Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.52, 2017

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

Fanos Tadesse [1] Yoseph Cherinet[1] Yemsrach Miressa[2] Yidnekachew Tadesse[3] Ayele Gizachew[4]
1.College of Veterinary Medicine and Agriculture, Addis Ababa University, Ethiopia
2.Akaki Woreda Livestock and Fisheries Development, Ethiopia
3.Horo Guduru zone Livestock development office
4.GIZ Ethiopia

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 clininicaly 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 higher rate in donkeys with clininicaly 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 four million donkeys are known to exist in the world of which 5.2 million were found in Ethiopian (Svandsen, 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 (Sarcoid), 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 ophthalmic case reports of 3,456 donkeys, (DHWP, 2003,2004,2005) where the risk factors and causative agents haven 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.

i.e. $$N > \frac{(Z_1 - \alpha/2 + Z1 - \beta)^2 [P_1 (1 - P_1) + (P_2 (1 - P_2)]}{(P1 - P2)2}$$

Where $Z_1 - \alpha = 50\%$ significance level

$Z1 - \beta = 80\%$ power level

$P1$ = Expected proportion in apparently healthy donkeys

P_2 = Expected proportion of clinically diseased donkeys eye

$$N_1 > \frac{7.89 \times [0.5 (1 - 0.5) + 0.8 (1 - 0.8)]}{(0.5 - 0.8)^2}$$

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 Conjuctival swab of both apparently healthy and donkeys with clinically diagnosed ocular case

Type of Bacteria	Number of isolates	% of isolates
Gram positive	**189**	**73.8**
Bacilli species	11	4.2
Streptococcus species	18	7.0
Coagulase negative staphylococcus	70	27.3
Corynebacterium species	51	19.0
Staphylococcus aureus	38	15.2
Gram negative	**67**	**26.2**
Eshertia coli	13	5.0
Pseudomonas species	54	21.0
Total	**256.00**	**100.00**

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%) Conynbacterium 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 *Eschertia 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 *Eschertia 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 was1.38 (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 ($p> 0.005$)

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

Type of Bacteria	Number of isolates	% of isolates
Gram positive	**104**	**78.8**
Bacilli species	7	5.3
Streptococcus species	11	8.3
Coagulase negative staphylococcus	53	40.1
Corynebacterium species	17	12.8
Staphylococcus aureus	*16*	12.1
Gram negative	**28**	**21.2**
Pseudomonas species	28	21.2
Total	**132**	**100**

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

Type of Bacteria	Number of isolates	% of isolates
Gram positive	**85**	**68.54**
Bacilli species	4	3.2
Streptococcus species	7	5.6
Coagulase negative staphylococcus	17	13.7
Corynebacterium species	34	27.4
Staphylococcus aureus	23	18.5
Gram negative	**39**	**31.4**
Eshertia coli	13	10.5
Pseudomonas species	26	20.9
Total	**124**	**100**

4. DISCUSSIONS

In the present study, detail investigation was carried out to isolate and asses the type of bacteria inhabiting the eye of apparently healthy donkeys and those with ocular problem brought to the DHWP mobile and stationery open air clinic. Samples were obtained from the conjunctiva. More over the quality and quantity of bacterial isolates from both apparently healthy donkeys' eye and donkeys with ocular diseases 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 yielded.

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.

Coagulse 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 very 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 became opportunistic 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 commonsals on mucous membranes and skins of animals and known as pyogenic bacteria causing a variety of suppurative conditions(Quinn, *et al.*,2002).This high relative recovery rate from clinically diseased eyes suggests its

pathogenesity is higher in under laying ocular pathology.

Staphylococci 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 (Kudiriene, *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 Haghkhah ,*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 (Haghkhah *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.

ACKNOLEDGEMENTS

I would like express my gratitude to the DHWP AAU Debre zeit branch office for the financial support and staff members of the DHWP and MVPH department laboratory for their coopration during the study

6. REFERENCES

Agajie, T, Tamirat, D, Pearson, R. and Temesgen, T. (2000): Socio economic circumstance of donkey. Use management in the rural and urban areas of central part of Ethiopia. *In*: Smith, Agajie T. and Move.L. (ed), proceedings of a work shop on promoting peri urban lively hoods through Better donkey welfare, October 2000, Debre zeit, Ethiopia. PP .16-25.

Andrew, S.E., Nguyen, A., Jones, G.L.and Brooks, D.E. (2003): Seasonal effects on the aerobic bacterial and fungal conjuctival flora of normal thoroughbred brood mares in Florida. *Veterinary ophthalmology*. 6: 45-50.

Blench, R. (1997): The history and spread of donkey in Africa. *In*: proceedings of Animal Traction Network for Eastern and Southern Africa (ATNESA) work shop. Trends in donkey population and the socio - economic context .PP. 22 – 30

Carter, G.R. and Chengappa, H.M. (1984): Essentials of Bacteriology and Mycology. Fourth edition, Charles C. Thomas, U.S.A. PP. 3-19.

Clark, B. (1974): Draft proposal to include the African wild ass .Ethiopian wild life conservation organization, Addis Ababa. Ethiopia. 200 pages

Cooper, S.C., Mc Lellan, G.J.and Rycroft A.N. (1999): conjunctival flora observed in 70 health domestic rabbits.

Veterinary Record. 149: 232 – 235.

Davidson, H. J., Rogers, D.P., Yearly, T.J., Store, G.G., Schoneweis, D.A.and Chengappa, M.M. (1994): Conjuctival flora of clinically normal pigs. *American journal of Veterinary Research*. 55: 949 – 951.

DHWP. (2003, 2004, 2005): Annual report of the donkey health and welfare clinical activities and extension education. Addis Ababa University, FVM, Debre Zeit.

Kudirkiene, E. Xilinsakas, H.and Sizugzsite,J. (2006): Micro flora of the dog eye. *Lithuanian Veterinary academy*, *Tilzes St* .34: 24-34

Feseha, G. (1998): Maximizing the use of donkey in the dry lands of Ethiopia; proceeding of the national work shop on challenges and opportunities for research and development in pastoral and agro pastoral areas of Ethiopia, 16 – 18 December 1998, Mekelle, Ethiopia.

Feseha, G. (1991): Use of equine in Ethiopia proceedings of fourth livestock improvement conference. Institute of Agricultural research, 13 – 15 November, Addis Ababa, Ethiopia. No 1: PP. 51 – 58

Feseha, G., Alemu, G., Firew, K., Abule, J., and Ketema Y. (1997): An over view of donkey Utilization and management in Ethiopia: Donkey power benefit. No1: PP .9-10.

Fielding, D. (1987): Donkey power in Africa Rural transport world. *Annual Review*. 63: 22 – 30

Gerding, P.and Kakoma, I. (1990): Microbiology of the canine and feline eye. *Veterinary clinic, North America small animals' practioner.* 20:615-625.

Getachew, M., Feshea, G., Andrew, F.T., Boja, E., Alemayehu, F.and Amare, B. (2002): Major diseases problems of donkeys. *In*: Pearson, A., Fielding, D., Tabbaa, D. Fourth international colloquium on working Equine. SPANA- AL Baath University, Hama, Syria, 20th – 26th April, PP.96 – 109.

Goddard, R.J. (2003): Sample size determination to detect difference of proportion. Health care research Unit, University of South amp 'ten.

Groves, C.P. (1986): Taxonomy, distribution, and adaptation of recent equids; *In*: meadov, R.H. and Uerpman, H.P. (ed). Equids in the ancient world. The John Hopkins University press, Maryland, USA. PP. 11 – 65

Haghkhah, M., Sarchahi, A .A. Molazem, M. (2005): Conjuctival flora in normal dogs *Journal of veterinary Research*.9: 79-83

Miller, P.E., Langen berg, J.A.and Harfmann F.A. (1985): The normal conjuctival aerobic bacteria flora of three species of captive crane. *Journal of zoo and wild life medicine*. 26: 545 – 549

MOA. (2004): Ethiopian Livestock population report. Addis Ababa ,Ethiopia.

Moore, C.P., Heller, N.and majors, L.J. (1988): Prevalence of ocular micro organisms in hospitalized and stabled horses. *American Journal of Veterinary Research*. 49: 773 – 777.

NAMSA. (2003): National metrology service agency. Addis Ababa, Ethiopia

Oppong, E.N. (1979): Disease of horse and donkey in Ghana (1909-1975).Bulletin Animal Health. *Production Africa*.27: 47-49

Payne, W.J.A. and Wilson, R.T. (1999): An introduction to Animal husbandry in the tropics. Fifth edition, Long mans, UK .PP. 546 – 575

Pearson, R.A., Nengomasha, E.and Kreck, R. (1997):Challenge in using donkeys for work in Africa. *In*: improving donkey utilization and management .Reader for ATNESA worhshop.1997.Debrezeit, Ethiopia .PP.7-15

Protsch, R. and Berger, R. (1973): Earliest radio carbonates for domesticated animals. *Science* .179: 235 – 239.

Quinn, P.J, Carter, M.E., Markey, B.and Carter, G.R. (1994): Clinical veterinary microbiology.Fourth edition. Wolf publishing, London .PP.43-45,327-344.

Quinn, P.J., Carter, M.E., Markey, B.K., Donnelly, W.J.C. and Leonard, F.C. (2002): Clinical veterinary microbiology. Fifth edition. Harcourt publishers limited, Edinburgh, London.PP.1-648.

Robins, S.I., Agell, M. and Kumer, U. (1981): Basic Pathology. Third edition. W.B Saunders Company, Philadelphia.PP.369-420.

Samuelson, PA., Anderson T. Gwin.R.M. (1984): Conjuctival fungal flora in horse, cattle dogs and cats. *Journal of the America Veterinary Association*.184:1240-1242.

SPSS. (2003): Statistical Packages for Social Science.User's guide for personal computers, version eight edition statistical analysis institute .Inc. Cary, NC.

Simona, N., Micaela S., Giovanni, B., Michele, C. and Francesca, M. (2007): Conjuctival fungal flora in health donkeys. *Journal of Veterinary ophatalmology department of Animal pathology and Veterinary clinical science, University of Pisa.Italy*.104: 207-220

Svendeson, E.D. (1997): The professional hand book of the donkey. Parasites abroad. *In*: Svendeson B.E. (ed).Third edition, Whittet books, London.PP.227-238.

Wilson, R. T. (1991): Equine in Ethiopia. *In*: A, Fielding and R.A. Pearson (ed), Donkeys, mules and Horses in Tropical Agricultural Development Proceeding of collonquium Held 3 – 6 Sept 1990, Edinburgh, Scotland (Center for Tropic veterinary medicine, university of Edinburgh). PP. 33-47