

# **Passive transfer and rate of decay of maternal antibody against African horse sickness virus in South African Thoroughbred foals**

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## **Conflict of interest**

No conflict of interest has been declared.

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## Summary

**Reasons for performing study:** African horse sickness is an insect transmitted, non-contagious disease of equids caused by African horse sickness virus (AHSV). Mortality can exceed 90% in fully susceptible horse populations. A live-attenuated (modified-live) cell culture-adapted (MLV) polyvalent AHSV vaccine is widely used to control AHS in endemic areas in southern Africa. Field studies detailing antibody responses of vaccinated horses are lacking.

**Objectives:** To determine antibody titres to the 9 known serotypes of AHSV in a cohort of brood mares that were regularly vaccinated with the MLV AHSV vaccine, and to measure the passive transfer and rate of decay of maternal antibody to the individual virus serotypes in foals.

**Methods:** Serum was collected from 15 mares before foaling and from their foals after foaling and monthly thereafter for 6 months. Antibody titres to each of the 9 AHSV serotypes were determined by serum-virus neutralisation assay.

**Results:** There was marked variation in the antibody response of the mares to individual AHSV serotypes even after repeated vaccination, with consistently higher titre responses to some virus serotypes. Similarly, duration of maternally-derived antibodies in foals differed among serotypes.

**Conclusions:** Data from this study confirm variation of the neutralising antibody response of individual mares to repeated vaccination with polyvalent AHSV vaccine. Virus strains of individual AHSV serotypes included in the vaccine may vary in their inherent immunogenicity. Passively-acquired maternal antibodies to AHSV vary markedly among foals born to vaccinated mares, with further variation in the duration of passive immunity to individual AHSV serotypes.

**Potential relevance:** These data are relevant to the effective utilization of live-attenuated AHSV vaccines in endemic regions, and potentially to the use of vaccines in response to future incursions of AHSV into previously free regions. Further studies involving a larger population will be required to determine the optimal time for vaccinating foals.

## Introduction

African horse sickness (AHS) is an insect-transmitted, non-contagious disease of equids caused by African horse sickness virus (AHSV) [1, 2]. AHSV is spread by hematophagous *Culicoides* midges that serve as biological vectors of the virus; *C. imicola* and *C. bolitinos* are the most important vectors in South Africa [3]. AHSV is a member of the family *Reoviridae* genus *Orbivirus* [4], of which 9 serotypes have been described [5, 6]. AHSV is endemic in sub-Saharan Africa but several epidemics have occurred beyond this region [7, 8]. As a consequence of its severity and because it is able to spread rapidly and without apparent warning from its historically endemic areas, AHS is listed by the World Organization for Animal Health (Office International des Epizooties [OIE]) as important to the international trade and movement of horses [9]. There is a significant but undetermined risk that AHSV could spread in the future to Europe and beyond, as recently has occurred with related bluetongue virus that also is transmitted by *Culicoides* midges [8, 10].

Vaccination is currently central to the control of AHS in endemic areas, and vaccination would likely be used to control any future incursions of AHSV into historically free regions such as Europe. Several AHS vaccines have been developed and used in the past including: a polyvalent live-attenuated virus (modified-live [MLV]) vaccine of adult mouse brain origin, a polyvalent cell culture-adapted MLV, and an inactivated cell culture propagated AHSV-4 vaccine. The inherent limitations of each of these vaccine types have previously been reviewed [11, 12]. There is clearly a need for safe and efficacious vaccines to facilitate the continued international movement of horses, but currently only a polyvalent cell-culture-adapted MLV AHSV vaccine is commercially available.

The MLV cell-culture-adapted polyvalent AHSV vaccine that is used widely throughout southern Africa was introduced in the 1960s [13]. Horses in the AHSV infected area in South Africa are vaccinated annually with this vaccine. The vaccine is administered in two doses, the first includes a cocktail of serotypes 1, 3, 4 (so-called “bottle 1”) and the second includes serotypes 2, 6, 7, 8 (“bottle 2”). Serotypes 5 and 9 are not included in this polyvalent vaccine because of reported cross-protection from serotypes 8 and 6, respectively [14]. The two vaccine doses are administered at least 21 days apart. Despite the widespread use of this polyvalent MLV AHSV vaccine in southern Africa, there are few published data of antibody titres in horses that have been vaccinated in the field with this

preparation and, further, there is no definitive information on the level and duration of maternal antibody in foals [14].

Given the importance of vaccination to the control of AHS, the objectives of this study were to determine antibody titres to each of the 9 serotypes of AHSV in a cohort of breeding mares that were regularly vaccinated with the MLV cell-culture-adapted AHSV vaccine, and to measure passive transfer and rate of decay of maternal antibody to the individual virus serotypes in their foals.

## **Materials and Methods**

### **Study population and blood collection**

Fifteen multiparous mares in late gestation (that foaled during the months of August and September, 2006) that were resident on a Thoroughbred stud farm in the surveillance zone of the AHS controlled area in the western Cape Province of South Africa were evaluated. This comprised all the mares on the farm that were due to foal during August and September, which are the months most distant to the expected peak AHSV transmission season. This area is subject to an active AHSV surveillance program that includes clinical surveillance and serological surveillance of negative sentinel horses. No cases of AHS have been detected within a radius of at least 30 km of this farm since the inception of the surveillance program in 1996. All 15 mares had been annually vaccinated with the polyvalent AHSV vaccine®<sup>a</sup> and, during the 2006 foaling season, 12 were vaccinated during the last trimester of pregnancy (according to normal farm practice) and 3 were not (Table 1). Mares were vaccinated with AHSV serotypes 2, 6, 7, 8 (bottle 2) in mid-June and serotypes 1, 3, 4 (bottle 1) in mid-July. Blood for harvesting of serum was collected from each mare in mid-August (28 days after the last vaccination). Sample size of the study cohort was, therefore, determined by availability and practicality. Serum was collected from the foals of these mares within 9 days (range, 3 to 9 days) after foaling and monthly thereafter until the foals were 6 months of age. The study was performed with full owner consent and was approved by the Research Committee of the Faculty of Veterinary Science as well as the Animal Use and Care Committee of the University of Pretoria under protocol V052/07.

## Serum neutralisation test (SNT)

Antibody titres to each of the 9 AHSV serotypes were determined by serum-virus neutralisation assay, essentially as previously described [5, 6, 15]. Briefly, each serum sample was inactivated at 56 °C for 30 min before testing and serum dilutions were made in Minimum Essential Medium <sup>b</sup> with 2g/l  $\text{NaHCO}_3$  <sup>c</sup>, gentamycin sulphate (Genta 50) <sup>d</sup> 0.05 mg/ml and 5% foetal calf serum <sup>e</sup>. An estimated 100 TCID<sub>50</sub> of cell-culture-adapted prototype strains of each AHSV serotype <sup>f</sup> were added to duplicate serial 2-fold serum dilutions (from 1:10 to 1:320), and plates were incubated for one hour at 37 °C prior to addition of a suspension of Vero ATCC CCL81 (African green monkey kidney) cells containing an estimated 480,000 cells/ml. The development of cytopathic effects was monitored daily for 4 to 5 days. Titres were determined as the reciprocal of the highest serum dilution that provided >50% protection of the cell monolayers.

## Data analyses

Data were entered into a Microsoft Access<sup>®</sup> database and statistical analyses were performed with Microsoft Excel<sup>®</sup>, Kinetica<sup>®</sup> 5.1 (Thermo Scientific) and SigmaPlot<sup>®</sup> software packages. The SNT titre distributions of the 9 AHSV serotypes in mare and foal sera were statistically described with box-and-whisker plots. Spearman rank order correlation was calculated between SNT titres of mare sera and those of the first serum collected from their respective foal. The biological half-lives in foals of maternal antibody to each of the 9 AHSV serotypes were estimated by the exponential decay equation  $T_{1/2} = -(\ln 2) / \beta$  where  $T_{1/2}$  is the decay time (days) and  $\beta$  is the regression coefficient. This analysis was performed on Ln transformed values from individual foals using least square means and mean antibody half-life for the cohort was calculated. Time until SNT titres became negative at a 1:10 serum dilution for each serotype was estimated using the Kaplan-Meier product-limit estimate of the survivor function [16].

## Results

Although the mares that were evaluated in this study had been annually revaccinated with polyvalent MLV AHSV vaccine, there were marked differences in the neutralising antibody titres to each of the 9 AHSV serotypes (Table 1; Figure 1). Highest SNT titres most consistently were detected against

AHSV serotypes 1, 4, 6 and 9, with a median titre value of at least 120. Titres were lower against other serotypes, specifically serotype 8 (median = 112), serotypes 2 and 3 (median = 56), serotype 7 (median = 28) and serotype 5 (median = 20). Neutralising antibodies were least consistently detected against serotype 5, with 5 mares (33.3%) testing negative to this virus including 2 mares (2/12) that had recently been vaccinated.

Antibody titres against each of the 9 AHSV serotypes were determined on sera collected from 15 foals between 3 and 9 days after birth (Table 2; Figure 1). Highest titres were detected against serotype 1 (median titre = 224), followed by serotypes 7 (median = 80) 4 (median = 40), 2, 3, 6 and 9 (all with median values of 28), and serotypes 5 and 8 (median = 20). One foal had no detectable SNT antibodies to any of the 9 AHSV serotypes. One foal had low titres to serotypes 1, 7 and 8 and was negative for the remainder.

The correlation between mare and foal SNT antibody titres was 0.50 ( $P < 0.00001$ ), indicating that AHSV titres of each mare-foal pair were proportionate. Mare #15 was seronegative to both serotypes 2 and 5, and she also had only low SNT titres to the other virus serotypes (range, 20 to 112). The foal from this mare also tested negative to all AHSV serotypes.

Analysis of the SNT titres to AHSV in 6 foals that were tested monthly for 6 months had an estimated mean half-life for neutralising antibodies to all 9 serotypes of 20.5 ( $\pm 2.6$  SD) days, with a range of 15.4 days for serotype 8 to 22.6 days for serotype 3. The overall product-limit estimate for the mean time until the SNT became negative at a 1:10 dilution was 96 days for all 9 serotypes, with a range of 62 days for serotype 5 to 128 days for serotypes 3 and 4 (Table 3). The survival curves for antibody duration to the individual serotypes were significantly different ( $P < 0.001$ ) (Figure 2).

## Discussion

In the current study, we monitored a cohort of 15 mare-foal pairs on a well-managed stud in the AHS surveillance zone to evaluate neutralising antibody titres in AHSV vaccinated horses. The mares were routinely vaccinated with a commercial polyvalent MLV AHSV vaccine, and the farm was shown to be free of AHSV infection since at least 1996.

There was marked variation in the SNT response of mares to the different AHSV serotypes contained in the vaccine. Specifically, serotypes 1, 4, 6 and 9 consistently induced the highest SNT titres in mares whereas the response to serotypes 5 and 8 was notably weaker and several horses had no demonstrable neutralising antibody to serotype 5. Serotype 5 is not included in this vaccine because of its purported cross-reactivity with serotype 8, however responses to serotype 8 also were consistently weak. In contrast, serotype 9 is also not included in the vaccine because of its cross-reactivity with serotype 6, yet high SNT titres were consistently detected against serotype 9. SNT titres to serotype 9 and serotype 6 were similar (usually within 1 dilution step). Howell (1962) previously demonstrated cross-neutralisation between serotypes 6 and 9 as well as between several of the other serotypes.

Considerable variation was evident in the antibody response of individual mares to the different AHSV serotypes. The study mares were all repeatedly vaccinated during prior seasons, suggesting that that repeated annual vaccination does not reliably induce an anamnestic response to all AHSV serotypes in every horse. Some mares developed high antibody titres to specific AHSV serotypes whereas titres to other serotypes remained low throughout the animal's life despite repeated vaccination. While this finding does not indicate that horses with weak SNT responses to individual virus serotypes are not immune to AHSV infection, it is consistent with field reports of severe AHS in some well-vaccinated horses [17]. Furthermore, the absent or low antibody titres to some serotypes in individual mares could impair passive protection of their foals.

Passive transfer of antibodies via colostrum is important for protection of foals against pathogens they encounter during the first few months of life. The survival of passively acquired antibodies against several equine pathogens has been documented [18, 19], but has not previously been determined for AHSV. Alexander and Mason (1941) showed that foals born to mares vaccinated with the neurotropic (mouse brain attenuated) MLV AHSV vaccine only had serum antibodies to AHSV after suckling colostrum from immune mares [20]. Although pre-suckling foal sera and periparturient (pre-foaling) mare sera were not analysed in the current study, antibody titres detected in the post-suckling samples strongly suggest transfer of maternal antibody from the dam to the foal for all 9 virus serotypes. The same authors also reported higher antibody titres in some foals as compared to those of their dam, which was also identified in the current study with serotypes 1 and 7 in particular.

Alexander and Mason (1941) did not calculate the half-life of maternal antibody to AHSV but reported that the duration of demonstrable antibodies in foals was correlated with the mare's SNT titre at the time of foaling. They further reported that antibodies were generally not detectable by 6 months of age. Typical half-lives of maternal antibody to other pathogens include 27 and 39 days, respectively, for IgGa and IgGb to influenza virus; 28 and 34 days, respectively, for IgGa and IgGb to tetanus toxoid [18] and 32 days for equine arteritis virus [19]. The mean half-life of 20.5 days for passively-acquired maternal neutralising antibody to AHSV in the current study was calculated based on 6 foals and is therefore imprecise. However, the estimate is similar although somewhat shorter than that to these other pathogens.

Data from this study confirm variation of the neutralising antibody response of individual Thoroughbred mares to repeated vaccination with an MLV polyvalent AHSV vaccine. Furthermore, the data suggest that virus strains of individual AHSV serotypes included in the polyvalent vaccine vary in their inherent immunogenicity. Lastly, passively-acquired maternal antibodies to AHSV vary markedly among foals born to vaccinated mares, with further variation in the duration of passive immunity to individual AHSV serotypes. Due to the limited geographical distribution and study population size, further studies are needed before definitive recommendations on the optimal timing of AHSV vaccination of foals in endemic areas can be made.

## **Manufacturer's details**

- a. Onderstepoort Biological Products, Onderstepoort, Gauteng, South Africa
- b. (Minimum Essential Medium) Highveld Biological, Modderfontein, Gauteng, South Africa
- c. (NAHCO<sub>3</sub>) Merck, Wadeville, Gauteng, South Africa
- d. (Genta 50) Virbac Animal Health, Centurion, Gauteng, South Africa
- e. Sigma-Aldrich, Johannesburg, Gauteng, South Africa
- f. ARC Onderstepoort Veterinary Institute, Gauteng, South Africa



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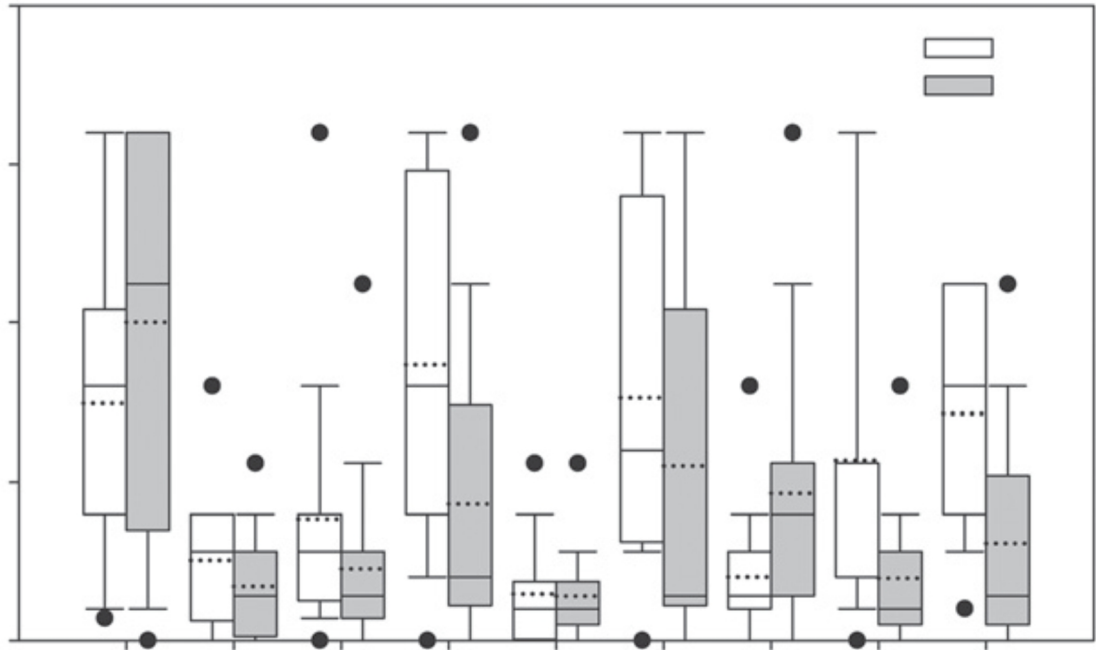


Figure 1: Box-and-whisker plot comparing SNT titres to the various AHSV serotypes from mares (n = 15) before foaling and their foals within 9 days after birth.

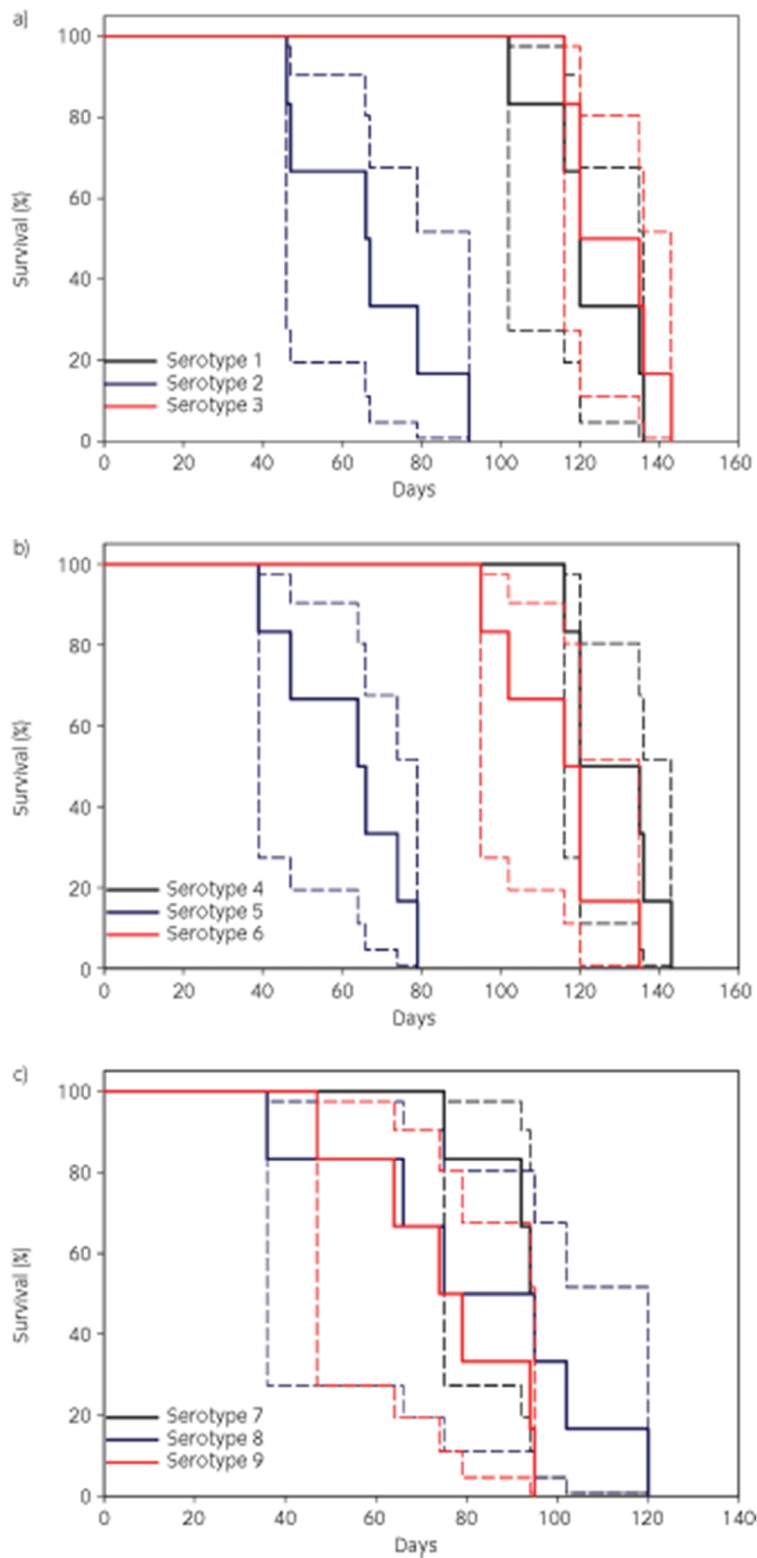


Figure 2: Survival curves: product limit estimates of persistence of maternal antibody to 9 AHSV serotypes in foals. Serotypes 1-3, 4-6 and 7-9 are represented in figures (a), (b) and (c), respectively. The dashed lines represent the 95% confidence intervals.

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**Table 1:** SNT titres to the 9 serotypes of AHSV of pregnant mares (n = 15) during the 2006 foaling season. Sera were collected on 15 August 2006. Animals identified in bold were not vaccinated during 2006 whereas the others were vaccinated in June and July of that year.

Dam no.	AHSV 1	AHSV 2	AHSV 3	AHSV 4	AHSV 5	AHSV 6	AHSV 7	AHSV 8	AHSV 9
1	160	80	160	160	20	320	56	112	160
2	320	160	320	320	80	160	160	224	224
<b>3</b>	<b>112</b>	<b>28</b>	<b>56</b>	<b>80</b>	<b>Neg</b>	<b>56</b>	<b>20</b>	<b>Neg</b>	<b>80</b>
4	160	80	80	320	80	160	80	320	224
5	160	56	80	224	14	80	14	80	224
6	320	56	40	160	40	80	28	112	224
7	224	40	112	320	28	320	56	112	160
<b>8</b>	<b>20</b>	<b>10</b>	<b>40</b>	<b>80</b>	<b>Neg</b>	<b>56</b>	<b>28</b>	<b>40</b>	<b>112</b>
9	80	10	80	224	28	320	20	112	224
<b>10</b>	<b>80</b>	<b>80</b>	<b>80</b>	<b>160</b>	<b>20</b>	<b>160</b>	<b>40</b>	<b>80</b>	<b>80</b>
11	14	Neg	Neg	Neg	14	Neg	Neg	112	20
12	224	80	56	320	112	320	56	320	160
13	160	56	14	80	Neg	56	20	40	80
<b>14</b>	<b>160</b>	<b>20</b>	<b>14</b>	<b>40</b>	<b>Neg</b>	<b>120</b>	<b>Neg</b>	<b>20</b>	<b>112</b>
15	40	Neg	20	112	Neg	80	20	20	56
Mean	148.0	50.4	76.8	173.3	29.0	152.5	39.8	113.6	142.6
Median	160	56	56	160	20	120	28	112	160

**Table 2:** SNT titres to the 9 serotypes of AHSV from foals (n = 15) tested between 3 to 9 days after foaling.

Foal no.	AHSV 1	AHSV 2	AHSV 3	AHSV 4	AHSV 5	AHSV 6	AHSV 7	AHSV 8	AHSV 9
1	320	56	56	56	28	320	112	10	80
2	320	56	112	224	28	320	224	40	112
3	320	14	28	40	10	112	80	Neg	28
4	28	Neg	Neg	Neg	Neg	Neg	28	10	Neg
5	320	56	56	160	40	56	320	80	80
6	320	28	28	56	20	28	112	40	56
7	224	40	56	112	56	224	112	56	112
8	20	Neg	20	28	10	28	56	14	10
9	224	28	224	320	56	320	160	80	160
10	160	80	28	28	10	28	28	10	28
11	40	Neg	Neg	Neg	28	10	Neg	56	Neg
12	224	112	40	224	112	160	80	160	224
13	160	28	14	28	10	20	28	10	10
14	320	10	14	20	10	28	56	20	14
15	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Mean	200	33.9	45.1	86.4	27.9	110.3	93.1	39.1	60.9
Median	224	28	28	40	20	28	80	20	28

**Table 3** Half-life and duration of neutralising maternal antibody in foals (n = 6) to individual AHSV serotypes (1 – 9)

AHSV serotype	Mean biological half-life in days (median.)	95% confidence intervals	Mean product limit estimate of antibody duration in days (median)	95% confidence intervals
1	21.5 (22.1)	26.0 – 16.8	121 (120)	111 - 132
2	18.4 (15.7)	41.7 - -9.8	66 (66)	52 - 81
3	22.6 (22.9)	29.5 – 15.2	128 (120)	119 - 137
4	23.3 (22.5)	28.4 – 18.0	128 (120)	120 - 137
5	23.3 (19.6)	58.6 - -21.7	62 (64)	49 - 74
6	20.6 (20.5)	29.9 – 10.5	115 (116)	103 - 126
7	21.1 (18.7)	37.5 – 2.8	96 (94)	84 - 108
8	15.4 (14.1)	19.4 – 11.1	75 (75)	59 - 106
9	18.7 (17.4)	28.1 – 8.5	76 (74)	61 - 90
Mean	20.5		96.3	