Updates on Diagnostic Approach and Treatment of Select Donkey and Mule Diseases

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Donkeys and mules have their own physiology with species-specific reference ranges, drug dosages, and management that cannot be extrapolated from horse medicine. Authors' address: School of Biosciences and Veterinary Medicine, University of Camerino, Matelica (MC), Italy; e-mail: fulvio.laus@unicam.it. *Corresponding and presenting author. © 2021 AAEP.

1. Introduction

The estimated global equine population totals 117 million animals, including 58 million horses, 50 million donkeys, and 9 million mules.1 Donkeys are mainly reared for milk production in several countries such as Italy2 because, thanks to its special properties, donkey milk is suitable for infants who cannot be breastfed and for people suffering from cow's milk protein allergies.3–5 Currently, donkeys are also used as pets for recreational purposes and pet therapy rather than draught or burden animals, according to different countries. On the contrary, mule population has decreased in Europe and the Mediterranean European countries in recent years, while in developing countries, these animals are still widely used as beasts of burden or working equids for draft purposes.1,6

The diagnostic approach and treatment of some diseases including tick-borne disease, gastric ulcer syndrome, intestinal parasite infections, and ocular disease in donkeys and mules are herein discussed on the basis of current knowledge, updated publications, and personal experience. This paper focuses on those diseases capable of disrupting the normal physiology of donkeys and mules even in the absence of, or with minimal, clinical signs. Because of their stoicism, donkeys and mules tend to show only mild clinical symptoms, exhibiting signs of discomfort and pain differently from horses. Behavioral, anatomical, and physiological differences among horses, donkeys, and mules have a strong impact on clinical management and must therefore be known and understood when approaching these equids.

2. Tick-Borne Diseases in Donkeys

Among the infections transmitted by ticks that may affect donkeys, Theileria equi and Babesia caballi represent the most common etiological agents; however, Anaplasma phagocytophilum and Borrelia burgdorferi s.l. are also reported to be possible sources of infection.7,8 In horses, tick-borne diseases are commonly described, while only one paper is available on
clinical signs in donkeys, this reporting important differences. In 2014, an epidemiological survey assessed the seroprevalence of tick-borne pathogens affecting donkeys in Italy. A total of 122 adult donkeys were tested for antibodies against *T. equi*, *B. caballi*, *A. phagocytophilum*, and *B. burgdorferi* s.l. by indirect immunofluorescent antibody test (IFAT), while the presence of pathogens was verified by using specific PCR protocols. According to IFAT results, 48/122 (39.3%) animals were positive for *T. equi*, 58/122 animals (47.5%) were positive for *B. caballi*, and 9/122 (7.4%) animals were positive for *A. phagocytophilum*, whereas no specific antibodies for *B. burgdorferi* s.l. were found in studied donkeys. Considering PCR results, 23/122 (18.8%) donkeys were positive for *Babesia/Theileria* spp., while no subject was positive for *A. phagocytophilum* or *B. burgdorferi* on PCR assays. On the basis of the survey by Veronesi and colleagues, a high exposure of Italian donkeys to piroplasm protozoans was found, thus confirming the results obtained in previous studies performed in donkey populations from other countries, including Turkey, Spain, and China, that showed a seroprevalence ranging from 17% to 93% for *B. caballi* and from 10% to 47% for *T. equi*. According to the study by Sgorbini and colleagues, the risk factors associated with piroplasmosis included advanced age and outdoor housing. These results agree with previous studies conducted on horses and are consistent with a high exposure to tick bites. Another study by Piantedosi and colleagues obtained similar results in donkeys tested for piroplasmosis infection by IFAT and found no evidence of symptomatic donkeys, as also observed by Veronesi and colleagues. Equine piroplasmosis is endemic in tropical and temperate areas and can occur as acute, subacute, chronic, and subclinical presentations, with subclinical being the most frequently observed. Animals surviving the acute phase may remain seropositive carriers with low levels of parasitemia, especially following *T. equi* infections, which is often responsible for overt clinical forms of piroplasmosis. On the contrary, *B. caballi* infections usually remain latent, although some animals can exhibit anemia, inappetence, pyrexia, edema, hemoglobinuria, reduced work efficiency, weight loss, and abortion in mares. Piroplasmosis in donkeys is an underestimated disease because of a natural resistance of these equids to infection and a prevalence of chronic asymptomatic forms typical of animals living in endemic areas. However, whenever observed, the clinical signs include anorexia, lethargy, disorders of intestinal motility (especially constipation), and splenomegaly. A study by Laus and colleagues investigated IFAT to detect immunoglobulin G antibodies against *T. equi* and *B. caballi* and PCR to detect *Babesia* spp. and *Theileria* spp. DNA in a donkey population in Central Italy. Clinical examination findings, hematological analyses, and serum bilirubin evaluations were compared to positive or negative immunologic status. A seroprevalence of 40.6% and 47.8% was found for *T. equi* and *B. caballi*, respectively. PCR results showed that 17.4% of the animals were found to be positive for *T. equi* and 3.6% for *B. caballi* by PCR analysis. Clinical examinations revealed 8.7% of the donkey population had only slight clinical signs consistent with chronic forms of the disease. Animals showed various degrees of anorexia, depression, pale mucus membranes, and poor body condition scores. The only symptom related to piroplasmosis was slight icterus, detected in 6 donkeys (Table 1). In contrast to a previous study performed on horses in Italy, no acute form of the disease was found. Despite the lack of specific clinical presentation, 58/138 donkeys had hematological and serum bilirubin alterations, and 96.6% of them were IFAT and/or PCR positive. Changes in erythrocyte number, packed cell volume, hemoglobin concentration, mean corpuscular hemoglobin, platelet number, and total bilirubin were significantly associated with test-positive animals even though they were asymptomatic. Therefore, piroplasm protozoans mostly cause subclinical infections in donkeys that potentially interfere with animal welfare and health other than productive and working abilities. The unique acute form of piroplasmosis in donkeys has been reported in an adult jack in Italy. This important difference between horses and donkeys should be taken into account when evaluating donkeys living in endemic areas. Current therapies, mainly based on the administration of imidocarb dihydrochloride, are derived from equine medicine and are not optimized for donkeys. Drug biodistribution appears to be different between donkeys and horses depending on the differences in plasma volumes that could result in increased kidney and liver toxicity in donkeys. Moreover, the severe side effects associated with the anticholinergic effect of the carbamidine derivatives, like intense abdominal pain, diarrhea, and dyspnea, are much more marked in donkeys than in horses. Prophylaxis is based on the use of topical antiparasitic agents that are not specifically registered for donkey species. Medications registered for horses, bovines, and sometimes companion animals (i.e., dogs) like synthetic pyrethroids are sometimes used as they have wide safety/handling margins, high repelling power, and high residuals lasting about 2 weeks for the pour-on formulations. Among pyrethroids, permethrin is the most popular; however, sprays and pour-on formulations based on deltamethrin, cyfluthrin, alphacypermethrin, and flumethrin are commercially available as well.

3. Intestinal Cyathostomin Infections in Donkeys and Mules

Worldwide, gastrointestinal parasite infestations represent a major challenge for equids including mules and donkeys. Clinical signs typically include diarrhea, ventral abdominal edema, pyrexia, colic,
Equids are usually coinfected with different nematode species. Cyathostomins (small strongyles) are considered as the most important intestinal parasite group in wild and domestic equids for their pathogenic potential at both larval and adult stages to negatively impact wellness. Therefore, these nematodes, characterized by a widespread distribution, are of major concern, and efficient control based on the appropriate management, diagnosis, and use of anthelmintic products is mandatory.

In past years, control of gastrointestinal parasites was based on regular and frequent administration of anthelmintic drugs as preventive treatment strategy. However, in light of the increasing evidence of anthelmintic resistance and changes in parasite distribution, a re-examination of recommendations for parasite control has been necessary. In response to this need, the American Association of Equine Practitioners (AAEP) has formed a task force charged with producing a comprehensive set of recommendations for helping veterinarians to improve strategies and programs for parasite control in horses. The most significant change has been the development of a new way of thinking with the goal of limiting parasite infections rather than eradicating all parasites from the equine gastrointestinal tract (GIT).

### Table 1. IFAT/PCR Positivity, Hematology, and Serum Bilirubin in Symptomatic Donkeys

<table>
<thead>
<tr>
<th>Donkeys</th>
<th>Positivity</th>
<th>Clinical Signs</th>
<th>Clinical Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Double IFAT</td>
<td>Depression</td>
<td>BCS &lt; 2, Inappetence</td>
</tr>
<tr>
<td>2</td>
<td>Double IFAT</td>
<td>Depression</td>
<td>BCS &lt; 2, Inappetence, Pale MM</td>
</tr>
<tr>
<td>3</td>
<td>Double IFAT</td>
<td>Depression</td>
<td>BCS &lt; 2, Inappetence, Pale MM</td>
</tr>
<tr>
<td>4</td>
<td>Double IFAT</td>
<td>Depression</td>
<td>BCS &lt; 2, Inappetence, Pale MM</td>
</tr>
<tr>
<td>5</td>
<td>Double IFAT</td>
<td>Depression</td>
<td>BCS &lt; 2, Icterus, Inappetence, Pale MM</td>
</tr>
<tr>
<td>6</td>
<td>Double IFAT</td>
<td>Depression</td>
<td>BCS &lt; 2, Icterus, Inappetence, Pale MM</td>
</tr>
<tr>
<td>7</td>
<td>Double IFAT</td>
<td>Depression</td>
<td>BCS &lt; 2, Icterus, Inappetence, Pale MM</td>
</tr>
<tr>
<td>8</td>
<td>PCR T. equi</td>
<td>Depression</td>
<td>BCS &lt; 2, Icterus, Inappetence, Pale MM</td>
</tr>
<tr>
<td>9</td>
<td>PCR T. equi</td>
<td>Depression</td>
<td>BCS &lt; 2, Icterus, Inappetence, Pale MM</td>
</tr>
<tr>
<td>10</td>
<td>PCR and IFAT (T. equi)</td>
<td>Depression</td>
<td>BCS &lt; 2, Icterus, Inappetence, Pale MM</td>
</tr>
<tr>
<td>11</td>
<td>PCR and IFAT (T. equi)</td>
<td>Depression</td>
<td>BCS &lt; 2, Icterus, Inappetence, Pale MM</td>
</tr>
<tr>
<td>12</td>
<td>PCR and IFAT (T. equi)</td>
<td>Depression</td>
<td>BCS &lt; 2, Icterus, Inappetence, Pale MM</td>
</tr>
</tbody>
</table>

Abbreviations: IFAT, indirect immunofluorescent antibody test; PCR, polymerase chain reaction; BCS, body condition score; RGB, red globe cells; WBC, white blood cells; PLT, platelets; Hb, hemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; TB, total bilirubin.
infection and egg shedding and guarantee animal welfare by minimizing the risk of parasitic disease. By doing this, there is a higher chance of maintaining efficacious drugs avoiding further development of anthelmintic resistance. To achieve these goals, it is fundamental to know the magnitude of egg shedding of individual horses by performing periodic fecal egg count surveillance.\(^{23}\) The modified McMaster fecal egg count procedure is the most extensively used technique; however, the technique\(^{a}\) is a new technique that has been developed by the University of Naples (Italy) and validated in several scientific papers as easy to use in clinical practice. The current guidelines established by the World Association for Advancement of Veterinary Parasitology\(^{24}\) and the AAEP suggest the assessment of the fecal egg count reduction (FERC) to know the percentage of efficacy of an anthelmintic substance. FERC can be calculated according to the formula FERC (%)=100[(C−T)/C], where C is the geometric mean of eggs per gram (EPG) before the treatment and T is the geometric mean of EPG after the treatment. The geometric mean was calculated by averaging the log counts (x+1) of the single EPG values, taking the antilogarithm, and then subtracting 1. At the present time, none of the three anthelmintic drug classes registered for horses (benzimidazoles, pyrimidines, and macrocyclic lactones) are currently available for treatment of parasites in mules, and only a few drugs are specifically licensed for use in donkeys.\(^{25,28}\) Therefore, mules and donkeys are usually treated with anthelmintic drugs at the same dosage, route, and intervals licensed for horses, despite the lack of scientific reports evaluating pharmacokinetics and efficacy related to their use in these animals.\(^{2,25,29}\)

Table 2. Main Pharmacokinetic Parameters of Ivermectin Following Oral Administration at 200 μg/kg BW in Mules, Horses, and Donkeys\(^{31}\)

<table>
<thead>
<tr>
<th>Species (Number of animals)</th>
<th>t(_{1/2}) (Day)</th>
<th>T(_{\text{max}}) (h)</th>
<th>C(_{\text{max}}) (ng/mL)</th>
<th>AUC(_{0-\infty}) (ng × Day/mL)</th>
<th>AUC(_{0-\infty}) (ng × Day/mL)</th>
<th>MRT (Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mule (n = 5)</td>
<td>2.74 (\pm) 2.02 (^{f})</td>
<td>16.8 (\pm) 9.96</td>
<td>42.31 (\pm) 10.20</td>
<td>135.56 (\pm) 43.71</td>
<td>163.93 (\pm) 61.82</td>
<td>6.07 (\pm) 4.36</td>
</tr>
<tr>
<td>Horse (n = 6)</td>
<td>6.53 (\pm) 9.92</td>
<td>4.05 (\pm) 2.16</td>
<td>61.28 (\pm) 10.73</td>
<td>164.96 (\pm) 30.07</td>
<td>–</td>
<td>7.34 (\pm) 1.30</td>
</tr>
<tr>
<td>Horse (n = 5)</td>
<td>4.25 (\pm) 0.24 (a)</td>
<td>9.22 (\pm) 5.71</td>
<td>44.0 (\pm) 23.1</td>
<td>132.7 (\pm) 47.3</td>
<td>–</td>
<td>4.78 (\pm) 0.64</td>
</tr>
<tr>
<td>Horse (n = 5)</td>
<td>2.93 (\pm) 0.4 (a)</td>
<td>3.60 (\pm) 0.96</td>
<td>51.3 (\pm) 6.1</td>
<td>137.1 (\pm) 35.9</td>
<td>–</td>
<td>4.2 (\pm) 0.4</td>
</tr>
<tr>
<td>Horse (n = 3)</td>
<td>2.76 (\pm) 0.2</td>
<td>3.3 (\pm) 0.7</td>
<td>82.3 (\pm) 12.4</td>
<td>200.92 (\pm) 22.67</td>
<td>–</td>
<td>6.5 (\pm) 2.0</td>
</tr>
<tr>
<td>Donkey (n = 3)</td>
<td>7.4 (\pm) 2</td>
<td>24.0 (\pm) 0.0</td>
<td>23.6 (\pm) 4.4</td>
<td>119.3 (\pm) 12.3</td>
<td>–</td>
<td>6.5 (\pm) 0.2</td>
</tr>
</tbody>
</table>

Abbreviations: (a), terminal half-life resulted from triexponential equation; AUC\(_{0-\infty}\), area under serum concentration-time curve from zero up to the last concentration ≥ LOQ; AUC\(_{0-\infty}\), area under serum concentration-time curve from time zero to infinity; C\(_{\text{max}}\), maximum concentration observed; MRT, mean residence time; T\(_{\text{max}}\), time of maximum concentration observed; t\(_{1/2}\), terminal half-life; \(^{f}\) harmonic mean; \(^{i}\) pseudo standard deviation.

Among anthelmintic compounds registered for equine species, ivermectin is a macrocyclic lactone commonly used due to its broad spectrum of activity against both endo- and ectoparasites.\(^{25,30}\) A recent study by Bazzano and colleagues\(^{32}\) investigated both pharmacokinetics and efficacy of ivermectin orally administered in mules at 200 μg/kg body weight (BW). Mules were found to have intermediate pharmacokinetic parameters between horses and donkeys, and these compounds seemed to be efficacious against cyathostomins (Table 2). Among anthelmintic drugs, macrocyclic lactones are characterized by a very long egg reappearance period, but recent reports have documented them shortened to just 4 to 5 weeks for both ivermectin and moxidectin on farms with high treatment frequencies.\(^{23}\) This is interpreted as emerging resistance in cyathostomins to this drug class. Thus, for macrocyclic lactones, it can be of value to run one set of posttreatment egg counts at around 4–6 weeks posttreatment to gauge the egg reappearance period status on the selected farm.\(^{23}\) The recurring onset of anthelmintic resistance together with the restrictions in the use of drugs in food-producing animals have enforced the search for sustainable alternative approaches for parasite control.\(^{32}\) Among the nutritional supplements used for the control of internal parasites in equine husbandry, promising results have been gained with the employment of plant-derived compounds.\(^{33,34}\) Although many plants have been listed as having anthelmintic activity in animals\(^{25,35}\) and the use of plant-derived anthelmintics would be preferable to synthetic drugs in dairy farming, scientific data demonstrating the real efficacy of these compounds against gastrointestinal parasites are scarce. In a recent study by Arfuso and colleagues,\(^{36}\) dairy donkeys were treated with the commercially available phytotherapeutic product.\(^{38}\) According to the manufacturer’s instructions, the product was administered two times at 2-week intervals (i.e., day 1, day 14) using the dose of one syringe (50 g) per donkey. Two administrations of phytotherapeutic supplement at fortnight interval were successful in reducing 56.9% intestinal strongyle egg shedding in naturally infected donkeys, causing no adverse reaction in treated animals throughout the experimental period. The FERC value obtained following phytotherapeutic supplementation was lower compared with other anthelmintic drugs tested in donkeys, such as ivermectin (96%)\(^{37}\) and eprinomectin (99%).\(^{38}\) The value was also lower when compared to the suggested cutoff values for interpreting results of strongyle FERC in horses.
Donkeys and mules can show similar ocular diseases to horses, but with a different prevalence. The incidence of ocular ulcer disease in a 3-year period in donkeys was 9% at the Veterinary Teaching Hospital of Camerino University (Italy; personal author data), much lower than that reported in horses (up to 76%). Corneal ulcer, infective kerato-conjunctivitis, uveitis, and eyelid trauma were the more common conditions reported. Ocular traumatic injuries are less frequent than in horses, likely because of their quieter attitude. Because of their stoicism, donkeys and mules do not show early clinical signs of ocular disease. Clinical examination of the eye can be more difficult in these equids than in horses. In fact, the lateral aspect of the frontal bone (the supraorbital ridge, forming the dorsal part of the orbit) is more laterally pronounced in donkeys and mules than in horses (Fig. 1), and consequently, the direct evaluation of the eye is uncomfortable since the procedure to lift the upper eyelid is more difficult and sometimes impossible to perform, especially in the donkey. This is also due to the stronger palpebral muscles. As a result, during the ultrasound examination of the eye, it is sometimes not possible to visualize the retrobulbar region due to the obstacle caused by this portion of the frontal bone when approaching the supraorbital acoustic window. It follows that careful clinical inspection of the eye in donkeys and mules often requires sedation, nerve blocks, and local anesthesia, which are not necessarily required in horses in similar conditions. Prolonged ocular topical treatment is also challenging in donkeys and mules. The difficulty opening the eye, their small size, and less experience with being handled makes prolonged ocular topical treatment challenging in donkeys and mules. For this reason, the application of a subpalpebral lavage system (SPL) in donkeys and mules is more often indicated than in horses. Furthermore, since sedation, nerve blocks (to overcome the strong palpebral muscles), and topical anesthesia are usually necessary for examination, SPL can be directly applied after diagnosis, if indicated. The technique for application of SPL in donkeys and mules is the same as in horses: the zygomatic process is slightly larger in donkeys and mules, and this should be taken into account when this part of the bone is palpated while looking for the supraorbital foramen for anesthetic injection.

6. Conclusions
In the present paper, the main features of piroplasmosis, equine gastric ulcer syndrome and ocular diseases in donkeys, have been highlighted. Epidemiology and clinical presentation of piroplasmosis and other tick-borne diseases show some differences between horses and donkeys; the latter having less severe symptoms despite high exposure to the parasites. Nonspecific clinical presentation or
asymptomatic forms of B. caballi and T. equi infections seem to be common in donkeys, and several clinical pathology alterations persist after natural infection. Therefore, even if clinically healthy, donkeys can be subjected to severe clinical pathological alterations. Acute presentations are very seldom observed in donkeys. Clinical monitoring of those donkeys that are chronically infected should be recommended because they represent a risk for transmission to other animals (as inapparent carriers) and their health status and productive performances could be altered. Decades of frequent anthelmintic use have selected for high levels of anthelmintic drug resistance in several parasite families like cyathostomin and Parascaris spp. populations, which emphasizes that the traditional approaches for parasite control are not sustainable anymore and that new strategies are needed. Preventive treatment should never be used, and a specific etiological diagnosis should be conducted before every treatment. Together with the recurring onset of anthelmintic resistance in equids, the increased public awareness of drug residues in animal products compels the scientific community to investigate novel strategies to control parasitic diseases in domestic animals. The results of investigations highlight a high prevalence of EGUS in a population of nonworking donkeys with no clinical signs of this disease. Furthermore, the squamous portion of the stomach was found to be much more frequently affected than the glandular region, as already reported for horses. This suggests that EGUS may be common in donkeys, but it is routinely overlooked. This suggests that subclinical forms of this syndrome could be present in apparently healthy donkeys. Thus, the effect of subclinical EGUS in donkeys needs to be thoroughly investigated in the future to exhaustively verify the impact on work or production performances. Several peculiarities of donkeys are present when compared to horses. Disease presentation can be very different, and the epidemiology of infectious and noninfectious diseases also needs to be specifically studied for donkeys to identify control plans to decrease the incidence and consequences of subclinical diseases.

Acknowledgments

Declaration of Ethics

The Authors have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The Authors have no conflicts of interest.

References and Footnotes


*Mini-FLOTAC®, University of Naples Federico II, Corso Umberto I, 40, Napoli, Italy.
*PARAXITEBIO®, BIOEQUIPE SRL, Lombardy, Italy. Composition includes standardized extracts of Cardus mariano, Eucalyptus globulus, Gentiana lutea, Urtica urens, and Mallotus philippinensis and analytical components including crude protein (0.62%), crude fat (0.22%), crude fiber (0.09%), crude ash (0.32%), moisture (91.32%), and nitrogenous extracts (7.43%).