Isolation and Identification of Aerobic Bacterial Flora from Healthy and Diseased Donkeys Eye of Central Ethiopia

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Abstract
The study was conducted from November 2012 to June 2013 to isolate aerobic bacteria residing in the eye of working donkeys. Forty five apparently healthy donkeys (APHDE) and 45 donkeys with clininacly diagnosed ocular disease (DCDO) were used for the study. Swabs were collected from the conjunctiva. Isolation and identification of the bacteria was under aerobic condition. Each of the samples collected yielded at least one bacterium species. A total of 256 bacteria species were recovered from both groups of donkeys. The majority 189/256 (73.8%) of the isolates were Gram- positive and the rest 67/256 (26.2%) were Gram- negative. Bacterial isolates identified in order of magnitude were coagulase negative staphylococcus species (27%), Pseudomonas species (22%), Corynbacterium species (19.9%), Staphylococcus aureus (15%), Streptococcus species (7%), Escherichia coli (5%), and Bacilli species (4%). There was no statistically significant difference (p>0.05) in the isolation rate of Staphylococcus aureus, Streptococcus species, Pseudomonas species and Bacillus species between APHDE and DCDO. The isolation rate of Coagulase negative staphylococcus species was higher in apparently healthy donkeys and this was statistically significant (p<0.05). Corynbacterium species and Escherichia coli were isolated at a higher rate in donkeys with clininacly diagnosed ocular disease and this was statistically significant (p<0.05). The result indicated the predominance of Gram- positives in both cases (p<0.05). Bacteria were recovered at a higher rate (1.47) in APHDE than the rate in DCDO (1.38).The quality of bacteria isolated in both cases was comparable except Escherichia coli which were not recovered from APHDE. Isolation of most bacteria both from APHDE and DCDO might suggest that the bacterium which resides in the conjunctiva have a chance to invade and cause opportunistic infections.

Keywords: aerobic bacteria, eye, donkey.

1. INTRODUCTION
Forty million donkeys are known to exist in the world of which 5.2 million were found in Ethiopian (Svendsen, 1997;Person et al., 1997).This represents more than 55% of the National equine population which is equivalent to 11.4% of the world and 37.4% of the African donkey population with this figure the country stand not only the biggest in Africa but also the second largest in the world next to china. According present regional classification of Ethiopia, 97% of the donkeys are found in three regions 44% Oromiya, 34% Amhara 19% in Tigray regional states (Feseha, 1998).

The Donkey has spent hundreds of years being used by man but despite this in the past little attempt has been made to study any aspect of donkeys (Svendsen, 1997). Recurrent draught in Ethiopia resulting in increase cattle mortality has also contributed to an increase in donkey usage as a draft and pack animals in both rural and urban areas. Donkeys account for 50% of the animas energy scenario in the country (Feseha et al., 1997). Donkeys are essential to the livelihoods of many families in Ethiopia providing relief from drudgery and diversifying household income. These animals play a crucial role in providing transport services both along rugged rural tracks and narrow up pave lanes of towns and villages.

Donkeys in Ethiopia, are subjected to a variety of health disorders including multi- parasitism, back sores and other wounds, hoof problems, ophthalmic problems, colic, obstruction and various infectious disease such as strangle, tetanus African horse sickness etc (Getachew et al., 2002). Donkeys are frequently very stoic, showing a muted pain response when compared to horses which could mean that some ophthalmic problems are much worse or more chronic when first examined. The donkey’s potential longevity can also contribute to different occurrence and management of some (ophthalmic) eye problems. Donkeys often graze with much of their head down (closer) to the ground; this can contribute to organic foreign body lodging and traumatic injuries in the eyes. This contributed the non-infectious cause of ophthalmic disease. The other ophthalmic problem of donkeys is caused by infectious agents such as bacteria, virus, fungus, recktesia, chlamydia and parasites which results in conjunctivitis, tumors (Sarcoi), cataracts, uveities, and others (Svendsen, 1997).Out of the health problem ophthalmic disease is the major in that it causes permanent blindness, temporary blindness, opacity which results in starvation (inability to locate feed) exited because of fear of the environment (the freedoms of the animal is disturbed).

Bacterial and fungal flora of the normal conjunctiva have been reported in cows (Samulson et al., 1984),
pigs (Davidson et al., 1994), birds (Miller et al., 1985) rabbits (Cooper et al., 1999) and fungal flora of healthy donkey eye (Nardoni et al., 2007). The common bacterial species isolated by different researcher from the above animals are streptococcus species, staphylococcus species, pseudomonas species, and Corynbacterium Species and Bacillus species. Even though extensive studies have been carried in the ocular bacterial flora in other domestic animals limited information is available on donkeys more over no work (research) has been done in Ethiopian except opthalmic case reports of 3,456 donkeys, (DHWP, 2003,2004,2005) where the risk factors and causative agents have not been identified.

Studies on donkey are infrequent; to the best of our knowledge no data have been in the literature concerning the nature and diversity of bacterial ocular flora of donkeys. The physical and/or mechanical damages mentioned above can lead to opportunistic bacterial and fungal infections resulting from the invasion of wounds flora.

Therefore the objectives of this study were:
- Isolation of various aerobic bacterial agents from apparently healthy donkey’s eye
- Isolation of various aerobic bacterial agents from clinically diseased donkey’s eye.
- To compare their proportion (Nature of isolates recovered from both groups)
- To recommended appropriate remedy based on the factors and underlying infectious agents associated.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted from November 2012 to April 2013 in three districts of central Ethiopia, namely Ada’a, Sebeta and Boset. These sites were previously selected as a working area by the mobile and stationary clinic of the DHWP based on their high equine population and poor economic status of the owner.

2.2. Study design and sampling

The design of the study was cross sectional. Systemic random sampling was done for apparent healthy donkeys eye (APHDE) and clinically diseased donkeys eye (DCDO) where every other donkey in the villages was sampled in respective order. Sampling was done with no discrimination of age, BCS, sex and color. To determine sample size to estimate the difference between proportions (Goddard, 2003) formula was used. Hence, the expected proportion of donkeys recovered with bacterial load in APHDE is set at 50% (P1) while for DCDO expected proportion is estimated at 80% (P2) recovery. The power of the study is set at 80%, 95% confidence interval and significance level of P<0.05 were used.

\[
N > \left( \frac{Z_{1-\alpha/2} + Z_{1-\beta}}{2} \right)^2 \frac{P_1(1-P_1) + (P_2(1-P_2))}{(P_1 - P_2)^2}
\]

Where
- \(Z_{1-\alpha} = 50\% \text{ significance level}
- \(Z_{1-\beta} = 80\% \text{ power level}
- P_1 = \text{ Expected proportion in apparently healthy donkeys}
- P_2 = \text{ Expected proportion of clinically diseased donkeys eye}
- N > 7.89 x [0.5 (1 – 0.5) + 0.8 (1 – 0.8)]

\(0.5 – 0.8\)^2

N1 > 36, hence N2 > 36; the total sample required is at least 36 + 36 = 72 donkeys. For the study a total of 90 donkeys (both sides = 180 swabs) were included from three districts of the study area.

2.3. Study Animal

Donkeys coming to the DHWP stationary and mobile clinic were the study population. The present study was conducted on 90 donkeys (45 apparently healthy and 45 clinically diseased) brought to the DHWP stationery and mobile clinic of the near by village. The group of animals investigated was working donkeys of mixed age, sex and BCS.

2.4. Study Methodology

Among the donkeys admitted to the DHWP stationery and mobile clinic during the period of November 2012 to June 2013, 45 APHDE and 45 DCDO were included in the study using a systemic random sampling every other appropriate donkey was sampled. Each sample animal was subjected to a thorough physical and clinical examination where history (previous exposure to ophthalmic problem, antimicrobial treatment, any concurrent disease, duration of the ion current illness) and sign including loss of appetite, fever, opacity, hyperaemia, oedema, and lacrimation were noted. Pen light and Ophthalmoscopy were used to examine the cornea, conjunctiva. Fluorescent die stain was used after sampling to cheek for any presence of lesion or ulcer on the cornea.
2.4.1. Sampling and sample processing
Before sampling sterile swabs and media were made for use. Then 5-7cm long sterile cotton swab was directed
and rubbed back and forth on the ventral conjunctiva and samples were collected. As soon as possible, samples
were inoculated in a transport media (Peptone water) and then inoculated to Brain heart Infusion agar (BHI) and
incubated for 24 hrs. Then the growth was streaked on 7% sheep blood agar and subcultures on blood agar and
macconkey agar. At least two cultures were made for each specimen. All cultures were incubated aerobically at
370c for 24 – 48 hrs, and colony morphology, color and status of hemolysis were recorded. Pure isolates were
subjected to Gram stain, Potassium hydroxide (KOH) test and tests that are used as primary identification and
secondary biochemical test adopting standard recommended procedures (Carter, 1984; Quinn et al., 2002).
Samples were processed at AAU FVM MVPH laboratory.

2.5. Data Analysis
Descriptive statistics (mean and percentage) was used to summaries the generated data. SAS as a statistical
package was used to see significance of association between isolates, nature and proportion of bacterial
population between APHDE and donkeys with DCDO (SPSS, 2003).

3. RESULTS
3.1. Descriptive analysis of bacterial isolates
All of the 90 samples (45 eye swabs from APHDE and 45 eye swabs from DCDO) collected for aerobic bacterial
isolates yielded bacteria. Except few bacteria which were isolated as pure culture, majority of the isolates existed
along with other bacteria. A total of 256 isolates were recovered: 189 (73.8%) were gram positives and 67
(26.2%) were gram negatives(table 1).

Table 1: Bacterial species isolated from Conjunctival swab of both apparently healthy and donkeys with clinically
diagnosed ocular case

<table>
<thead>
<tr>
<th>Type of Bacteria</th>
<th>Number of isolates</th>
<th>% of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive</td>
<td>189</td>
<td>73.8</td>
</tr>
<tr>
<td>Bacilli species</td>
<td>11</td>
<td>4.2</td>
</tr>
<tr>
<td>Streptococcus species</td>
<td>18</td>
<td>7.0</td>
</tr>
<tr>
<td>Coagulase negative staphylococcus</td>
<td>70</td>
<td>27.3</td>
</tr>
<tr>
<td>Corynebacterium species</td>
<td>51</td>
<td>19.0</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>38</td>
<td>15.2</td>
</tr>
<tr>
<td>Gram negative</td>
<td>67</td>
<td>26.2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>13</td>
<td>5.0</td>
</tr>
<tr>
<td>Pseudomonas species</td>
<td>54</td>
<td>21.0</td>
</tr>
<tr>
<td>Total</td>
<td>256.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Isolation rate of bacteria from APHDE and DCDO were shown in table (2, 3). Percentages of overall
isolated bacteria were Staphylococcus Species (43%), Pseudomonas Species. (21%) Corynebacterium Species.
(20%), Streptococcus Species.(7%), Escherichia coli (5%), and Bacilli Species (4%). There was no statistically
significant difference (p>0.05) in the isolation rate of Staphylococcus aureus, Streptococcus species,
Pseudomonas species and Bacillus species between APHDE and DCDO. The isolation rate of CNS was higher in
apparently healthy donkeys and this was statistically significant (p<0.05). Corynebacterium species and
Escherichia coli were isolated at higher rate in clinically diseased donkey eyes and this was statistically significant
(p<0.05).Major proportions of Gram positive isolates were recovered from both apparently healthy and clinically
diseased donkeys eye except Escherichia coli among gram negatives which were not isolated from apparently
healthy donkeys eye. The overall recovery rate of bacterial isolate in APHDE was 1.47(132/90) and that of
DCDO was 1.38 (124/90). Age, sex and health status considered had no impact on the frequency of recovery of
the bacteria isolates (P< 0.005). Gram positive were recorded more often than Gram negative bacteria (p> 0.005)
In the present study, detailed investigation was carried out to isolate and assess the type of bacteria inhabiting the eye of apparently healthy donkeys and those with ocular problems brought to the DHWP mobile and stationary open air clinic. Samples were obtained from the conjunctiva. More than the quality and quantity of bacterial isolates from both apparently healthy donkeys’ eye and donkeys with ocular disease were compared. One hundred eighty eye swabs from both left and right eyes (90 for APHDE and 90 for DCDO) were cultured in this study, and a total of 256 bacteria were isolated.

Out of 256 bacteria isolated, 189 (73.8%) were Gram positive and 67 (26.2%) were Gram negative. Of 132 bacteria isolated from apparently healthy donkeys, 104 (78.78%) were gram positive and 28 (21.22%) were gram negative. From 124 bacterial isolates from donkeys with ocular disease, 85 (68.54%) were gram positive and 39 (31.46%) were gram negative. This might indicate the role of Gram-positive bacteria as a potential cause of ocular problems in donkeys.

Coagulase negative staphylococcus was one of the predominant recovered bacterial isolates in the study. Comparable proportions of bacteria were recorded from dogs with clinically diseased and apparently healthy eye (Haghkhan et al., 2005). It was isolated at a higher rate in APHDE and this was statistically significant. CNS occurs as commensals on the mucous membranes of animals and occasionally causes opportunistic infections although they are usually regarded as non-pathogenic (Quinn et al., 2002).

Pseudomonas species were isolated at equal rate 21% from APHDE and 21% from DCDO which were the dominant bacteria among gram negatives and the second from both gram positives and negatives. Moore et al., 1988 has reported similar finding in horse. Pseudomonas is commensals on mucous membranes, skin and faeces of animals and cause eye infection (Quinn et al., 2002).

Corynebacterium species were isolated at a rate of 12.8% from apparently healthy donkeys and 27.4% from donkeys with ocular problem and this difference was statistically significant. Comparable proportions of bacteria were recorded from horse (Moore, et al., 1988; Andrew, et al., 2003). Corynebacterium are commensals on mucous membranes and skins of animals and known as pyogenic bacteria causing a variety of supplicative conditions(Quinn, et al., 2002). This high relative recovery rate from clinically diseased eyes suggests its

### Table 2: Over all bacterial species isolated from eye swabs of APHDE from all the study districts

<table>
<thead>
<tr>
<th>Type of Bacteria</th>
<th>Number of isolates</th>
<th>% of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive</td>
<td>104</td>
<td>78.8</td>
</tr>
<tr>
<td>Bacilli species</td>
<td>7</td>
<td>5.3</td>
</tr>
<tr>
<td>Streptococcus species</td>
<td>11</td>
<td>8.3</td>
</tr>
<tr>
<td>Coagulase negative staphylococcus</td>
<td>53</td>
<td>40.1</td>
</tr>
<tr>
<td>Corynebacterium species</td>
<td>17</td>
<td>12.8</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>16</td>
<td>12.1</td>
</tr>
<tr>
<td>Gram negative</td>
<td>28</td>
<td>21.2</td>
</tr>
<tr>
<td>Pseudomonas species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 3: Over all bacterial species isolated from eye swabs of DCDO from all the study districts

<table>
<thead>
<tr>
<th>Type of Bacteria</th>
<th>Number of isolates</th>
<th>% of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive</td>
<td>85</td>
<td>68.54</td>
</tr>
<tr>
<td>Bacilli species</td>
<td>4</td>
<td>3.2</td>
</tr>
<tr>
<td>Streptococcus species</td>
<td>7</td>
<td>5.6</td>
</tr>
<tr>
<td>Coagulase negative staphylococcus</td>
<td>17</td>
<td>13.7</td>
</tr>
<tr>
<td>Corynebacterium species</td>
<td>34</td>
<td>27.4</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>23</td>
<td>18.5</td>
</tr>
<tr>
<td>Gram negative</td>
<td>39</td>
<td>31.4</td>
</tr>
<tr>
<td>Eshertia coli</td>
<td>13</td>
<td>10.5</td>
</tr>
<tr>
<td>Pseudomonas species</td>
<td>26</td>
<td>20.9</td>
</tr>
<tr>
<td>Total</td>
<td>124</td>
<td>100</td>
</tr>
</tbody>
</table>
Pathogenesis is higher in underlying ocular pathology. Staphylococcus aureus were isolated at a rate of 12.12% from apparently healthy donkeys and 18.5%, the second dominant from donkeys with ocular problems. Similar study indicated staphylococcus aureus was isolated from dog with ocular problems at a recovery rate of 20.5% which was relatively at a higher rate compared to the present study (Kudriene, et al., 2006). Many other workers have isolated staphylococcus aureus from the eye of both apparently healthy and clinically diseased domestic animals as in horse Moore, et al., 1988; in dog Haghkhan, et al., 2005). Staphylococcus aureus is the main inhabitant of the mucous membranes of animals. It can be involved as opportunistic bacteria following pathologic role of stress conditions such as viral infections and other cause of infection in immunosuppressed hosts (Robins et al., 1981; Quinn, et al., 2002).

Streptococcus Species were isolated from apparently healthy donkey at a rate of 7.03% and 5.6% from donkeys with ocular problem. This result is comparable to those recovered from the dog (Gerding, et al., 1990). They are the normal flora of the skin and mucus membranes existing with no harm when the eye is normal and tend to cause disease in ocular pathology (Quinn et al., 2002).

Escherichia coli were isolated as a second dominant bacterium from gram negatives at the isolation rate of 10.46% from clinically diseased eyes (ocular problem). Similarly it was isolated from clinically diseased dog eye. (Haghkhan et al., 2005).

Bacillus species were isolated at a rate of 5.5% from apparently healthy donkeys and 3.2% from clinically diseased donkeys’ eye. Several researchers have isolated from different species of animals (Haghkhan et al., 2005, Moore, et al., 1988). Most of the numerous Bacilli species are saprophytes and they are widely distributed in air, soil and water (Quinn et al., 2002). The presence of Bacilli species usually reflects contamination during either acquisition or handling of specimens. They are usually ignored when isolated from clinical materials except Bacillus anthracis (Carter, 1984).

5. CONCLUSION

In the present study, an attempt was made to isolate and identify the major bacteria from the eye (conjunctiva) of apparently healthy donkeys and donkeys with clinically diseased eye. The result showed that a variety of bacteria which may be resident and or transient. The majority of the bacteria are Gram positive in both APHDE (at rate of 1.47) and DCDO (at rate of 1.38). Isolation of most bacteria both from APHDE and donkeys with ocular problems might suggest that the bacterium which resides in the conjunctiva have a chance to invade and cause opportunistic infections.

The conjunctiva is exposed to the surrounding environment where most of the bacteria isolated in this study are naturally found in the soil, water, feces of animals and as a normal flora of the skin and mucous membrane. Hence from this we can concluded that the microbial built up of the conjunctive have diverse nature which causes disease when the immune status of the mucous membrane is disrupted.

Due to resource limitation the present study did not include antibiogram sensitivity pattern of the isolates identified to species level; isolation of other micro organisms including anaerobic bacteria, viruses and fungal species expected to reside in the conjunctiva of the eye. Furthermore, the result of the present study was compared with works done in horses, dogs and other domestic animals; but these don’t represent donkeys.

ACKNOWLEDGEMENTS

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6. REFERENCES


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