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Prevalence and therapeutic management of chlamydiosis in goats

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Abstract
This study aimed to assess the prevalence of Chlamydia abortus in goats from three different areas of western Maharashtra, India, with a focus on flocks with a history of abortion and weak offspring. Blood samples were collected from 150 goats for diagnosis using an iELISA test, and prevalence was evaluated based on area, sex, and age. Alterations in hematological and total protein values were estimated in seropositive goats, and DNA was extracted for PCR testing. Seropositive goats were then divided into three groups, each receiving a different antibiotic and supportive therapy for ten days. The study found that Meropenem @ 10mg/kg BW i/v was more effective in mitigating anti-chlamydial antibody titres in seropositive goats than Oxytetracycline @ 10mg/kg BW i/v and Tylosin @ 10mg/kg BW i/m. These findings suggest that Meropenem may be a promising treatment option for Chlamydia abortus infections in goats, although further research is needed to confirm these results.

Keywords: Chlamydia spp., seroprevalence, antibiotics, goat, abortion

Introduction
India leads in goat population and milk production, providing income to small farmers and tribes through nutrient-rich milk and meat. *(Annual report 2021-22, DAHD, India). C. abortus is a leading cause of goat abortion.* Abortions persist for 2 to 3 years until almost all the females abort and later animals become asymptomatic carriers, leading to economic losses owing to low productivity and poor health [1]. Chlamydiosis is well-studied in a few areas in India, but little is known about its status in other states due to the difficulty associated with handling this fastidious intracellular pathogen [2]. The natural transmission route is thought to be primarily through ingesting or inhaling infected materials, such as when grazing in contaminated pastures [3]. Diagnosing Chlamydia spp. can be done via antigen detection methods like, immunohistochemical staining [4], immunofluorescence and ELISA [5], but they may not identify the specific species or serotype involved. PCR is used for the confirmation of the organism. Conventional PCR protocols for C. abortus DNA detection target the 16S-23S rRNA region of pmp genes [6]. Macrolides, fluoroquinolones, and tetracyclines are used to treat chlamydial infections. Repeated use of tetracyclines could result in antibiotic resistance against them [7]. This study aims at finding out the prevalence of Chlamydiosis in goats in western Maharashtra region and alternative antibiotic therapy for its management.

Materials and Method
A total of 150 blood samples were collected from the Shirwal, Dahiwadi and Baramati regions of western Maharashtra. Samples were collected from the flocks having history of abortion, stillbirths and weak kids. Age, sex and area from where sample is taken were recorded. Each blood sample was divided into EDTA tube and clot-activator tube for serum collection. Blood samples were stored at -4 ºC and serum samples were stored at -18 ºC till further use. Indirect Enzyme Linked Immunosorbent Assay (i-ELISA) test was performed using PrioCHECK^{TM} Ruminant Chlamydia spp. Ab. kit manufactured by Thermo Fischer Scientific using serum samples. All the steps for test were followed according to user manual provided with kit. The iELISA plate was read using plate reader at 450nm absorbance. Anti-chlamydial antibody titre in serum was calculated in each sample using following formula:

$$S/P\% = \frac{(OD_{Sample} - OD_{m\,NC})}{(OD_{m\,PC} - OD_{m\,NC})} \times 100$$

Where,
S/P% stands for standard to positive ratio
OD_{Sample} stands for optical density of Sample
OD_m NC stands optical density of Negative control
OD_m PC stands for optical density of Positive control
The percentage values after using above formula were compared with following table provided in user manual

Table 1: The percentage values after using above formula were compared

S/P value	Interpretation
Titre ≤ 25	Negative
25 < Titre ≤ 35	Positive +
35 < Titre ≤ 60	Positive ++
60 < Titre ≤ 100	Positive +++
Titre > 100	Positive ++++

Samples with values above the 25 were considered positive. Values for positive samples were recorded. Hematological parameters such as hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and DLC (Differential leucocyte count) using automated hematology analyzer i.e. Abacus Junior Vet.5 (Diatron, Hungary) and serum total protein values were calculated using Erba Chem 7 analyser for positive samples. Blood samples of positive animals were tested for presence of Chlamydia spp. DNA using PCR. 16SIGF and 16SIGR primers having sequence 5'TACCTGGTACGCTCAATT3' and 3'ATAATGACTTCGGTTGTTATT5' (Taheri *et al.* 2021) [8] respectively were used to target 16S-rRNA gene with 436 bp length. Results were compared with standard.
Positive animals were divided into 3 groups for treatment purpose. 18 animals were divided into 3 groups having 6 animals each. Each group was treated with different antibiotic for 10 days. Group I was treated with Oxytetracycline @ 10 mg/kg BW i/v, Group II with Tylosin @ 10mg/kg BW i/m, and Group III with Meropenem @ 10mg/kg i/v. Each group was also given supportive therapy, including rehydration, analgesia, and nutrition.
Serum was collected from treated animals and anti-chlamydial antibody titre was again tested for evaluation of antibiotic efficacy in mitigating titre.

Results and Discussion

Out of 150 goat serum samples tested with iELISA 18 showed positive results with antibody titre which can be considered as positive as per the user manual provided with kit. The seroprevalence of Chlamydiosis in study regions was found 12%. These results are in agreement with Zakaria *et al.* (2020) [9] and Al-Ahmed *et al.* (2020) [10] who found 17.6% and 13.08% seroprevalence in Khorasan Razavi province located in north-eastern Iran and Al-Basra province, Iraq respectively. Shirwal, Baramati and Dahiwadi had seroprevalence of 10%, 8% and 18% respectively. Results are in accordance with Borujeni *et al.* (2019) [11] who found 6.67, 3.33, 5.17, 5, 13.11 and zero percent seroprevalence in Shoushtar, Izeh, Dezful, Hendijan, Ahvaz and Susangerd cities of Khuzestan province. Area had no significant effect on impact on prevalence of disease. Females were more vulnerable to the infection than males showing 12.40% prevalence on the other hand only 9.52% males were found seropositive. Results were in agreement with Esmaeili *et al.* (2015) [12] who found 27.2% females and 11.3% males seropositive for Chlamydiosis. This could be due to females are more likely to come into contact with the bacteria during breeding and the cervix may be more easily exposed to contaminated materials during this process. There may be pregnancy or lactation stress as well. Out of four age groups 1-2 year age group had highest prevalence of Chlamydiosis i.e. 15.38%. Remaining three which were 0-1, 2-3 and >3 had prevalence of 8.69%, 12.50% and 7.40% respectively. These results agreed with Esmaeili *et al.* (2015) [12] who found highest seropositivity in 2 years age group of sheep. The reason maybe they sexually mature at 1-2 years of age and their reproductive system gets well developed for infection to set in. Blood samples of all seropositive animals were used for DNA extraction and used in PCR for detection of 16S-rRNA gene of Chlamydia abortus at 436 bp but none showed the positive results. This may be due to absence of organism in the blood as the bacteria is found in aborted fetal and placental tissues Rodolakis *et al.* (1998) [13]. No significant changes were observed in hematological and serum total protein values. Oxytetracycline (@10mg/kg BW i/v) which is Tetracycline class antibiotic, due to its exaggerative use in treating Chlamydiosis over years, the organism may develop resistance against it. Tylosin (@10mg/kg BW i/m) a macrolide class antibiotic showed good results. Oxytetracycline and Tylosin inhibit growth of chlamydiae and help limit additional placental damage, but they do not completely eliminate the infection or reduce the severity of existing placental damage, so additional abortions and stillbirths are possible (Nietfeld, 2001) [14]. Meropenem (@10mg/kg BW i/v), a carbapenem class antibiotic significantly decreased the antibody titre in infecetd goats so it can be suggested that use of this antibiotic as an alternative.

Table 2: Steps and conditions of thermal cycling for 16SIGF/R primer pair

Primers (Forward and Reverse)	Cycling conditions				
	Initial denaturation	Denaturation	Annealing	Extension	Final extension
16SIGF 16SIGR	95 ºC for 3 minutes	94 ºC for 30 seconds	70 ºC for 30 seconds	72 ºC for 45 seconds	72 ºC for 10 minutes
		Repeated 45 cycles			

Table 3: Treatment groups

Groups	No. of Animals	Treatment given	Duration
I	06	Oxytetracycline @ 10mg/kg BW I/V	10 days
II	06	Tylosin @ 10mg/kg BW I/M	10 days
III	06	Meropenem @ 10mg/kg BW I/V	10 days

Table 4: Overall prevalence of Chlamydiosis in all regions

Total No. of samples	No. of Positive samples	No. of Negative samples	Prevalence (%)
150	18	132	12

Table 5: Region-wise prevalence of Chlamydiosis

Areas	Total no of samples (n=150)	No. of Positive Samples (n=18)	Percent positivity (%)
Baramati	50	4	8
Dahiwadi	50	9	18
Shirwal	50	5	10

Table 6: Sex-wise prevalence of Chlamydiosis

Sex	Total No. of samples	No. of positive samples	Percent positivity
Female (does)	129	16	12.40%
Male (bucks)	21	2	9.52%

Table 7: Age-wise prevalence of Chlamydiosis

Age group	Total No. of Samples (n=150)	No. of Positive samples (n=18)	% Positivity
0-1	23	2	8.69%
1-2	52	8	15.38%
2-3	48	6	12.50%
>3	27	2	7.40%

Table 8: Haematological and serum total protein values of seropositive animals (Mean ± S.E.)

		Mean ± S.E. value	Reference value
Hb (g/dl)		8.57 ± 0.41	8-12
TEC (x 10^6 /µl)		15.56 ± 0.78	8-18
PCV (%)		23.13 ± 2.41	22-38
TLC (x 10^3/µl)		16.51 ± 1.92	4-13
DLC (%)	Neutrophils	52.55 ± 3.86	30-48
	Eosinophils	1.00 ± 0.42	1-8
	Lymphocytes	45.05 ± 3.97	50-70
	Monocytes	1.66 ± 0.34	0-4
Serum Total protein (gm/dl)		6.26 ± 0.43	6.4-7

Table 9: Pre and Post treatment antibody titres in seropositive animals

Groups	Antibiotics used	No. of animals	S/P value (Mean ± S.E.)		'p' value
			Before	After	
I	Oxytetracycline	6	45.66^{a} ± 3.56	33.33 ± 2.18	0.005**
II	Tylosin	6	72.66^{b} ± 10.16	48.50 ± 4.59	0.017*
III	Meropenem	6	56.33^{ab} ± 6.14	47.00 ± 6.60	0.001**
F value			3.610*	3.012^{NS}	

*Means bearing different superscripts within the column differed significantly.

*Significant at $p<0.05$, * = significant, ** = Highly significant

Table 10: PCR results

No. of samples tested with PCR	No. of positive samples with PCR
18	0

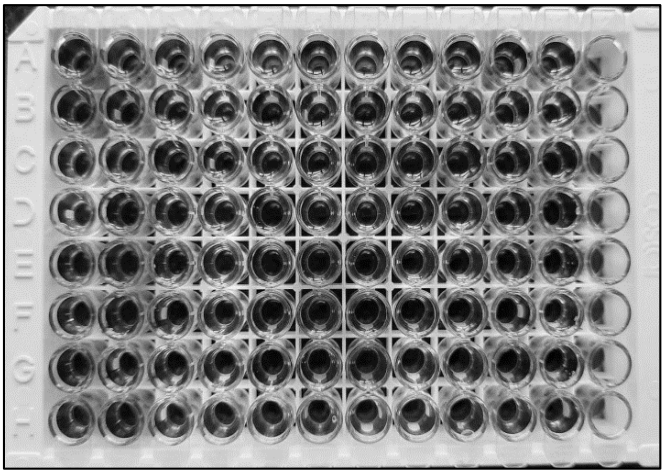

Fig 1: i-ELISA reaction plate showing IgG antibodies against Chlamydia abortus. Intesity of yellow color and titre of antibodies is directly proportional. Well A1 and B1 are positive control. Well C1 and D1 are negative control.

Fig 2: Lane L: 50 bp ladder, Lane P: Positive control (*Chlamydia abortus*), Lane N: Negative control, Lane 1-10: Blood DNA isolates

Conclusion

It can be concluded that sex, age and area has no significant impact on occurrence of the disease. Organism cannot be detected using blood samples. Meropenem @ 10mg/kg BW i/v is more efficient than Oxytetracycline @ 10mg/kg BW i/v and Tylosin @ 10mg/kg BW in mitigating the antibody titre.

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