STUDIES ON THE EFFECTIVENESS OF TREATMENT AND ON CARRIER STATUS OF CONTAGIOUS CAPRINE PLEUROPNEUMONIA (CCPP) IN EXPERIMENTALLY INFECTED GOATS

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SUMMARY

In this study, a randomized clinical trial was carried out in twenty-nine goats after experimental infection. Three experimental groups each with eight goats and one control group with five goats were used in this study. Antimycoplasmal drugs i.e. tetracycline, tylosin and streptomycin were assessed on the basis of clinical cure, pathological lesions and carrier status of *Mycoplasma capripneumoniae* infection for the three experimental groups. On day 60 post-infection, the surviving goats were slaughtered and pathological lesions were assessed. Detection of *Mycoplasma capripneumoniae* in the slaughtered goats was based on isolation and on a combined polymerase chain reaction / restriction enzyme analysis (PCR/REA). A simple scoring index based on drug efficacy, pathological lesions, isolation, availability and relative costs of treatment was constructed. All eight goats treated with streptomycin recovered and the risk ratio of recovery was 2.5 with 60% drug efficacy (p value=0.035) and all had pleuritic adhesions while only 37.5% had pneumonic lesions. In the tetracycline treated group only one of the eight goats did not recover, therefore, the risk ratio of recovery was 2.2 with 54.3% efficacy (p=0.217). In this group pneumonic and pleuritic lesions were observed in only one goat. Goats treated with tylosin had 62.5% (5/8) recovery percentage and the risk ratio of recovery was 1.6 with 36% efficacy (p=0.592). All goats showed pneumonic lesions and 80% had pleuritic adhesions. Isolations of *Mycoplasma capripneumoniae* was observed in the untreated goats, tetracycline and tylosin treated groups. The PCR/REA test detected *Mycoplasma capripneumoniae* in spontaneously recovered goats and animals from each treatment group. In view of the findings of this study it can be concluded that treatment with tetracyclines and tylosin does not seem to eliminate carrier status but carrier status in goats treated with streptomycin is doubtful based on the inability to isolate Mccp. This study demonstrates that streptomycin was the best drug followed by tetracycline and tylosin.

INTRODUCTION

Contagious caprine pleuropneumonia (CCPP) is a serious diseases of goats (Cottew, 1979) and the presence of the disease in Tanzania was confirmed by Msami *et al.* (1998). Tanzania has 11 million goats (Ministry of Agriculture and Cooperatives, 1995) and the occurrence of the disease could adversely affect the development of the goat industry. The causative agent *Mycoplasma capricolum capripneumoniae* (Mccp) belongs to a group of six mycoplasmas referred to as *Mycoplasma mycoides* cluster. However, identification of Mccp and diagnosis of CCPP is associated with problems of slow growth and also members of Mycoplasma mycoides are closely related hence they exhibit similar biochemical features and serological cross-reactions (Ernø, 1987). Due to the diagnostic problems mentioned above, the epidemiology of the disease and particularly with regard to the carrier state is not well known. In contagious bovine pleuropneumonia (CBPP) of cattle carrier status is associated with sequestra formation but evidence of sequestration in CCPP infection is conflicting (Wesonga *et al.*, 1998; Semuguruka, W. D. personal communication, 1999). Varying degrees of responses and carrier status have been reported by Abubakr *et al.* (1984) using oxytetracycline or tylosin and Rurangirwa *et al.* (1981) using dihydrostreptomycin. Following the recent CCPP outbreak in Mpwapwa District of Tanzania, goats which had apparently recovered after treatment with oxytetracycline showed lesions characteristic of CCPP on slaughter (Minga, U.M. personal communication, 1999). Previous studies on carrier state may not have reflected actual absence of Mccp due to difficulties in the cultivation of the organisms (Bölske *et al.*, 1996). Further studies involving improvement of the cultural methods and utilization of better diagnostic methods could assist in verifying the presence of Mccp in chronic cases (Wesonga *et al.*, 1998). In this regard more information about effect of treatment on clinical cure, pathological lesions and carrier state is required and this could be achieved by conducting a comparative study in one trial to assess the effectiveness of different treatments. The increase in sensitivity afforded by the use of PCR in this study could allow detection of chronically infected animals. Demonstration of carrier status could assist in explaining the maintainance of infection between outbreaks and therefore contribute further knowledge which may be used for planning control strategies.

MATERIALS AND METHODS

Study area and experimental animals

The treatment trial lasted from December 1999 to mid March 2000 and was carried out at the Isolation Unit of the Animal Diseases Research Institute (ADRI) in Dar-es-salaam. Pathology and histopathology work was also done at ADRI. Mycoplasma isolation and molecular biology procedures were carried out at the Faculty of Veterinary Medicine, Sokoine University of Agriculture. Thirty goats of a local breed aged between 1-3 years were purchased from a private farm in Mbagala area of Temeke District of Dar-es-salaam. The animals were eartagged, body weights and sex recorded. The farm had no previous history of CCPP infection and the animals were ascertained to be free of Mccp antibodies by the modified growth inhibition test (Black, 1973). Nasal swabs were also collected before the trial but Mccp was not isolated. The animals were housed in isolation and fed indoors on hay and given drinking water ad lib. The animals were dewormed with ivermectin (Ivomec, MSDAGVET, I ml contains 10 mg ivermectin) subcutaneously at 0.4 mg/kg body weight, kept under observation for 14 days and allowed to adapt to the new environment. During this period one goat died of starvation due to change of feed from lush grass to hay while the rest of the goats adapted well.

Experimental infection

A laboratory isolate obtained from a previous outbreak and identified as Mccp by growth inhibition test was used. The inoculum contained 10^9 colony forming units (cfu) per ml and was prepared according to Harbi *et al.* (1981) and all the twenty-nine goats were infected with 10 mls on the same day intra-tracheally as described by Smith, (1964).

Post-infection monitoring and Treatment trial

All the animals were clinically examined twice daily recording their body temperatures and respiration rates. Animals showing temperatures above 40°C and/or respiration rates above 40 per minute and other respiratory signs were taken for the treatment trial. Three drugs were among the recommended drug in the treatment of mycoplasmas. The experimental animals were randomly allocated to the treatment groups using calculator-generated random numbers. The treatment started when the first signs of the disease, which was a rise in body temperature was observed and was administered as follows:

-Tetracycline treatment group; 8 goats were treated with oxytetracycline (Oxytetracycline, Alfasan, 1 ml contains 100mg oxytetracycline) intramuscularly

(i.m.) at 10 mg/kg body weight daily for 6 days.

-Tylosin treatment group; 8 goats were treated with tylosin (Tylap, Laprovet, 1 ml contains 200mg tylosin) i.m. at 20mg/kg body weight daily for six days.

-Streptomycin treatment group; 8 goats were treated with dihydrostreptomycin (Streptopen, Alfasan, 1ml contains 200,000 I.U. procaine benzyl penicillin and 20mg dihydrostreptomycine) i.m. at 40 mg dihydrostreptomycine/kg body weight single dose.

-Control group; The remaining 5 goats were not treated and acted as control group.

Post-treatment monitoring

One of the ADRI staff was requested to mix animals treated with different drugs in the same rooms. The investigator who assessed the clinical signs was therefore blinded with respect to the type of treatment administered to the different animals. The animals were examined daily to monitor changes in the body temperatures and respiratory signs of CCPP observed after the experimental infection. After experimental infection, nasal swabs were collected at an interval of 14 days up to the end of the study. The laboratory staff who collected the samples was blinded with respect to the type of drug administered to each particular source animal. The swabs were preserved in 0.5ml phosphate buffered saline (PBS) to be examined for Mccp by the combined PCR/REA test. Animals that died during the trial were necropsied and examined for CCPP lesions. Samples for isolation of mycoplasma and the combined PCR/REA test were collected aseptically from the lung tissue, mediastinal and bronchial lymph nodes. On day 60 post infection (p. i.), the 22 surviving goats were slaughtered and examined for CCPP lesions. Lung tissue, bronchial and mediastinal lymph nodes were taken for Mccp detection by mycoplasma isolation and PCR/ restriction enzyme analysis.

Mycoplasma isolation

Isolation was done by a H25P broth and agar medium which was a modification of the pyruvate enriched medium described by Thiaucourt *et al.* (1992). Mycoplasma isolation was carried out as described by Bölske (1995). The isolates were identified by growth characteristics and colony morphology (Cottew and Yeats, 1978) and confirmed by the growth inhibition test as described by Black, (1973).

Molecular biology techniques

DNA extraction and PCR as described by Johansson *et al.* (1997) was adopted. A 15% polyacrylamide gel electrophoresis was performed according to Sambrook *et al.* (1989). 1Kb molecular weight marker was run in

parallel track. The polyacrylamide gel was stained by silver nitrate as described by Herring *et al.* (1982). Restriction enzyme analysis of the PCR product was carried out by taking 8ml of the PCR product as described by Johansson *et al.* (1997). The digested PCR product was separated by polyacrylamide gel electrophoresis and stained by silver nitrate as described above.

Statistical data analysis

Data management was handled by Microsoft Excel and was analyzed by StatXact-4 version 4.0.1 (1998). The Fisher-Freeman-Halton test (Hilton *et al.*, 1994) was used to test statistical independence between type of treatment and outcome and p-values were given as equalities without reference to a levels or statistical significance as recommended by Rothman and Greenland (1998). Epi-Info version 6.0 was used to calculate relative risk (RR), drug efficacy and 95% confidence interval (CI).

Drug score index.

In a further attempt to compare the three drugs an index was constructed based on the method of internal consistency (Goodes and Hall, 1952). The following items were considered: drug efficacy, absence of CCPP lesions, lack of isolation, availability at the farmer's level in Tanzania and relative cost of intervention in Tanzania. Drug availability and relative cost of intervention were included because they may limit the use of the drug. The scoring was done by the following schedule: Strongly favourable=1 Moderately favourable=2 Favourable=3

Unfavourable=4 Strongly unfavourable=5

RESULTS

Assessment of clinical cure

Table 1 shows the mortality and clinical recovery percentage in the different treatments groups. The proportion of the treated animals which clinically recovered ranged from 62.5% to 100%. The associated risk ratios (RR), drug efficacies and Fisher¢s two-sided exact p values are given in Table 2. In comparing the effect of streptomycin treatment to that of no treatment a risk ratio of 2.5 (CI 95% 0.9-7.3) was obtained implying that those treated with streptomycin were more likely to recover than those receiving no treatment. The drug efficacy of streptomycin was 60% (two-sided Fisher's exact p value=0.035). In comparing tetracycline treated and the untreated control groups the strength of association as given by the RR was 2.2 (p=0.2168). Tylosin fared worst compared to the other drugs with a risk ratio) of 1.6 however, the observation was statistically found to be insignificant (p=0.5921).

Table 1. Clinical recovery percentages of the different treatment groups

Treatment group	Total goats	deaths	recovered	recovery percentage
Oxytetracycline	8	1	7	87.5
Tylosin	8	3	5	62.5
Streptomycin	8	0	8	100
Control	5	3	2	40
Total	29	7	22	75.9

Overall Fisher's exact test: p=0.064.

Table 2. Comparison of the different treatment groups

Treatment	Relative risk (RR)	95% CI for (RR)	DRUG efficacy	95% CI for drug efficacy	P*
Streptomycin	2.5	0.9-7.3	60%	-17.0-86.3	0.035
Tetracycline	2.2	0.7-6.6	54.3%	-38.0-84.9	0.217
Tylosin	1.6	0.5-5.2	36%	-112.5-80.7	0.592

P* = Fishers exact two sided p value.

RR= risk ratio

Assessment of the Pathological lesions: Gross pathological changes

A summary of the major pathological findings is shown in Table 3. No sequestra were observed in all the 29 goats. In the slaughtered animals extensive pathological lesions were observed in the groups treated with tylosin and streptomycin. In the group of goats treated with tylosin all the goats had pneumonic lesions and 80% had pleuritic lesions. In the group of goats treated by streptomycin, 37.5% of 8 goats had pneumonic lesions and all had pleurisy. In the group of goats which were treated with tetracycline only one goat had pneumonic and pleuritic lesions. The occurrence of pathological lesion was statistically associated with the type of treatment (p=0.0003).

Table 3. Pathological lesions in goats slaughtered after the trial.

Treatment group	Group size	No. with lesions	No without lesions	Location of lesion (%)	
				Pneumonic	Pleurisy
Tetracycline	7	1	6	1 (14.28)	1 (14.28)
Tylosin	5	5	0	5 (100)	4 (80)
Streptomycin	8	8	0	3 (37.5)	8 (100)
Control	2	2	0	1 (50)	2 (100)
Total	22	16	6	10	15

Fisher¢s exact p value=0.0003

Isolation of mycoplasma

The number of isolations in the different treatment groups are shown in Table 4. Overall Mccp was isolated from 15% of the treated goats. The results further show that 60% of the isolates were obtained from lymph nodes and 40% from the lungs. The highest isolations were observed in the group of goats treated with tylosin (40% of the goats) whereas in the group of goats treated with tetracycline, 14.3% of the goats were positive. No isolations were made in the group of goats treated with streptomycin. In the slaughtered goats, isolations were statistically associated with the type of treatment (p=0.0153)

Table 4. Mycoplasma isolations in goats slaughtered after the trial.

Treatment group	Number of Goats slaughtered	Mycoplasma isolations		Number of Goats positive (%)
		Lung	Lymph Node	
Tetracycline	7	0	1	1 (14.3)
Tylosin	5	1	1	2 (40)
Streptomycin	8	0	0	0
Control	2	1	1	2 (100)
Total	22	4	6	7 (22.7)

Fisher¢s exact p value=0.0153

Polymerase chain reaction/ restriction enzyme analysis

The combined PCR/REA test results in the different treatment groups are shown in Table. 5. The highest proportion of positive goats was observed in the group of goats treated with tylosin and the lowest proportion in the group of goats treated with tetracycline. Detection of Mccp by PCR/REA test was statistically not associated with the type of treatment (p=0.1914). All the nasal swabs were negative. The constructed simple drug superioty index is shown in Table 6. This simple index shows that tylosin is overall dominated by the other two drugs. Streptomycin had the lowest score followed by tetracycline.

Table 5. PCR/restriction enzyme analysis results in slaughtered. goats

Treatment group	Number of Goats slaughtered	PCR/REA test positive		Number of Goats positive (%)
		Lung	Lymph Node	
Tetracycline	7	0	1	1 (14.3)
Tylosin	5	1	1	2 (40)
Streptomycin	8	2	2	2 (25)
Control	2	1	2	2 (100)
Total	22	4	6	7 (31.8)

Fisher¢s exact p value=0.1914

Table 6. Drug superiority index scores.

Criterion	Streptomycin	Tetracycline	Tylosin
Drug efficacy	2	3	4
Absence of lesions	5	2	4
Lack of isolation	1	2	3
Availability at farmers level	3	2	5
Cost of the full course of the drug	1	2	4
Cost of veterinary service	1	4	4
Total score	13	15	24

DISCUSSION

The Risk ratio of 2.5 taken together with the low p-value indicates that streptomycin is effective against CCPP and these results are unlikely to have risen by chance. A risk ratio of 2.2 and efficacy of 54.3% for tetracycline would indicate a moderate therapeutic effect. Tylosin faired worst with a risk ratio of 1.6 and 36% drug efficacy, which indicate a relatively small biological effect. In the present study no sequestra was observed in the twenty-nine goats including the twenty-two goats that survived and persisted through the chronic form of the disease. This is in contrast to descriptions in the contagious bovine pleuropneumonia of cattle where sequestra formation is associated with the use of antibiotics (Masiga *et al.*, 1996). Formation of sequestra in CCPP has been reported by other workers (Kaliner and MacOwan, 1976; Wesonga *et al.*, 1998) but both observations were made in untreated goats and from this study therefore we could rightfully say that sequestra formation is not a common phenomena in CCPP infected and treated goats. In the present study all the goats treated with tylosin and streptomycin showed pathological lesions and had more extensive changes in the lungs and pleuritic adhesions than in goats treated by tetracycline. In the group of goats that were treated with tetracycline only 14.3% of the goats had pneumonic and pleuritic lesions. Similar findings have been reported by Rurangirwa *et al.* (1981) who observed CCPP lesions in all the experimentally infected goats treated with streptomycin. This finding has differed from Abubakr *et al.* (1984) who reported that 40% of CCPP infected goats treated by tylosin and 40% of similar goats treated by tetracycline showed CCPP pathological lesions. This apparent difference could be explained by differences in the quality of the drugs since formulations and sources were not the same. The difference could also be explained by the difference in virulence of the microorganisms involved in the two studies. Strain difference in virulence is a phenomena documented by McMartin *et al.* (1980) who reported virulence difference in reference strains F38 and G69. However, in this study the same isolate was inoculated into the different treatment groups. In streptomycin treatment occurrence of lesions could probably be minimized by increasing the duration of treatment since timely and effective treatment could likely reduce the extent of the pathological lesions. This study also intended to determine carrier status amongst treated goats, the findings revealed that Mccp was isolated from 25 percent of the surviving treated goats. These goats are carriers by definition. Similar findings have been reported by Abubakr *et al.* (1984) who isolated

Mccp from 26.6% of treated goats. In the slaughtered goats Mccp was isolated from 40% and 14.3% of the groups which were treated with tylosin and tetracycline respectively. No isolations were made in the group of goats treated with streptomycin but Mccp was detected by PCR. The failure to isolate Mccp in streptomycin treated goats is in agreement with Rurangirwa *et al.* (1981) who could not re-isolate Mccp in experimentally infected goats treated with streptomycin. Treatment with tetracycline and tylosin does not eliminate completely carrier status. The existence of carrier state in goats treated with streptomycin is doubtful based on the inability to isolate Mccp in this study and that of Rurangirwa *et al.* (1981). The reported recurrences of CCPP cases and further deaths after antibiotic treatment may probably be due to carrier status. Movement of carrier goats by nomadic migration or trade may have been responsible for the introduction of the disease in new areas as it has been observed in the recent outbreaks of CCPP in Dar es salaam and Morogoro regions (Kusiluka *et al.*, 2000). In the simple scoring index the research objectives i.e. drug efficacy, absence of pathological lesions and lack of isolation were considered. Detection of Mccp by the combined PCR/REA test has been excluded in developing the index since the test detects DNA fragments not necessarily viable microorganisms. The simple drug superiority index indicates that streptomycin is the best drug followed by tetracycline.

CONCLUSIONS AND RECOMMENDATIONS

On the basis of results obtained from the present study streptomycin appears to be a better drug than tetracycline and tylosin and should therefore be the preferred drug when treatment is being considered. The study established the existence of carrier status in goats treated with tetracycline and tylosin. Carrier status was also confirmed in spontaneously recovered goats. Treatment alone is unlikely to be effective in controlling this disease but it might reduce the morbidity and mortality and permit goats in an affected herd to be slaughtered while still in reasonable body condition. Strict movement control has to be enforced if any epidemic is to be brought under control. Vaccination may offer more possibilities in the control of contagious caprine pleuropneumonia. Due to the small sample size limitation of the study, the findings should be further validated by carrying out a large scale field trial.

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