

Passive transfer of maternal immunoglobulin isotype antibodies against tetanus and influenza and their effect on the response of foals to vaccination

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Keywords: horse; foals; vaccination; maternal antibodies; immunoglobulin; influenza; tetanus

Summary

Influenza and tetanus-specific antibodies of the IgG sub-isotypes are positively transferred to foals via colostrum and inhibit their response to inactivated influenza vaccines and tetanus toxoid. High titres of influenza antibodies of IgGa and IgGb subisotypes and tetanus antibodies of the IgGa, IgGb and IgG(T) subisotypes were detected in postsucking serum samples collected from foals born to mares that had received booster doses of multicomponent vaccines during the last 2 months of gestation. Thereafter, titres declined in an exponential manner but were still detectable in all foals at age 26 weeks, regardless of whether they had been vaccinated prior to age 26 weeks. Mean $\pm$ s.e. half-life of decline of influenza IgGa antibodies (27.0 ± 2.3 days) was significantly shorter than that of influenza IgGb antibodies (39.1 ± 2.7 days; $P < 0.005$). Tetanus IgGa and IgGb antibodies declined with half-lives of 28.8 ± 3.0 and 34.8 ± 5.1 days, respectively. Titres of tetanus IgG(T) antibodies were substantially higher than those of influenza IgG(T) antibodies in postsucking samples and remained so through age 26 weeks, declining with a half-life of approximately 35 days. Postsucking titres of tetanus and influenza antibodies of the IgA isotype were low and declined rapidly to undetectable levels.

Yearlings showed significant increases in titre of influenza IgGa, IgGb and IgG(T) subisotype antibodies but no increase in influenza IgA antibodies in response to 2 doses of multicomponent vaccines containing tetanus toxoid and inactivated influenza A-1 and A-2 antigens. Yearlings also showed strong tetanus IgGa, IgGb and IgG(T) subisotype responses to one dose of vaccine and a substantial further rise in titre in response to administration of a second dose 3 weeks later, but failed to show an increase in titre of tetanus IgA antibodies. The influenza and tetanus IgGa, IgGb and IgG(T) subisotype responses of 6-month-old foals to vaccination followed the same pattern as those shown by yearlings but titres were generally lower. In contrast, 3-month-old foals failed to show increases in titre of either influenza or tetanus IgG subisotypes in response to 2 doses of vaccine and generally needed 1–3 additional booster doses of vaccine to achieve titres similar to those achieved by yearlings after 2

doses. Based on the finding that maternal antibodies exert a significant inhibitory effect on the response of foals to tetanus toxoid and inactivated influenza antigens, it is recommended that primary immunisation of foals born to vaccinated mares should not commence before age 6 months.

Introduction

Tetanus and influenza are important infectious diseases of horses and are commonly targeted in equine immunisation programmes (Wilson *et al.* 1995). Administration of booster doses of vaccines during the last 2 months of gestation is practised routinely on breeding farms in order to improve protection of mares during the periparturient period and to protect foals passively, through transfer of maternal antibodies via colostrum, against challenge with specific infectious agents (Wilson 1999). Passively acquired maternal antibodies also have the potential to interfere with the response of foals to vaccines administered during the first few months *postpartum*. While maternal antibody interference has been well documented in several domestic animal species, there have been few studies in foals. Therefore recommendations for primary vaccination of foals have rarely been based on research data documenting whether or not vaccines are able to prime the immune response of foals in the presence of maternally-derived antibodies.

For many years it has been routine practice in North America to begin vaccinating foals at age 3 or 4 months in order to complete the primary series before weaning (Ardans 1982; Wilson *et al.* 1995). This approach was based on the assumption that maternal antibodies will have decayed to low or undetectable levels by age 3 months and was reinforced by a study documenting rapid disappearance of passively acquired influenza haemagglutination inhibition antibodies in foals (Liu *et al.* 1985). The rationale underlying this approach was brought into question by studies documenting failure of foals from vaccinated mares to respond serologically to primary vaccination against influenza (van Oirschot *et al.* 1991; van Maanen *et al.* 1992; Cullinane *et al.* 1994, 2001; Conboy *et al.* 1997; Holland *et al.* 1999). Of even greater concern was the finding that some foals vaccinated against influenza in the presence of maternal antibodies appeared to develop a tolerance-like phenomenon (Cullinane *et al.* 1994,

2001). These foals failed to mount a detectable serological response even after administration of 5 or 6 doses of inactivated influenza vaccine with the last doses administered at more than age one year when they would be expected to respond.

Natural or experimental infection of horses with equine influenza virus induces protective immunity for at least one year (Hannant *et al.* 1988). The response to infection is characterised by local production of IgA, in addition to circulating IgG_A and IgG_B antibodies (Nelson *et al.* 1998). Previously infected horses may resist challenge even when circulating antibody levels have waned (Hannant *et al.* 1988). In contrast, immunity following parenteral vaccination with inactivated influenza vaccines is incomplete and short-lived (Mumford *et al.* 1983; Wood *et al.* 1983) and is strongly correlated with the level of circulating antibody, as measured by the single radial haemolysis (SRH) test (Townsend *et al.* 1999; Morley *et al.* 2000; Newton *et al.* 2000). In addition, some ineffective inactivated influenza vaccines induce only IgG(T) responses and fail to induce the complement-fixing IgG_A and IgG_B subisotype responses that are detected in the SRH test (Nelson *et al.* 1998).

Limited information is available regarding immune correlates for protection against tetanus in the horse. Tetanus toxoid is regarded as a potent antigen and is known to induce strong antibody responses that probably confer protection. Antibodies of the IgG(T) subisotype are thought to be important in protection against bacterial exotoxins such as those elaborated by *Clostridium tetani* (Roberts 1975; Patton *et al.* 1978). However, the isotype-specific responses of horses to tetanus toxoid have not been described, and information regarding passive transfer and persistence of maternal antibodies of different immunoglobulin isotypes in foals is lacking.

The objectives of this study were, therefore, to determine: 1) the immunoglobulin isotype profile of maternal tetanus and influenza antibodies passively acquired by foals; 2) the half-life of decline of passively acquired antibodies of different subisotypes; 3) isotype-specific responses of foals to vaccination with inactivated adjuvanted vaccines against influenza and tetanus and 4) the effect of maternal antibodies and age on these responses.

Materials and methods

Experimental animals

This study was conducted over a 2 year period and involved 13 University-owned foals of Thoroughbred, Quarter Horse, Arabian and mixed breeding (*Groups 1* and *2*) and 5 privately-owned unvaccinated Quarter Horse yearlings (*Group 3*) that served as controls. Dams of foals and yearlings had received booster doses of vaccines against tetanus, influenza, Eastern equine encephalitis (EEE), Western equine encephalitis (WEE), EHV-1 and EHV-4 during the last 2 months of pregnancy. Foals were assigned to groups based on alternating order of their birth and were vaccinated starting at either age 3 months (*Group 1*) or 6 months (*Group 2*). Groups were further subdivided (*1A*, *1B*, *2A*, *2B*) so that the 4 foals in *Group 1A* and the 3 foals in *Group 2A* received vaccines from manufacturer A¹ during year 1, while the 3 foals in *Group 1B* and 3 in *Group 2B* received vaccines from manufacturer B² during year 2. The 5 control yearlings received vaccines from manufacturer A during year 2.

Sampling and vaccination protocol

Blood samples were collected from all foals into evacuated tubes by jugular venipuncture during the first week *postpartum* and again during weeks 4, 8, 12, 16, 19, 22, 26, 29–30, 33, 36, 40 and 43. Samples were allowed to clot at room temperature and were then centrifuged. Serum was pipetted into screw top plastic storage vials and stored frozen at -20°C until assayed. Primary immunisation of foals in *Groups 1A* and *1B* was initiated at age 12 weeks, with subsequent doses at 16 and 26 weeks, whereas foals in *Groups 2A* and *2B* were vaccinated at age 26, 30 and 40 weeks. Additional doses of vaccine were administered as indicated in figure legends.

Primary vaccination of yearlings in *Group 3* commenced at age 1 year with a second dose 4 weeks later. Blood samples were collected from yearlings at the time of administration of each dose of vaccine and again 3 weeks after administration of the second dose. Inactivated i.m.-administered multivalent vaccines containing tetanus, influenza A-1, influenza A-2, EEE and WEE antigens (Encevac TC-4 with Havlogen¹, or Fluvac EWT²) and inactivated EHV-1/4 vaccine (Prestige with Havlogen¹, or EHV-1/4²) were used.

Antibody assays

Serum was assayed for influenza A-2-specific and tetanus toxoid-specific antibodies of the IgG_A, IgG_B, IgG(T) and IgA isotypes by ELISA using the technique described previously (Nelson *et al.* 1998). Briefly, purified equine influenza virus (A/equine/2/Kentucky/81) was coated onto 96 well polystyrene plates (Immulon)³ at 10 haemagglutinin (HA) units per well. The plate was blocked with 1% teleostean gelatin⁴ in PBS (gelatin/PBS). Samples were diluted in gelatin/PBS and incubated in plates in triplicate. Each plate included control wells consisting of a serum sample negative for influenza-specific antibodies, and a series of dilutions of a standard positive equine anti-influenza virus serum of known titre (determined by comparison with a negative serum control in this ELISA system). Plates were incubated subsequently with equine immunoglobulin isotype-specific monoclonal antibody supernatants (anti-IgG_A [WS29], anti-IgG_B [WS13], anti-IgG(T) [WS30], or anti-IgA [WS15]) diluted in PBS to give a final antibody concentration of 0.01 mg/ml (Lunn *et al.* 1998). Plates were washed prior to the addition of peroxidase-conjugated goat anti-mouse IgG and IgM(H+L) (Affinipure)⁵. Colour was developed by addition of 3,3',5,5'-tetramethylbenzidine substrate⁶, and the reaction stopped by the addition of H₃PO₄. The A₄₅₀ of each well was determined spectrophotometrically with an ELISA reader (EE 311SX Microplate Autoreader)⁷. Titres of samples were determined by comparison with a standard curve constructed using the dilutions of the positive control serum. The ELISA procedure for assay of tetanus-specific antibodies was identical to that used for the influenza assays except that the ELISA plates were coated with tetanus toxoid instead of influenza virus. Tetanus toxoid concentrate culture filtrate at a concentration of 1 g/ml⁸ was diluted 1:3000 and 50 µl of this dilution was used to coat each well of the ELISA plate.

Data analysis

The influenza and tetanus immunoglobulin isotype data from

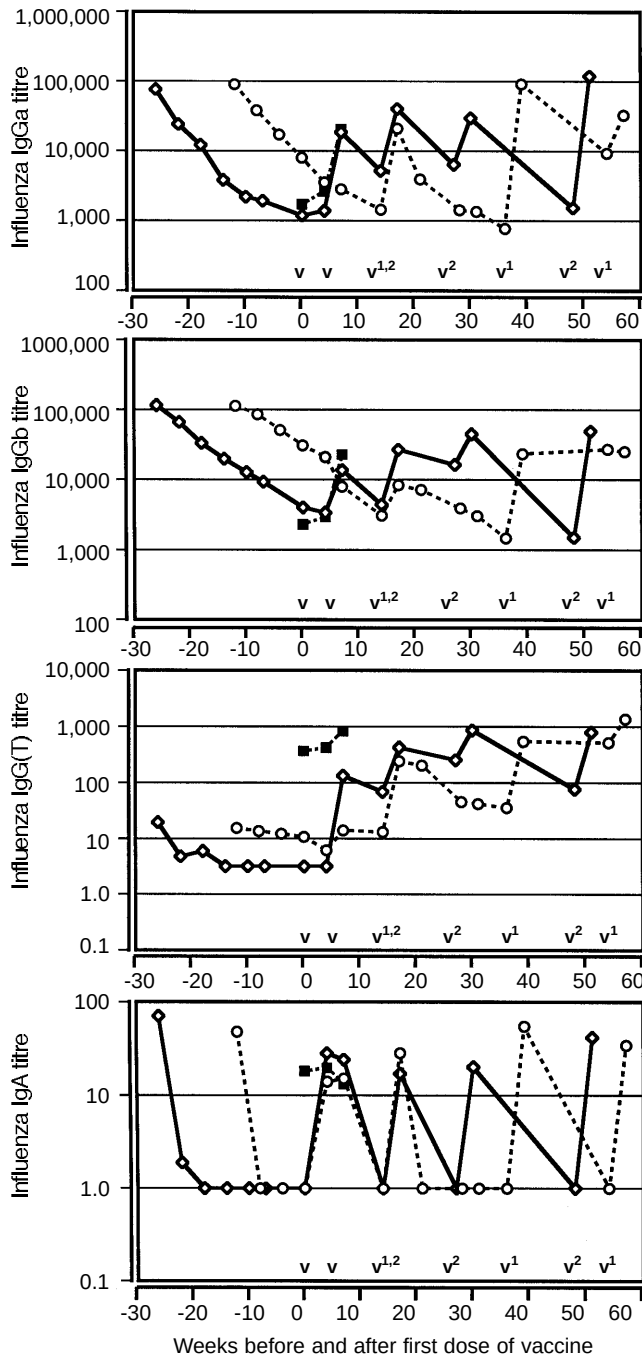


Fig 1: Geometric mean titres of influenza virus-specific IgGa, IgGb, IgG(T) and IgA antibodies in 2 groups of foals and one group of yearlings. Foal data shows decline of maternally transferred antibody, followed by response to vaccination starting at either age 3 months (○; n = 7) or 6 months (◇; n = 6). Yearlings were studied for 7 weeks only (■; n = 5). Data are arranged so that the initial dose of vaccine in all animals was given at week 0. Vaccination times are indicated by 'V' when all 3 groups were vaccinated, with superscripts when a subset of groups were vaccinated (i.e. V¹ indicates only Group 1 received a vaccine at this time point).

Group 1 foals to age 12 weeks and from Group 2 foals to 16 weeks were used to evaluate passive transfer and decay of maternal antibodies of each isotype. Half-life of decline of titre was determined by iterative least squares regression analysis of

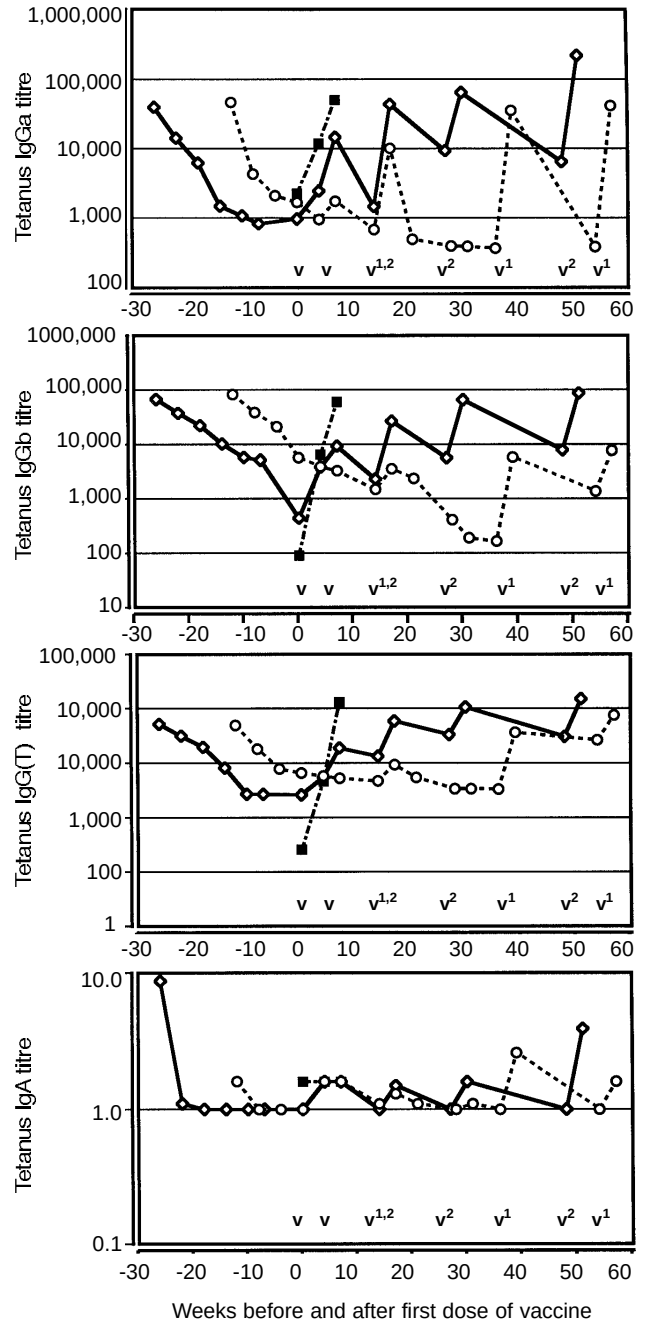


Fig 2: Geometric mean titres of tetanus-specific IgGa, IgGb, IgG(T) and IgA antibodies in 2 groups of foals and one group of yearlings. For details see Figure 1.

the data for each foal using a commercial pharmacokinetic computer program (ESTRIP)⁹, assuming first order elimination. Data from individual foals was used to calculate mean ± s.e. half-life of decline for the influenza and tetanus IgGa and IgGb subisotypes. Individual foal data were unsuitable for calculation of half-lives of decline for influenza and tetanus IgG(T) antibodies. Consequently, geometric mean tetanus IgG(T) titre for each sampling time through 12 weeks was calculated and analysed by iterative least squares regression analysis to estimate the half-life of decline of tetanus IgG(T) antibodies.

Geometric mean titre for each antibody isotype on each

sampling day was determined for both influenza and tetanus separately for foals in *Groups 1A, 1B, 2A and 2B* and for yearlings to permit qualitative comparison of the responses of each age *Group* to vaccines A and B. Geometric mean and mean $\pm$ s.e. of the logarithmically transformed data for all *Group 1* and *Group 2* foals was also calculated. Data for *Group 1* foals, *Group 2* foals and yearlings were then arranged relative to the day that the first and subsequent doses of vaccines were administered and statistical comparison between *Groups* of mean L_n titres of each isotype was made using a 2 tailed unpaired *t* test. Significance was ascribed when the *P* value was ≤ 0.05 .

Results

Decline in maternally-derived antibodies

High titres of influenza and tetanus antibodies of the IgGa and IgGb isotypes were observed in postsucking serum samples collected during the first week *postpartum* (Figs 1, 2). Thereafter, titres declined in an exponential manner but were still detectable in all foals at age 26 weeks, regardless of whether they had been vaccinated prior to 26 weeks. Mean $\pm$ s.e. half-life of decline of influenza IgGa antibodies (27.0 ± 2.3 days) was significantly shorter than that of influenza IgGb antibodies (39.1 ± 2.7 days; $P < 0.005$). Half-lives of decline of tetanus IgGa (28.8 ± 3.0 days) and IgGb (34.8 ± 5.1 days) antibodies were not significantly different. Low titres of influenza and tetanus IgA antibodies were detected in postsucking serum samples and declined rapidly to undetectable levels in most foals by age 4 weeks.

Postsucking influenza IgG(T) titres varied between foals, but were generally low and declined to undetectable levels in most foals by age 12 weeks (Fig 1). Titres of tetanus IgG(T) antibodies in postsucking samples were substantially higher than titres of influenza IgG(T) antibodies and remained so to age 26 weeks (Fig 2). There was substantial variation between individual foals with regard to the rate of decline of tetanus IgG(T) antibodies. IgG(T) titres in *Groups 1A* and *2A* foals sampled in 1996 declined much more slowly than did titres in *Groups 1B* and *2B* foals born in 1997. Estimated half-life of decline, based on regression analysis of geometric mean IgG(T) titre, was 35 days.

Response of yearlings to vaccination

Titres of influenza and tetanus antibodies of all isotypes were low in yearlings before administration of the first dose of vaccine (Figs 1, 2). Mean titres of influenza IgGa and IgGb increased approximately 10-fold and influenza IgG(T) doubled after administration of 2 doses of vaccine. Unlike influenza, where 2 doses of vaccine were generally needed to induce a strong primary response, yearlings mounted tetanus IgGa, IgGb and IgGT responses after administration of one dose of vaccine and showed a substantial further increase in titre after administration of the second dose. Titres of tetanus IgGa, IgGb and IgG(T) increased 22-fold, 670-fold and 500-fold, respectively, after administration of 2 doses of tetanus toxoid. In contrast, influenza and tetanus IgA titres did not increase in response to 2 doses of vaccine.

Comparative responses of 3- and 6-month-old foals and yearlings to vaccination

Comparison of the responses of all 3-month-old (*Group 1*) foals, all 6-month-old (*Group 2*) foals and yearlings to each

dose of vaccine highlighted differences related to age or maternal antibody status (Figs 1, 2). As would be expected based on decline of maternally-derived antibodies with time, 3-month-old foals had significantly higher titres of influenza IgGa, influenza IgGb, tetanus IgGb and tetanus IgG(T) antibodies than did yearlings and significantly higher influenza IgGa and IgGb titres than did 6-month-old foals at the time of administration of the first dose of vaccines. Conversely, poor passive transfer of influenza antibodies of the IgG(T) and IgA isotypes resulted in titres that were significantly lower in 3- and 6-month-old foals than in yearlings at the time of administration of the first dose of vaccines. With the exception of IgA, titres of all influenza isotypes were numerically higher in yearlings than in foals and higher in 6- than in 3-month-old foals, 3 weeks after completion of the primary series of 2 doses of vaccine (Fig 1). Significant differences between 6-month-old foals and yearlings were not observed, except with regard to tetanus IgGb titres 3 weeks after administration of the second dose of vaccine. In contrast, influenza IgG(T), tetanus IgGa, tetanus IgGb and tetanus IgG(T) titres were all significantly higher in yearlings than in 3-month-old foals, 3 weeks after administration of the second dose of vaccines.

Differences between 3- and 6-month-old foals with regard to their serological responses to the first 2 doses of vaccine were at least partially obscured by the superimposed effect of maternal antibodies (Figs 1, 2). However, suboptimal priming in 3-month-old foals was evidenced by the finding of significantly lower titres of tetanus IgGa, IgGb, and IgG(T) antibodies in 3-month-old foals, 3 weeks after administration of the third dose of vaccine. Tetanus IgGb titres of 3-month-old foals remained significantly lower than those of 6-month-old foals, 3 weeks after administration of the fourth and fifth doses of vaccine, and tetanus IgG(T) titres of 3-month-old foals were also significantly lower after administration of the fourth dose of vaccine, suggesting that it takes several doses of vaccine for foals vaccinated at the younger age to catch up with those vaccinated at an older age. Influenza IgGa titres in 6-month-old foals were significantly higher than titres in 3-month-old foals, 3 weeks after administration of the fifth dose of vaccine approximately one year after administration of the first dose. Rather than reflecting inhibition of induction of the primary response by maternal antibodies in 3-month-old foals, this difference probably reflects interference resulting from the high IgGa antibody titres induced by the fourth dose of vaccine.

The pattern of influenza and tetanus IgA responses in 6-month-old foals was identical to that observed in 3-month-old foals but distinctly different from that observed in yearlings. Whereas foals showed a rapid but transient increase in IgA titre in response to all doses of vaccine except the second dose in the primary series, yearlings failed to show any detectable response. IgA titres in yearlings were higher than those of foals prior to administration of the first dose of vaccine and remained relatively constant after administration of subsequent doses.

Comparative influenza and tetanus isotype responses of foals to vaccines A and B

Influenza IgGa, IgGb and IgA antibody isotype responses of 3-month-old foals to vaccines A and B were qualitatively similar, although the first 3 doses of vaccine B induced higher mean titres of IgGb than did vaccine A. The pattern of influenza IgG(T) responses of 3-month-old foals to vaccine A differed from those

to vaccine B. Two doses of vaccine B induced an increase in mean IgG(T) titre and a third dose administered at age 26 weeks induced a further marked anamnestic response. In contrast, none of the 3-month-old foals seroconverted in response to the first 2 doses of vaccine A and there was only a minor increase in IgG(T) titre after administration of the 3rd dose.

The pattern of influenza IgGa responses of 6-month-old foals to vaccines A and B were qualitatively similar, although responses to vaccine B were consistently higher than those induced by vaccine A. The pattern of influenza IgGb and IgG(T) responses of 6-month-old foals to the 2 vaccines appeared to differ. Whereas foals vaccinated with vaccine A showed a minimal increase in mean titre of either isotype after administration of the first 2 doses of vaccine in the primary series, foals given vaccine B showed a marked increase in titre in response to the second dose of vaccine administered at age 30 weeks.

Tetanus isotype responses to vaccines A and B were similar in both 3- and 6-month-old foals, with the possible exception of the IgG(T) response. The tetanus IgG(T) responses of foals in both groups were difficult to interpret because they were partially obscured by the high level of maternal antibodies transferred and the much slower rate of decline of these antibodies in *Groups 1A* and *2A* foals than in *Groups 1B* and *2B* foals.

Discussion

Passive transfer of antibodies via colostrum is important for protection of foals against pathogens they may encounter during the first few months *postpartum*. Several studies have documented passive transfer of specific antibodies against a number of equine pathogens, but few have described transfer of specific antibodies of the major immunoglobulin isotypes and subisotypes (McGuire and Crawford 1973; Galan *et al.* 1986). While presucking serum samples were not available for the foals in our study to document with certainty that specific antibodies were absent from the serum of foals before sucking, results strongly suggest that specific antibodies of the IgGa, IgGb, IgG(T) subisotypes and the IgA isotype were passively transferred to foals via colostrum. Sheoran *et al.* (2000) reported that IgGb, IgGc, IgG(T) and IgA were absent from the serum of foals before sucking and that presucking concentrations of IgGa were extremely low (Sheoran *et al.* 2000).

The high titres of influenza IgGa and IgGb, and tetanus IgGa, IgGb and IgG(T) antibodies in our foals during the first week suggest that these subisotypes were all well absorbed from colostrum. This conclusion is supported by a recently published study that showed the relative concentrations of IgGa, IgGb, and IgG(T) in the serum of foals after ingestion of colostrum to be in a ratio approximating that of the relative concentrations of the respective isotypes in colostrum (Sheoran *et al.* 2000). The finding of high postsucking titres of tetanus IgG(T) antibodies and low titres of influenza IgG(T) antibodies in the serum of foals in our study suggests that colostrum concentrations of tetanus IgG(T) were higher than those of influenza IgG(T). These results seem appropriate from a biological standpoint in that antibodies of the IgG(T) subisotype are thought to play an important role in protection against tetanus (Roberts 1975), whereas antibodies of the IgGa and IgGb subisotypes are thought to be involved in protection against influenza (Nelson *et al.* 1998). The finding of low titres of influenza and tetanus IgA antibodies in postsucking serum samples and the rapid disappearance of this isotype from foal serum is consistent with results of a study by Sheoran *et al.*

(2000), who documented poor absorption of IgA from colostrum and rapid clearance from foal blood. Rapid decline in titres of IgA may reflect redistribution of absorbed IgA to mucosal surfaces as has been demonstrated for antibodies to *Streptococcus equi* (Galan *et al.* 1986).

Mean half-lives of decline of influenza and tetanus antibodies of the IgGa and IgGb isotypes were in the same range as those reported for passively acquired specific antibodies to a variety of pathogens measured in foals using other serological tests (Ferguson *et al.* 1979; Kendrick and Stevenson 1979; Gibbs *et al.* 1988; van Maanen *et al.* 1992; Hullinger *et al.* 1998; Breathnach *et al.* 1999). In addition, the half-lives of decline of tetanus and influenza antibodies of a particular subisotype (IgGa or IgGb) were similar to each other. The finding of a significantly shorter half-life of decline for influenza IgGa than influenza IgGb antibodies is in agreement with published results (Sheoran *et al.* 2000), and suggest that IgGa antibodies are consumed or decay more rapidly than do IgGb antibodies. This finding further suggests that duration of passive protection against a particular pathogen may be influenced to some extent by the predominant IgG subisotype of specific antibody present in colostrum.

Of the factors that influence duration of persistence of maternally-derived antibodies at detectable levels, the amount of antibody absorbed by the foal, the rate of consumption of antibody and the sensitivity of the assay procedure used to detect antibody are the most important. The high sensitivity of the ELISA procedures used in this study and the high titres of specific antibody acquired passively by experimental foals permitted documentation of persistence of maternally-derived influenza antibodies of the IgGa and IgGb subisotypes and tetanus antibodies of the IgGa, IgGb and IgG(T) subisotypes in most foals until at least age 6 months. This is longer than has previously been estimated by some workers (Liu *et al.* 1982, 1985) and highlights the potential for maternal antibodies to interfere with the response of foals to vaccines administered up to age 6 months and beyond.

Two doses of vaccine A induced marked increases in titre of influenza antibodies of the IgGa, IgGb and IgG(T) subisotypes in the yearlings vaccinated in our study. These results differ from those reported by Nelson *et al.* (1998) who found that naïve ponies failed to develop IgGa or IgGb responses but showed a marked increase in titres of antibodies of the IgG(T) subisotype in response to vaccination (Nelson *et al.* 1998). Since IgGa and IgGb responses appear to be important in protecting vaccinated horses against influenza, the results of our study suggest that vaccine A may be more effective in inducing protective immunity than the vaccine used by Nelson *et al.* (1998). Differences between the vaccines with regard to antigen preparation and presentation, including the adjuvant used, probably account for the observed differences in subisotype responses. This conclusion is also supported to some extent by apparent vaccine-associated differences in the magnitude of the IgG(T) response of the foals vaccinated with vaccines A and B in our study.

The isotype-specific responses of horses to tetanus toxoid have not previously been reported. IgG(T) is the predominant immunoglobulin isotype generated in horses hyperimmunised with tetanus toxoid for antitoxin production, a finding that formed the basis for designating this subisotype of IgG as IgG(T) (Roberts 1975). IgG(T) is thought to be important in neutralising toxins such as tetanus toxin, and in the response of horses to internal

parasites (Roberts 1975; Patton *et al.* 1978). Since both vaccines used in this study induced substantial IgG(T) responses in vaccinates, it is probable that both succeeded in inducing a protective response. The role of IgGa and IgGb in protecting horses against tetanus toxin has not been defined.

This study demonstrated clearly failure of 3-month-old foals to develop an appropriate serological response to either influenza or tetanus after primary immunisation with 2 doses of inactivated adjuvanted multi-component vaccines from 2 different manufacturers. These results are consistent with previous reports of vaccination studies in 3-month-old foals (van Oirschot *et al.* 1991; van Maanen *et al.* 1992; Cullinane *et al.* 1994, 2001; Conboy *et al.* 1997; Holland *et al.* 1999). The small numbers of foals in each age group in this study did not permit us to determine whether a 'threshold' level of antibody was required to inhibit the response to vaccination, regardless of age. In contrast to the results of other studies (Cullinane *et al.* 1994, 2001), our study failed to demonstrate evidence for the development of tolerance of foals to either influenza or tetanus antigens. Although an additional 2 doses of vaccine were required for Group 1 foals to achieve levels of influenza antibody similar to those found in yearlings given 2 doses of vaccine, these foals appeared to be fully capable of mounting IgGa, IgGb and IgG(T) responses to influenza vaccine.

This study is the first to document maternal antibody interference with the response of foals to a commercially available tetanus toxoid. Not only did 3-month-old foals in our study fail to seroconvert after 2 doses of vaccine, mean titres did not achieve levels approaching those of yearlings until after foals had received 5 doses of vaccine. In contrast, yearlings seroconverted after the first dose of toxoid and showed a further marked increase in titres of tetanus IgGa, IgGb and IgG(T) after the second dose. This result is in agreement with published reports in which tetanus antibodies were measured using a mouse tetanus toxin-antitoxin neutralisation test and confirms that tetanus toxoid is a potent antigen in horses (Jansen and Knoetze 1979; Liefman 1980). Whereas the response of 6-month-old foals to tetanus toxoid in our study was qualitatively similar to that of yearlings, the magnitude of responses was substantially lower, suggesting persistence of inhibitory effects of maternal antibodies on the response to tetanus toxoid beyond age 6 months.

Based on the finding that maternal antibodies exert a significant inhibitory effect on the response of foals to tetanus toxoid and inactivated influenza antigens, it is recommended that primary immunisation of foals born to vaccinated mares should not commence before age 6 months and should include 3 doses in the primary series. A 2 dose primary series of tetanus toxoid appears appropriate for naïve yearlings and older horses.

Acknowledgements

This work was supported in part by a gift from Bayer Corporation, Shawnee, Kansas, and a grant from the Center for Equine Health, School of Veterinary Medicine, University of California-Davis with funding provided by the Oak Tree Racing Association, the State of California pari-mutuel wagering fund, and contributions from private donors. The authors wish to thank the Bickford and Melton families for permitting us to use their horses and Connie Littrell, Nettie Ehrke, Trish Vogt, Ken Taylor, Dr Janet Roser and the staff of the Animal Science Horse Barn for help with collection of blood samples.

Manufacturers' addresses

¹Bayer Corporation, Shawnee, Kansas, USA.

²Fort Dodge Laboratories, Fort Dodge, Iowa, USA

³Dynatech Laboratories Inc, Chantilly, Virginia, USA.

⁴Sigma Chemical Co, St. Louis, Missouri, USA.

⁵Jackson ImmunoResearch Laboratories, Inc, West Grove, Pennsylvania, USA.

⁶Kirkegaard & Perry Laboratories Inc., Gaithersburg, Maryland, USA.

⁷Bio-Tek Instruments, Winooski, Vermont, USA.

⁸East Coat Biologics, Inc., North Berwick, Maine, USA.

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