Research Article

Study on the prevalence of anaplasmosis and efficacy of different antiprotozoal drugs in goats at district Mirpurkhas, Sindh –Pakistan

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Abstract

This study was conducted to determine the prevalence of Anaplasmosis and efficacy of different antiprotozoal drugs in goats around the Mirpurkhas. A total of 300 goats were examined for the occurrence of Anaplasmosis. *Anaplasma marginale* was the only species identified from all positive goats and the highest prevalence of Anaplasmosis was recorded in Samaro (30%) and the lowest (8%) in Sindhri, district Mirpurkhas. The overall prevalence of anaplasmosis was recorded at 13.33%. The rate of infection was higher in females (17%) compared to males (9%). The age wise prevalence was recorded 14% in two years of age and 12% in 2 years and above of age. The most prominent clinical features in positive cases of anaplasmosis were recorded increased body temperature, respiration and pulse rate, with loss of condition and icterus. The disease positive animals were divided into four groups; A, B, C and D in order to determine the efficacy of different antiprotozoal drugs. The Imidocarb, Diminazine and Oxytetracycline drugs were administered to A, B and C groups respectively, on the standard dose by the recommendations of manufacturers. While the D group was kept as an untreated positive control. It was observed that maximum efficacy against *Anaplasma marginale* was achieved by the administration of Imidocarb (80%), followed by Diminazine (60%) and Oxytetracycline (30%). All animals of the infected control group (D) were remained positive and shown the increasing intensity of the infection and one goat was died in control group D due to the severity of the infection.

Keywords: Anaplasmosis; Antiprotozoal drug's efficacy; Goats; Prevalence

Introduction

Goats are small ruminants and maintain an especially valuable ecological and economical place in Asian agriculture. The importance of goats with a variety of different breeds and the degree of their

adaptation is a unique and wide range of distribution throughout the ecosystem [1]. About 90% of the world's goat population is found in developing countries. New Zealand, Australia, India, Pakistan and China are the major goat producing countries in the world [2]. In Pakistan, there are 68.4 million heads of a goat and they are producing annually 25.8,000 tons of hair and 671,000 tons of mutton [3]. Hemo-protozoan diseases, especially Anaplasmosis, Trypanosomiasis, Babesiosis and Theileriosis are major impediments to the health and production of livestock farming [4]. However, epidemiological studies revealed that demographic, location and environmental factors affect the risk of infection.

The protozoan parasites are the major threat to the animal's health in tropical and subtropical areas of the world. Protozoan diseases have been remained economically important due to affecting national and international trade [5, 6]. These are also responsible for various degrees of morbidity, mortality and economic losses [7]. *A.marginale*, a subspecies with high pathogenicity has been proven capable of infecting goats [8]. Clinical signs in infected goats are reported as fever, weight loss, anorexia, hematuria, anemia and icterus [9]. The most effective method to control the parasitic diseases is through the strategic use of drugs that are available in the market, however, they are expensive and have toxic side effects, marginal efficacy and drug-resistant [10]. Sometimes animals survive after a long convalescent period without treatment, but others may develop renal failure and shock which leads to death. Generally, anaplasmosis in goat is diagnosed on the basis of clinical signs and blood smears examination [11].

There was no comprehensive study conducted on the prevalence of anaplasmosis and efficacy of antiprotozoal drugs in goats.

Therefore, this study was designed to determine the prevalence of anaplasmosis and to evaluate the efficacy of different antiprotozoal drugs in goats of Mirpurkhas Sindh, Pakistan.

Materials and methods
Study area
Mirpurkhas is situated in the east of Sindh province and it is the fourth largest city of the province with an estimated population of 488,590. It comprises of many villages that depend on their livelihood on the rearing of small ruminants especially goats [3].

Collection of samples
A total, 300 goats of all ages and of either sex were randomly selected for the study. The blood smears were prepared for the examination and diagnosis of anaplasmosis in selected goats of Mirpurkhas.

Blood smears
Blood smears were made randomly from each goat flock depending on the number of goats and the history of the animals with weakness, anemia, inappetence and presence of the ticks on the different parts of the body. The Ear was cleaned with spirit swab before the puncturing ear vein and blood smears from each animal were prepared by using a disposable 23G needle. From each animal, 03 thick and 03 thin blood smears were prepared between 12 PM to 5 PM according to the procedure described by Tick Fever Research Center, Queensland [12].

Thin blood smears preparation
Thin smears were prepared by puncturing ear vein with a sterile needle to obtain a small quantity of the blood for the preparation of blood smears.
1. A drop of blood not larger than a pin's head was placed at the center of the slide.
2. A spreader slide at an angle of $35^0$was placed in contact with the drop of blood then pushed gently to down till the thin blood smear was prepared.
3. Then, the slide was dried and fixed immediately in 100% methyl alcohol.

4. All slides were properly labeled with a permanent marker, kept in a slide box and brought to the laboratory for further process.

Thick blood smears

Thick blood smears were prepared by placing a drop of blood on the center of the slide. Then at the angle of 45°, a spreader slide was pushed down gently to prepare thick smears. The prepared thick smears were fixed as done for thin smears and processed to the laboratory.

Staining of the blood smears

The staining procedure for all slides was carried out by the procedure as suggested by Chatterjee [13].

Giemsa's staining

Giemsa solution (Midiline chemical division Merck, Darmstadt, Germany) prepared bottles were purchased from the market. Slides were kept at horizontal on slide frame and few drops of Giemsa stain were poured on and then left for 15 minutes. Then, slides were gently washed in running tap water and kept upright position until slides dried. Now, these slides were ready for microscopic examination.

Microscopic examination and Identification of blood protozoa

All thin blood smear slides were examined under binocular electric microscope oil emersion 100x- objective lens. The Anaplasma *spp*. were identified and confirmed with the help of the key developed by Adam [14].

Therapeutic trials

Identified infected goats showing clinical signs divided into four groups A, B, C and D (Control group), and each group was consisting of ten animals. The efficacy of three antiprotozoal drugs with the recommended dose (Imidocarb 0.1/kg body weight intramuscular, Diminazine 0.3ml/kg body weight intramuscular and Oxytetracycline 1ml/kg body weight intramuscular) was evaluated against positively infected goats. The blood smears were prepared from the infected cases after the treatment on day 14th and 30th and in positive cases, the 2nd and 3rd booster doses of treatment were given accordingly to check the efficacy of drugs, as shown in (Table 1).

Results

The study was randomly carried on 300 goats from different areas in and around Mirpurkhas. Data shows the overall prevalence rate of 13.33%. The highest prevalence rate (30%) of anaplasmosis was recorded in Taluka Samaro and the lowest (8%) in Taluka Sindhri (Table 2).

In (Table 3) *A. marginale* was the only species identified as a small, round dark red inclusion body in the margin of Red blood cells similarly with the same characteristics as described by Urquhart [15]. Animals with anaplasmosis positive blood smears had shown clinical signs of increased body temperature, respiration, heart rates (Table 4), inappitance, ictrus and mild lymph nodes enlarged (Table 5).

Prevalence

As depicted in (Table 6), the gender-wise prevalence rate of Anaplasmosis as 9% and 17% in male and female goats respectively.

Age wise prevalence

The age wise prevalence of anaplasmosis was observed as 14% in goats of up to 2 years of age and 12% in above than 2 years of age (Table 7).

Drug Efficacy

The drug efficacy was evaluated on the basis of the disappearance of the clinical signs and the absence of *Anaplasm marginale* from examined blood smears after the treatment. The Table 7 indicates the efficacy of antiprotozoal drugs against Anaplasmosis in infected goats. The results showed the efficacy of 80%, 60% and 30% with Imidocarb, Diminazine and Oxytetracycline, respectively, (Table 8). However, the efficacy showed that two animals by Imidocarb, four animals by Diminazine and

seven animals by Oxytetracycline did not recover as shown in (Table 9).

Control group

All control group animals (Group D) infected with Anaplasmosis remained positive during the study period (Table 10), however after 30 days one goat expired.

Table 1. Drug names, dosage and routes of administration

S. No.	Drug	Trade name	Dosage and administration	Manufacturer
1.	Imidocarb di propionate (120mg/ml)	Imizol	0.1/kg body weight intramuscular	ICI, Pakistan
2.	Diminazine di aceturate (35mg/ml)	Diminazene	0.3ml/kg body weight intramuscular	Star Laboratories (Pvt) Lahore, Pakistan
3.	Oxytetracycline (200mg/ml)	Rasomycin LA	1ml/10kg body weight intramuscular	Star Laboratories (Pvt) Lahore, Pakistan

Table 2. The Taluka-wise prevalence rate of *Anaplasmosis*

S. No.	Name of Taluka/locality	No: of Animals examined	No: of Animals infected	Prevalence rate (%)
1	**Kot Ghulam Muhammad**	50	06	12
2	**Samaro**	50	15	30
3	**Hussain Bux**	50	05	10
4	**Sindhri**	50	04	08
5	**Sahab Belaro shakh**	50	05	10
6	**Jhudo**	50	05	10
	Total (%)	300	40	13.33

Table 3. *Anaplasma marginale spp.* identified from goats

S. No.	Species identified	Total number of animals examined	No: of animals infected	Prevalence rate (%)
1	*Anaplasma marginale*	300	40	13.33

Table 4. Body temperature, pulse rate and respiration rate in infected goats

	Body temperature in ^{0}F	Heart rate/ minute	Respiration rate/ minute
Mean	105.2	112	84
SD	2.22	10.88	8.22
Normal*	102-103	70-90	10-30

*Rodostitis*et al.* (2002)

Table 5. Major clinical signs observed in infected goats

Clinical signs	Major observations
Appetite	Inappetence
Lacrimation	Absent
Body condition	Slightly thin
Nasal discharge	Mild
Dehydration	Normal
Body coat	Normal dry
Mucous membrane color	Icteric
Muscle tremors	Mild
Lymph node	Mild enlarged
Defecation	Constipation/dry feces
Urine	Bright yellow

Table 6. Gender wise prevalence rate of anaplasmosis

Sex	No. of animal examined	No. of animals infected	Prevalence percentage (%)
Male	150	14	9%
Female	150	26	17%

Table 7. Age-wise prevalence of Anaplasmosis

Age group	No. of animals examined	No. of animals infected	Prevalence rate (%)
Up to 2 years	150	22	14%
Above than 2 years	150	18	12%

Table 8. Efficacy percentage of imidocarb, diminazine and oxytetracycline against anaplasma

Group	Drugs	No. of animals treated	No. of animals recovered	Efficacy (%)
A	Imidocarb	10	08	80
B	Diminazine	10	06	60
C	Oxytetracycline	10	03	30
D	Control group	10	00	00

Table 9. Efficacy of imidocarb, diminazine and oxytetracycline against *A. marginale*

Groups	Drugs (Brand Name)	Age	Animal recovered	Remarks
A	**Imidocarb**	3m-5y	08 M/F	Complete recovery
		1y	02 M*	No recovery
B	**Diminazine**	3m-5y	06 M/F	Complete recovery
		3m-5y	04 M/F	No recovery
C	**Oxytetracycline**	3m-5y	03 M/F	Complete recovery
		3m-5y	07 M/F	No recovery
D	**Control**	3m-5y	10 M/F	Remained + ve

m=months, y=years, M=male and F=female * one goat died in this group

Table 10. Days wise efficacy of imidocarb, diminazine and oxytetracycline in anaplasmosis in goats

Goat No.	Group-A Imidocarb			Group-B Diminazine			Group-C Oxytetracycline			Group-D Non-medicated (control)		
	Days			Days			Days			Days		
	1	15	30	1	15	30	1	15	30	1	15	30
1	+	+	-	+	+	-	+	+	-	+	+	+
2	+	+	-	+	+	-	+	+	-	+	+	+
3	+	+	-	+	+	-	+	+	-	+	+	+
4	+	+	-	+	+	-	+	+	+	+	+	+
5	+	+	-	+	+	-	+	+	+	+	+	+
6	+	+	-	+	+	-	+	+	+	+	+	+
7	+	+	-	+	+	+	+	+	+	+	+	+
8	+	+	-	+	+	+	+	+	+	+	+	+
9	+	+	-	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+	+	+
Efficacy (%)	0	0	80	0	0	60	0	0	30	0	0	0

+ = indicates the presence of Anaplasmosis in the blood

 - =indicates the absence of Anaplasmosis in the blood

Discussion

This study was carried out on randomly selected 300 goats of district Mirpurkhas. The overall prevalence of anaplasmosis was 13.33%. The prevalence of Anaplasmosis was recorded the highest 30% in samaro and 8% as the lowest in Sindhri. This is in agreement with the results reported from Cyprus [16], who recorded a prevalence of Anaplasmosis 18% in goats. There was also a recorded prevalence of Anaplasmosis as 13.89% and 8.33% in sheep and goats respectively, from Khyber Pakhtunkhwa, Pakistan [17].

The gender wise prevalence rate of Anaplasmosis in goats was recorded as (9%) and (17%) in male and female goats respectively. The findings of this study are in contrast with Lee [18], who reported that a higher prevalence of Anaplasmosis in male goats (52.14%) as compared to females (47.85%). This difference may be due to the increased opportunities of exposure to ticks carrying *Anaplasmaspp.*in a whole herd.

The age wise prevalence of Anaplasmosis observed in our study was (14 %) in up to 2years of age whereas, above than 2years of age was observed (12%). Arunconcluded that the overall incidence of anaplasmosis was found 9.2% in sheep above 2 years of age [19]. Similar results of anaplasmosis in goat were observed by Lee [18], who reported 4.9% and 9.5% in aged < 1 year and ≥ 1 year, respectively. The variation in prevalence percentage might be due to the difference in goat breed, management, feeding and housing as well as the age group of animals used in the study.

Goats, which were found positive had shown the clinical signs of fever, inappetence and constipation (dry feces), anemia (pale conjunctiva and oral mucous membrane), lethargy, rough coat, early tiredness and loss of the body weight before the treatment, while all goats which recovered had shown gross improvement and activeness and weight gain. These clinical signs are in close agreement with the

Chochlakis [16] and Dold and Cocks [20], who observed the similar clinical signs of Anaplasmosis in infected and recovered goats in Cyprus and South Africa, respectively.

The *A. marginale* was the only species identified from all positive samples which is in close agreement with the Sajid [21] and Talat [22], who also identified the *A. marginale* causing the Anaplasmosis in goats in Pakistan.

Results of the present study revealed that the efficacy of Imidocarb, Diminazine and Oxytetracycline was 80%, 60% and 30% respectively. It was observed that maximum efficacy against anaplasmosis was recorded with Imidocarb and Diminazine, while the minimum efficacy was recorded with Oxytetracycline; these results are in the closed agreement described by Zaugg [23].

In control infected group (Group-D), one goat with Anaplasmosis was expired while the intensity of the clinical signs (Anemia, anorexia) was changed from moderate to severe within 30 days and all positive goats showed health deterioration by the severity of the infection. Further, we found that control group (D) anaplasma positive goats (day 30th) deteriorated health with an increase in the severity of the clinical signs which were similar as previously reported by Chakrabarti [24].

Conclusion

The anaplasma infection is an endemic infection present in all goat farming areas of district Mirpurkhas. The rate of infection was higher in the female in comparison to male animals. The higher Anaplasmosis cases were observed in goats of two years than other age groups. All infected goats treated with Imidocarb @ 0.1ml/kg body weight i/m and Diminazine 0.3ml/kg body weight i/m showed good response against Anaplasma infection in goats.

Authors' contributions
MI Memon conceived and designed the project and S Ram performed the experiment. HK Kunbhar and NA Korejo analyzed the data while, RA Leghari contributed to materials, tools and field supervision. MR Memon, SA Soomro and S Parveen helped in data analysis and proofreading of the manuscript.

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