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# Evolution of equine infectious anaemia in naturally infected mules with different serological reactivity patterns prior and after immune suppression

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ABSTRACT

Information on equine infectious anaemia (EIA) in mules, including those with an equivocal reaction in agar gel immunodiffusion test (AGIDT), is scarce. For this, a study was conducted to evaluate the clinical, viral loads and pathological findings of two groups of naturally infected asymptomatic mules, respectively with a negative/equivocal and positive AGIDT reactivity, which were subjected to pharmacological immune suppression (IS). A non-infected control was included in the study that remained negative during the observation period. Throughout the whole study, even repeated episodes of recrudescence of EIA were observed in 9 infected mules, independently from their AGIDT reactivity. These events were generally characterised by mild, transient alterations, typical of the EIA acute form represented by hyperthermia and thrombocytopenia, in concomitance with viral RNA (vRNA) peaks that were higher in the Post-IS period, reaching values similar to those of horses during the clinical acute phase of EIA. Total tissue viral nucleic acid loads were greatest in animals with the major vRNA activity and in particular in those with negative/equivocal AGIDT reactivity. vRNA replication levels were around 10–1000 times lower than those reported in horses, with the animals still presenting typical alterations of EIA reactivation. Macroscopic lesions were absent in all the infected animals while histological alterations were characterised by lymphomonocyte infiltrates and moderate hemosiderosis in the cytoplasm of macrophages. On the basis of the above results, even mules with an equivocal/negative AGIDT reaction may act as EIAV reservoirs. Moreover, such animals could escape detection due to the low AGIDT sensitivity and therefore contribute to the maintenance and spread of the infection.


## 1. Introduction

Equine infectious anaemia (EIA) is a viral disease that affects all domestic members of the genus Equus spp. (horse, donkey and mule) and is characterised by intermittent fever, progressive anaemia, emaciation and death in severe cases. Clinical forms of EIA are acute, chronic and inapparent, with the latter being the most frequent. Animals in the chronic and inapparent phase may experience a recrudescence of the infection as a consequence of severe stress, hard work or the presence of other diseases (Quinlivan et al., 2007). EIA virus (EIAV), a Lentivirus of the Retroviridae family, is responsible for the infection that is characterised by a restricted tropism for the equine monocyte-macrophage lineage with productive replication occurring only in the differentiated tissue macrophages (Oaks et al., 1998).

During the surveillance programme conducted in Italy, between 2007 and 2012, that used the ELISA as a screening test and agar gel immunodiffusion test (AGIDT) as a confirmatory test, prevalence in mules was found to be significantly higher than in horses and donkeys (Sala et al., 2012). In addition, equivocal AGIDT reactions were more frequent among this hybrid species (Scicluna et al., 2013).

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Studies on the carrier status with negative and equivocal AGIDT reactions are respectively described for horses, by McConnell and Katada, (1981) and by Issel and Adams (1982), in which the authors succeeded in the transmission of EIAV from such animals. Similar investigations were also conducted to evaluate the correlation of the AGIDT serological reactivity pattern with plasma viral loads at different time intervals and in the tissues of experimentally infected horses (Harrold et al., 2000; Hammond et al., 2000). In addition, in a study conducted by Cook et al. (2003), the authors report that the acute form of the disease in the horse is characterised by the typical EIA alterations of hyperthermia and thrombocytopenia which are observed when the plasma viral RNA (vRNA) copies exceed concentrations of $\log_{10}$ 7.7/ml, defined as the pathogenic threshold (Cook et al., 2001). Different was the evolution observed in donkeys as these animals maintained low viremic levels in the absence of clinical signs (Cook et al., 2001).

Clinical and virolgical studies on EIA in mules are limited to two publications, in which the authors, in one of them (Spyrou et al., 2003), describe in detail the evolution of the natural and experimental infection observed in two subjects for each condition, for a time period that was however insufficient to completely elucidate this. Also, the presence of EIAV in these animals was reported only qualitatively. Considering the relatively high EIA prevalence levels in the Italian mule population and their elevated density in restricted areas where they are used as working animals, the scientific literature on the importance of the role that they might play as EIAV reservoirs is to date scarce. In view of this, an observational study was conducted to assess the clinical, pathological and virological parameters of naturally EIA infected mules having equivocal/negative AGIDT reactions, according to the scoring system proposed by Issel et al. (1999). In addition, the results obtained from these animals were compared to those having a clearly positive AGIDT reaction. As all the study animals were presenting an inapparent form of the infection, they were subjected to a pharmacological immune suppression (IS) as described by Craigo et al. (2007) for the reactivation of the disease. Further to the serological findings, already described by Scicluna et al. (2013), this paper reports the clinical evolution of EIA, in relation to the plasma vRNA levels, with the aim to investigate the correlations among these characteristics and evaluate the results in relation to the risk such animals may represent in the epidemiology of EIA. Viral replication was assessed in terms of vRNA copies in plasma, as well as, total viral nucleic acid loads (viral DNA and RNA) and presence of gross and histological lesions in tissues of various organs collected at the end of the study period (SP).

## 2. Materials and methods

### 2.1. Experimental animals and design

Ten mules, arbitrarily identified as they were enrolled from 1 to 10, were acquired from five distinct EIA outbreaks that had occurred in five neighbouring provinces of Central Italy. The age, sex and serological status for EIAV, of the experimental animals recorded on recruitment, are reported in Table 1. The ten animals were divided into two groups on the basis of their AGIDT reactivity observed at the start of the experiment: five mules had either equivocal or negative reactions (Group N) while the remaining animals presented a clear precipitation identity band (Group P), (Scicluna et al., 2013). A negative control, Mule 11, was included in the study that was however kept isolated from the infected animals, but subjected to the same experimental conditions adopted for the other ten mules. Clinical examination and sampling of the negative control were carried out with the same frequency and using the same methods as those described for the infected animals. To ensure its persistent negative status, the EIAV tests on this animal were extended to 30 days after the end of the SP.

The study design was approved by the competent authorities (Italian Ministry of Health – identification No IZS 02/10 RC). Animal husbandry and experimental procedures adopted throughout the SP, that lasted for a minimum of 84 days, were conducted under veterinary supervision and in compliance with the European Union Regulations in force for the use of animals in experiments. Biosecurity measures, as prescribed by the National Regulations, were also adopted to ensure that no EIAV transmission occurred from these animals.

### 2.2. Pharmacologically induced immune suppression

The mules included in the study underwent IS to induce EIAV reactivation. The treatment protocol, described by Kono et al. (1976) and Tumas et al. (1994), started on day 56 from the start of the SP using dexamethasone (Rapison®) at 0.11 mg/kg body weight/die. The duration of the pharmacological administration for each animal was based on the Delayed Type Hypersensitivity (DTH) reaction obtained by the inoculation of *Phaseolus vulgaris* agglutinin® (PHA – Sigma) with the same procedure described by Baus et al. (1996).

### 2.3. Clinical examination

The general condition of the animals, including the clinical signs considered as characteristic of an acute form of EIA,

**Table 1**
Details of the characteristics of the study group: identification, age, gender, serological results observed on recruitment and at the end of OP.

| Mule identification no. | Age | Gender | Serological results (ELISA/AGIDT/IB[a]) | | AGIDT reactivity groups[b] | Duration of IS treatment in days |
|---|---|---|---|---|---|---|
| | | | On recruitment | At the end of SP | | |
| 1 | 12 | F | +/2/+ | +/2/+ | P | 10 |
| 2 | 2 | F | +/3/+ | +/3/+ | P | 8 |
| 3 | 30 | F | +/0/+ | +/0/+ | N | 8 |
| 4 | 22 | F | +/3/+ | +/3/+ | P | 8 |
| 5 | 9 | F | +/1/+ | +/2/+ | N | 8 |
| 6 | 8 | F | ±/0/+ | −/0/+ | N | 8 |
| 7 | 11 | C | +/1/+ | +/1/+ | N | 8 |
| 8 | 17 | F | +/1/+ | +/1/+ | N | 8 |
| 9 | 11 | F | +/2/+ | +/4/+ | P | 8 |
| 10 | 7 | F | +/3/+ | +/4/+ | P | 8 |

[a] Serological results for ELISA and IB are reported qualitatively as positive (+) or negative (−). The AGIDT reactivity is reported as a score as described by Issel et al. (1999).
[b] AGIDT reactivity group: N mules with negative (score 0) or equivocal (score 1) reactivity and P mules with clear reactivity (score 2–5).