PHARMACOLOGICAL REACTIVATION OF EQUINE INFECTIOUS ANAEMIA VIRUS IN NATURALLY INFECTED MULES: CLINICAL, HAEMATOLOGICAL AND SEROLOGICAL RESPONSES - Part 2

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Part 1 of this study reports the introduction, materials and methods and the first part of the results

Results

Platelet count (Figure 2): thrombocytopenia was not observed for mules 6, 7 and 8. For mules 1 to 5 and 9 to 11, thrombocytopenia occurred between day 8 and 28 P.IS.. The duration of thrombocytopenia was variable, lasting for one day in mules 2, 9 and 11, two days for mule 10, three days for mule 3 and 4, five days for mule 5 and seven days for mule 1. Values inferior to the baseline were generally very low even when short-lived.

	Figure 2 - PLT counts lower than baseline value (100, 000 X microlitre)							
	Mules							
D.P.IS.	1	2	3	4	5	9	10	11
8				5				
14	75	10	13	7	11	8	13	
15	43		17	6	8		7	
16	58		62		5			
17	75				96			
18	45		69	99	78			
19	90							
20	95							
24			95					
28			17					18

Temperature (Figure 3): increase in rectal temperature > 39°C was registered for mules 1 to 5 and 10, between day 9 and 17 P.IS, while for mule 11, between 21 and 24 days P.IS.. for mules 3, 4 and 11.

	Figure 3 - Temperatures > 39°C						
	Mules						
D.P. IS.	1	2	3	4	5	10	11
9						40.1	
10						40.5	
11		39.5	39.5	39.7		40.6	
12				39.9	40.1	39.1	
13	39.5				39.9		
14	41.1				40.4		
15	41				40.1		
16			39.6		39.3		
17			39.7	39.2			
21							39.1
22							
23							40.8
24							40.3

Temperature remained below 39°C for mules 6, 7, 8 and 9.

Highest temperature of 41°C was observed in mules 1, 10 and 11, while the duration of the fever varied, just one day for mule 2, three days for mules 1 to 4 and 11, four days for mule 10 and five days for mule 5. Hyperthermia was discontinuous

The other clinical signs for which the mules were monitored, apart from fever and thrombocytopenia, were present only in a mild to an unapparent and were observed in all mules. These were concomitant to fever and thrombocytopenia or following these two events.

Serological results are presented are relative to the samples at weekly intervals from day -7 to 28 P.IS.

C-Elisa (Figure 4): all animals, except for mule 6, reacted in the Elisa using p26 as antigen. Mules with generally low titres were 3, 5 and 7 , while mule 11 was positive only on day 28 P. IS.. The remaining mules (1, 2, 4, 8, 9 and 10) had medium to high titres. Rise in titres were registered on day 14 for mule 7 and on day 21 for mules 3, 8, 9 and 10, with a sharp increase for the last two mules, as for also mules 5 and 11 but on day 28, especially for the latter, which from negative become positive. Decrease for mule 4 was observed on day 14 P.IS.. Although when recruited mule 6 had also been positive in the C-Elisa, the animal remained constantly negative for this method throughout the whole observation period.

	Figure 4 - Elisa titres										
	Mules										
D.P.IS.	1	2	3	4	5	6	7	8	9	10	11
-7	48	192	0	576	12	0	6	48	24	192	0
0	192	192	6	576	12	0	6	24	48	192	0
7	48	192	6	576	12	0	0	12	48	192	0
14	48	192	0	384	12	0	6	12	0	192	0
21	48	192	12	192	12	0	12	48	576	576	0
28	48	192	12	192	192	0	48	48	576	576	48

Agid (Figure 5): reactivity for the two methods was nearly equivalent for all mules at all time intervals. In particular, when reactivity was weak, positivity was more evident in OIE, on the contrary when reactivity was strong positivity was more evident in Coggins (data not shown).

	Figure 5 - Reactivity of serum samples in Agid										
	Mules										
D.P. IS.	1	2	3	4	5	6	7	8	9	10	11
-7	2	3	0	3	1	0	1	1	2	3	0
0	2	3	0	3	1	0	0	1	2	3	0
7	2	3	0	3	1	0	1	1	2	2	0
14	2	3	0	3	1	0	0	1	2	3	0
21	2	3	0	3	1	0	1	1	4	4	0
28	2	3	0	3	2	0	1	1	4	4	2

For ease of interpretation of the readings in Agid, the reactivity was transformed in scoring as follows:
0 – absence of the precipitation line, 1 - precipitation line bends in well, 2 - precipitation line touching sample well, 3 - precipitation line close to sample well 4 - precipitation line equidistant between sample and antigen wells 5 - precipitation line touching antigen well.
Mules 3 and 6 were totally negative during all the P. IS. period, while mules 7, 9 and 10 showed an increase in reactivity on day 21 while mule 5 and 11, registered an increase on day 28, as observed in Elisa, especially for the latter mule, which from negative become positive.
IB (Figure 6): the reactivity of the mules to p 26 and gp 45 and 90 is described in Figure 6.

Figure 6 - Description of reactivity of mules in IB

mule		mule	
1	Strong pos for all proteins on all days	7	Strong pos for p26 medium for gp 45 & 90 with increase on day 28 for gp 90
2	Strong pos for all proteins on all days	8	Strong pos for p26 & gp 90, medium for gp 45 with increase on day 14 and for gp 90 on day 21
3	Strong pos for all with increase of p26 on day 21	9	Strong pos for p26 & gp 90, medium for gp 45 with increase from day 14 for gp 45
4	Strong pos for all with decrease on day 14	10	Strong pos for gp 90 with increase from day 21 for p 26
5	Strong pos for all on all days	11	Weak pos only for p26 increase from day 14 for gp 90 on day 28 for p26
6	Medium pos for p26 & gp 90 & weak pos for gp 45 for all days		

An effective increase in the reactivity of the mules in IB was noted for p26 for mule 10 and 11 on day 21 and 28 respectively, for gp 45 for mule 8 and 9 on day 14 and 21 respectively and for gp 90 for mule for 7 and 11 on day 21 and 28 respectively.
The only positivity detected in the duplex EHV 1 and 4 Real Time PCR was for the nasal swab of mule 6 on day 14. For this mule neither fever nor thrombocytopenia was observed Furthermore no increase in reactivity was registered in IB, the only method for which the animal was positive.

Discussion
The pharmacological immune suppression of the naturally EIAV naturally infected mules was effectively efficient as verified by the response to the DHT following the administration of PHA with the PHA/SS ratio resulting below one for all mules by the end of the drug administration.
Following, immune suppression seven of the experimental animals presented both fever and thrombocytopenia in, while thrombocytopenia for one mule. As can be observed in figure 2 and 3, fever and thrombocytopenia were concomitant for most mules presenting these alterations. Other clinical signs for which the mules were monitored were practically mild to unapparent.
Following the end of the pharmacological immune suppression an increase in serological reactivity for EIAV occurred in only six of the experimental animals. While for two mules the increase in the serological response was observed at least in one of the serological methods, four mules registered an increase in reactivity in all the three methods employed. Although the three methods had as common denominator the p26, the increase for this protein was mostly registered in Elisa and Agid, than in IB, which is reported as more sensitive than the other two (Issel, C.J. *et. al*., 1988). This could be due to the fact that not all animals are capable during the reactivation of the infection of immediately producing antibodies against linear epitopes of the protein, which in the IB is presented in its denatured state.
Other point is that the method which is least sensible for the detection of the serological reactivity to EIAV is the Agid followed by the C-Elisa as can be seen in Figure 4, 5, and 6. as also observed by other authors, Issel, C.J. *et. al*., (4), Leroux C. *et. al.,* (6) and Scicluna *et.al*. (7) This result of relevant importance in the appropriate use of the three methods in a surveillance and control programme. In fact in the choice of a screening test to be employed in a control programme, among other characteristics to be considered sensibility is fundamental for the success of the control programme. This places the choice of the C-Elisa in advantage of the Agid as a screening test in monitoring the EIA infection.
In comparing the clinical response to the results of the serological reactivity of the eight animals which had registered fever and/or thrombocytopenia, only four of the animals registered an increase of the serological reactivity. These results do not reveal any evident correlation between the two responses. In fact, in literature, in similar experiments conducted in EIV infected horses for the study of the humoral response following immune suppresion, an increase in the production of gp 90 was observed but using other serological methods as the virus-neutralization described by Howe H. *et. al*., (8). Therefore, in our study other serological methods are being undertaken to investigate deeper the humoral response obtained in these mules during the P.IS.
Virological investigations will also be carried out on biological samples collected during the P.IS. to study among other points the viral load which occurred during the P.IS. period as well as the characteristics of the viral strain of each animal in view of the fact that they came form different outbreaks even if correlated geographically. Other factors which will also be considered in the analyses of this study is the breeds from the mules descended.
This study has been conducted to information on the potential epidemiological role of these animals in the diffusion of the virus both during the chronic/inapparent and the acute/viremic phases of the EIA infection. The study characteristics of the viral strain of each animal in consideration of the different serological and clinical pattern observed for each mule and also in view of the fact that they came form different outbreaks, even if correlated geographically, might better explain some aspects till now unclear.
This study is the first report of the pharmacological reactivation of the EIA infection in naturally infected mules which apparently induces a mild to unapparent clinical form characterised by fever, thrombocytopenaia and an increase in serological reactivity only for some of the experimental animals.

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