2005; Tchaptchet et al., 2012).

Inactivated *P. acnes* is commercialised under the name of EqStim® (Neogen Europe Ltd) and recommended for intravenous (i.v.) injection (3 injections, 2–3 days apart). *Proprionobacterium acnes* administration is usually well tolerated, with occasional and transient pyrexia, anorexia and lethargy (Flaminio et al., 1998). High fever has been anecdotally reported when used intramuscularly (Tizard et al., 1992). The use of inactivated *P. acnes* is recommended as prophylactic treatment or as adjunct to antibiotic therapy to improve immune defences in the treatment of equine respiratory diseases or prior to stressful events such as weaning and transportation (Rush, 2001, 2002; Rush and Flaminio, 2000). Twenty eight horses with clinical signs of respiratory diseases received 3 i.v. injections of *P. acnes* (days 1, 3 or 4, and/or 7), whilst 15 horses remained untreated and acted as controls. All enrolled animals received conventional treatment as determined by the examining clinician. The diagnosis of affected horses was viral pneumonia (60.5%), bacterial pneumonia (25.6%), Strangles (9.3%), chronic guttural pouch infection and pharyngitis (2.3% each). A mild febrile reaction was measured in some treated animals. However, the frequency of recovery at the end of the study was significantly increased (p-value = 0.045, Fisher's exact test) in the treated group (78.6%; 22/28) when compared to controls (46.7%; 7/15). The decrease in disease severity during the study was also significantly improved (p-value < 0.001) in the treated group (Evans et al., 1988). These results were later confirmed by a second study regrouping 25 horses treated with *P. acnes* (i.v. injection on days 1, 3 or 4 and 7) alongside conventional treatment for ERDC compared with 20 controls horses that received conventional treatment only. Ninety six percent (24/25) of *P. acnes* treated horses showed complete recovery or improvement 14 days after the start of the treatment when compared with 35% (7/20) in the control group (Vail et al., 1990). This effect was attributed to stimulation of respiratory macrophage functions induced by *P. acnes* administration. *P. acnes* was also tested in horses subjected to transport stress. One to 2 doses were administered i.v. on days −5 and/or −2 prior to transportation. In total, 217 horses were treated and compared with 233 control animals, in the context of 10 separate groups/shipments. The overall frequency of illnesses on arrival (as assessed by the presence of clinical signs of respiratory disease and treatment requirement) was significantly reduced (p-value < 0.0001; Fisher's exact test) in the treated groups (10.6% affected) when compared with control groups (31.8%). Similar results were reported when animals were clinically examined 7 days after arrival (18.4% and 60.9%, respectively; p-value < 0.0001)(Nestved, 1996).

In foals treated with *P. acnes* (3 i.v. injections 24 h apart, initiated 3–4 days after birth, and repeated at 1 month of age), no modification of IFN gamma mRNA expression and synthesis was measured after the first series of treatment in neonatal foals. However, the IFN gamma response was increased at around 1 month of age, after the second series of treatment (Sturgill et al., 2011b). Immunostimulation in the young foal may be of interest to limit or prevent *R. equi* infection. In the experimental study previously described in this review, Ryan et al. (2010) reported a reduced proliferation of *R. equi* in monocytes and macrophages purified from horses treated with *P. acnes* (3 injections i.v., days 0, 2 and 8) when compared with controls (Ryan et al., 2010). Performance of the different immune-modulators commercially available have rarely been compared. The levels of phagocytosis and oxidative

burst wassignificantly greater (p-value < 0.05) on day 24 in neutrophils purified from iPPVO treated foals when compared with neutrophils from *P. acnes* treated foals. *R. equi* proliferation and cytokine mRNA expression was not significantly different in PBMNC-derived macrophages or bronchoalveolar lavage macrophages purified from iPPVO or *P. acnes* treated foals (Ryan et al., 2010). However, no differences were measured in terms of phagocytosis and oxidative burst (day 24 of the study).

Several studies have evaluated the impact of *P. acnes* treatment on endometritis in mares and subsequent pregnancy rate improvement. Persistent endometritis in mares has anatomical, physiological and immunological predisposing factors, such as uterine inflammation. Phagocytosis by uterine neutrophiles is believed to be reduced in susceptible mares (Troedsson et al., 1993). *P. acnes* administration (i.v., days 1, 3 and 7) following the diagnosis of endometrial inflammation improved significantly cytological evaluation for endometritis in barren mares (only 1/12 positive mare in the treated group when compared with 15/16 in the control group, p-value < 0.0001, Fisher's exact test) (Zingher, 1996). In a double blind randomised study, mares diagnosed with persistent endometritis received 3 i.v. doses of *P. acnes* (days 0, 2 and 6) alongside conventional treatment. Results indicated that pregnancy rate and frequency of live foals were increased in *P. acnes* treated mares, if breeding occurred during the interval from 2 days before to 8 days after treatment (Rohrbach et al., 2007).[7] There are anecdotal reports of successful use of *P. acnes* for the resolution of Strangles (*S. equi* infection), corneal ulceration and fungal infection, although no data were provided (Evans et al., 1990). The use of *P. acnes* to prevent and/or treat papillomatosis in horses as also been anecdotally reported (Tizard et al., 1992).

4. General conclusion

In conclusion, there are an increasing amount of studies and reports that support the efficient use of non-specific immune-modulators such as iPPVO or *P. acnes* as adjuncts to conventional management of ERDC (Table 1). Their activity is mostly based on non-specific stimulation of innate immune response. They may not provide protection against direct infection or transmission of respiratory pathogens but they seem to contribute to the reduction of the disease severity, subsequently reducing the frequency of complications and improving the rate of recovery. In the case of iPPVO, its anti-herpetic function may provide a valuable contribution in prevention of equine herpesviruses infection.

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