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Case-control study to identify the causative agents of ophthalmia and conjunctivitis in goats in Savannakhet province of Lao PDR

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ABSTRACT

Pinkeye is a highly contagious disease of goats with different aetiologies. Surveys in Lao PDR have identified eye lesions typical of pinkeye as a common condition, however, this has not been confirmed diagnostically, and the responsible pathogens have not been identified. A matched case-control study was implemented in 70 goat holdings from Savannakhet province, Lao PDR, to detect agents causing pinkeye and conduct phylogenetic analysis of the identified pathogens. Fifty eye swabs from goats with infected eyes (cases) and 50 paired samples from unaffected cohorts (controls) were collected from 25 holdings. Samples were tested using quantitative PCR assays targeting known pinkeye pathogens at the genus and species levels. The prevalence of pathogens in case and control goats was as follows: *Mycoplasma conjunctivae* (94% and 74% respectively, $P = 0.006$, OR = 5.5), *Chlamydia pecorum* (4%, 10%), *Moraxella ovis* (30%, 30%), *Moraxella bovis* (0%, 0%) and *Moraxella bovoculi* (0%, 0%). *M. conjunctivae* was present in a high proportion of goats in both groups revealing that Lao goats are carriers of *M. conjunctivae*. However, the mean $\log_{10}$ genome copy number/μL of DNA extract was significantly higher in case goats than control goats ($P < 0.05$). Thus, *M. conjunctivae* is likely the principal causative agent of pinkeye in Lao goats with carrier status converting to clinical infection following corneal damage or other causative factors. *M. conjunctivae* detected in samples from different goats and districts showed low genetic diversity. Identifying the causes of pinkeye in Lao goats will assist in designing appropriate treatment and control strategies.

1. Introduction

Lao People's Democratic Republic (Lao PDR/Lao) is a landlocked Southeast Asian country bordering China, Vietnam, Cambodia, Thailand and Myanmar. The goat industry is a smaller livestock sector but one that is rapidly growing due to demand for goat meat from neighbouring Vietnam (Burns et al., 2018). Disease has been identified as a major constraint on goat production in Lao PDR (Gray et al., 2019; Olmo et al., 2022) with several major endemic syndromes reported by farmers as the most prevalent and important. These include pinkeye-like eye infections, orf-like mouth and foot lesions, diarrhoea and lameness (Jayasekara et al., 2023; Phengvilaysouk et al., 2022; Windsor et al., 2017). The cause(s) of these syndromes are not clear and the existing diagnostic capacity in Lao PDR is insufficient to address disease causation in goats, hindering the development of effective control and treatment. As part of a multidisciplinary project on goat production and marketing in Lao PDR and Vietnam (ACIAR project LS/2017/034), studies were undertaken to investigate the causation of several of these syndromes. This paper reports the investigation into the causation of pinkeye-like eye lesions in Lao goats.

Eye lesions in animals can be infectious or non-infectious in origin. The most common infectious ocular disease type is infectious keratoconjunctivitis, known commonly as pinkeye. Pinkeye occurs in cattle, sheep and goats; however, causative pathogens vary between cattle and sheep/goats. In cattle, the disease is also known as infectious bovine keratoconjunctivitis and *Moraxella bovis* is the responsible pathogen (Constable et al., 2017). In goats, the disease is known as infectious keratoconjunctivitis while *Mycoplasma conjunctivae* (Constable et al., 2017) and *Chlamydia pecorum* (Nietfeld, 2001) are considered as the major causative agents. Further, other pathogens such as *Moraxella ovis*

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and *Mycoplasma agalactiae* can also cause eye infections in goats (Underwood et al., 2015).

There is no information available on prevalence of pinkeye causing pathogens in Lao goats. The only information available from a previous survey is that 11.9% (8/67) of goat farmers from Savannakhet province have reported the occurrence of sore eyes in their goats (Phengvilaysouk et al., 2022). Therefore, a study was designed with an overall aim of determining the most common causes of eye infection in Lao goats using molecular diagnostic methods and to characterize detected pathogens phylogenetically. The scope of the study included 5 likely causes of pinkeye in goats namely *M. conjunctivae*, *C. pecorum*, *Mor. ovis, Mor. bovis* and *Moraxella bovoculi*. The study was restricted to these probable pathogens in line with the resources available. The presence of other *Mycoplasma* and *Chlamydia* strains was tested with the use of genus specific assays, although *M. conjunctivae* and *C. pecorum* would be the most likely species detected. The hypothesis under test was that eye infection in Lao goats is caused predominantly by *M. conjunctivae* and *C. pecorum* alone or in combination.

2. Materials and methods

The study was approved by the Animal Ethics Committee of the

Fig. 1. Examples of clinical signs showed at the time of sample collection; a) lacrimation b) partially closed eye with lacrimation c) mucopurulent ocular discharge d) conjunctival hyperaemia, cloudy cornea and mucopurulent ocular discharge e) corneal opacity with lacrimation and ocular discharge f) opaque cornea with purulent ocular discharge (Photos courtesy of Thaixiong Xaikhue).

University of New England, Australia (approval number: AEC20–006).

2.1. Selection of goat holdings for inclusion in the study

Smallholder goat holdings (N = 70) were selected from 7 villages (Na Po, Nhon Nhang, Xa Loi, Nhonsomphou, Songkhone, Sebanghiang and Kanglouang) of 3 districts (Phin, Songkhone and Sepon) of Savannakhet province in which ACIAR project LS/2017/034 was operating. Savannakhet province is the province with the highest goat population in Lao PDR.

2.2. Study design and sampling

Power analysis determined that a minimum of 40 paired samples was required for a case-control study where each case (goats with infected eyes) was matched by a control (unaffected flock mate) with power of the test of 0.8 to detect differences in between cases and controls at $P = 0.05$, when the predicted proportion of positive samples in the controls is 0.1 and hypothesised odds ratio for presence of risk factor in cases vs control is 5 (Thrusfield, 2007). To further increase the statistical power of the case-control comparison, 50 matched samples were used for this study.

The case-control study commenced in January 2022 and was completed in June 2022 when 50 paired samples had been collected (N = 100). Sample collection was by a project team of Lao scientists who visited each of the project goat holdings monthly, during which a range of project activities was conducted. The sample and survey data were recorded using the CommCare® mobile acquired data platform (Dimagi Inc., US) and uploaded real-time to a server.

Cases were identified as goats exhibiting any of the following clinical signs namely; inflammation/reddening of the eye, ocular discharge, excessive lacrimation, corneal opacity or corneal ulceration. Examples of goats with a range of clinical signs in eyes at the time of sample collection are shown in Fig. 1. From each case, a conjunctival swab sample was taken from the inner part of the lower eyelid of affected eyes using a commercially available sterile polypropylene swab with virus transport medium supplied from Pacific Laboratory Products, Australia. Similarly, a conjunctival swab was also collected from a matched healthy goat of the same breed, close in age to the case and from the same or adjacent herd. Samples were held in transport medium in an ice-box containing ice/ice bricks until transfer to a refrigerator at 4℃ until final storage up to 7 – 10 days later at −80℃ at National Animal Health Laboratory (NAHL) in Vientiane. The number and source of samples collected is summarised in Supplementary Table 1. Briefly the 50 paired samples were collected from 25 holdings in 7 different villages in 3 districts of Savannakhet province as described above.

2.3. DNA extraction

Total genomic DNA was extracted from eye swabs at the NAHL, Vientiane, Lao PDR using the Isolate II genomic DNA kit (Bioline, UK), according to the manufacturer's protocol with minor modification. A process control was included in all extractions to control for cross-contamination between samples during this step. Extracted DNA samples were air-freighted on dry ice to the Elizabeth Macarthur Agricultural Institute (EMAI), Menangle, NSW, Australia where they were stored at −80°C until PCRs were carried out.

2.4. PCR and gel electrophoresis

All PCR and electrophoresis work was undertaken at EMAI in Australia. Quantitative real time probe-based PCR assays were used to screen samples to obtain prevalence data. Conventional PCR assays and Sanger sequencing were used to characterise pathogens in positive samples. In-house diagnostic assays (from EMAI) and previously published assays were used. Details of primers and probes used are provided in Supplementary Table 2. An appropriate positive and negative control as well as, the DNA extraction process control were included in each PCR assay. All previously published assays were validated and optimised before being used to test actual samples.

Initially all DNA samples (N = 100) were screened with probe-based PCR assays. A Ct value $\leq$ 40 with a clear amplification plot was considered as a positive PCR result. A representative set of positive DNA samples with Ct value < 30 for the pathogen specific quantitative assays were used in pathogen specific conventional PCRs for sequencing and phylogenetic analysis. On this basis 32 and 16 samples were tested with genus *Mycoplasma* and genus *Chlamydia* specific conventional assays, respectively. *Mor. ovis* conventional PCR was performed on all DNA samples.

The cycling conditions for all probe-based PCR assays were as follows: initial denaturation cycle at 95°C for 10 min, followed by 45 cycles of 15 s at 95°C and 1 min at 60°C. TaqMan® Environmental Master Mix (Applied biosystems, UK) was used for all probe-based PCR reactions and were performed on a QuantStudio™ PCR system (Thermo Fisher Scientific, US) and the fluorescence data obtained was analysed using QuantStudio™ Design and Analysis Desktop Software (Version 1.5.2; Thermo Fisher Scientific, US). MyTaq™ Red Mix (Meridian Bioscience, US) was used for all conventional PCR assays. The cycling conditions for the conventional PCR assays were as follows: initial denaturation at 95°C for 1 min, 30 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 15 s for primer pairs Chuni1F/Chuni2R and ISRdownF/ISRupR; and 55°C for 15 s for primer pairs 16SF2/23 R and GPO1F/MGSOR, extension at 72°C for 10 s and final extension at 72°C for 1 min.

Target gene copy (GC) numbers for the different pathogens were also calculated based on an internal standard curve comprising 4 × 10-fold dilutions in duplicate of known target sequence concentrations derived from plasmid DNA.

2.5. DNA sequencing and phylogeny

Electrophoresis of conventional PCR products was carried out in 2% agarose gel (Bioline, UK) using 1X TBE buffer and GelRed® (Biotium, US) to confirm the presence of specific bands. The DNA bands were visualized with the BioRad gel documentation system and analysed with Image Lab Software (Version 6.1; BioRad, US). Amplicons showing a clear and specific band were sequenced bidirectionally with the Sanger method. Chromatograms were visualized and aligned and consensus sequences were prepared using Geneious Prime Software (Version 2022.2.2; Biomatters, US). Consensus sequences were compared with the GenBank database using BLASTN to identify the pathogen species. For phylogenetic analysis, consensus sequences were aligned with homologous reference sequences from GenBank using Geneious Prime and phylogenetic trees were reconstructed with MEGA version 11 (Tamura et al., 2021) using the Maximum Likelihood method and 100 bootstrap replications. Finally, the obtained DNA sequences were submitted to GenBank.

2.6. Statistical analysis

All samples were assayed in duplicate for intra-run repeatability and mean values were used. The prevalence of pathogens in goat herds was calculated based on results from probe-based PCR assays. Data analysis and visualization were performed with JMP® 17.0.0 (SAS Institute, US) and MS Excel. GC values were then $\log_{10}$ transformed ($\log_{10}$ GC + 1) prior to statistical analysis. Comparison of prevalence of infection between the two groups was by Pearson Chi square analysis where $P < 0.05$ was indicated significant differences, and odds ratio for presence of the organism in the case group tested as described by (Thrusfield, 2007) for matched case-control studies. Comparison of mean $\log_{10}$ GC numbers between case and control goats was by two sample T test.

3. Results

3.1. Clinical signs, age and sex distribution

The main clinical sign in the sampled cases was eye discharge (45/50), however, the type of eye discharge for individual cases was not recorded. Only three goats had completely opaque cornea and none had corneal perforation, haemorrhage or visible entropion. Only five goats exhibited more than one clinical sign and five goats were also suspected of being completely blind in the affected eye. All of those samples were from native Lao goats. The majority of cases were from the age group of 7 – 12 months old (56%), 38% were aged 1 – 6 months old and only 6% were aged over 12 months of age. Regarding sex, 74% of cases were females and 26% were males.

3.2. Pathogen detection and prevalence in samples

None of the negative or DNA extraction controls amplified in the PCR assays indicating no cross-contamination of samples during DNA extraction and PCR assay. Genus *Mycoplasma*, genus *Chlamydia*, *M. conjunctivae*, *C. pecorum* and *Mor. ovis* were identified when screening all samples with probe-based quantitative PCR assays. None of the samples were positive for *Mor. bovis* or *Mor. bovoculi*. *Mycoplasma* spp. (from generic PCR) and *M. conjunctivae* were the most common organisms detected and were detected in both case and control samples. A considerable number of samples from both groups were also positive for *Mor. ovis*, whereas very low numbers of samples were positive for *Chlamydia* spp. and *C. pecorum*. Cases had a significantly higher prevalence of detection than controls in the case of *Mycoplasma* spp. and *M. conjunctivae* with odds ratios > 5 (Table 1). Differences between case and control prevalence for all the other pathogens tested were not significant.

3.3. Pathogen levels in case and control samples

In goats positive for genus *Mycoplasma*, the $\log_{10}$ GC was significantly higher in case than control goats ($P = 0.0001$) being 28-fold higher on the untransformed scale (Table 2). Similarly, the $\log_{10}$ GC value of *M. conjunctivae* was significantly higher in positive case than positive control goats ($P = 0.0001$) being 20-fold higher on the untransformed scale (Table 2). However, the $\log_{10}$ GC values of *Mor. ovis*, genus *Chlamydia* and *C. pecorum* were not significantly different between case and control groups ($P > 0.05$) (Table 2). Dot plots showing the variation in $\log_{10}$ GC between individual samples are shown in Fig. 2.

Most goats (65%) had only a single pathogen identified in the eye swab (Supplementary Fig. 1) with this being either *M. conjunctivae* or *Mor. ovis*. A smaller number (25%) had two pathogens detected with 3 combinations observed; *M. conjunctivae* and *Mor. ovis, M. conjunctivae* and *C. pecorum* or *Mor. ovis* and *C. pecorum*. Only 2% of samples had 3 pathogens detected in them.

Table 1
Pathogen prevalence and odds ratios based on 50 case and 50 control samples.

Pathogen	Prevalence (%)				Odds ratio	
	Control	Case	Overall	*P*	OR	95% CI
G. *Mycoplasma*	84	100	92	0.0032	Infinity	-
M. conjunctivae	74	94	84	0.0064	5.5	1.46 – 20.76
Mor. ovis	30	30	30	1.0000	1	0.43 – 2.35
G. *Chlamydia*	4	12	8	0.1404	3.3	0.63 – 17.07
C. pecorum	10	4	7	0.2397	0.4	0.07 – 2.03
Mor. bovis	0	0	0	-	1	-
Mor. bovoculi	0	0	0	-	1	-

P = Probability, OR = Odds ratio, CI = Confidence interval

Table 2
Mean $\log_{10}$ GC values in eye swab samples that were positive for the presence of the listed pathogens.

Pathogen	Mean ± SE $\log_{10}$ GC/μL		95% CI		*P*
	Control	Case	Control	Case	
G. *Mycoplasma*	3.52 ± 0.27	4.96 ± 0.24	2.99 – 4.04	4.48 – 5.45	0.0001
M. conjunctivae	3.02 ± 0.24	4.32 ± 0.21	2.54 – 3.50	3.89 – 4.75	0.0001
Mor. ovis	3.47 ± 0.31	4.08 ± 0.31	2.84 – 4.10	3.45 – 4.71	0.17
G. *Chlamydia*	1.62 ± 0.47	0.67 ± 0.27	0.47 – 2.78	0.01 – 1.34	0.13
C. pecorum	0.99 ± 0.42	0.97 ± 0.66	−0.08 – 2.07	−0.73 – 2.67	0.97

SE = Standard error, GC = Gene copy number, CI = Confidence interval, *P* = Probability

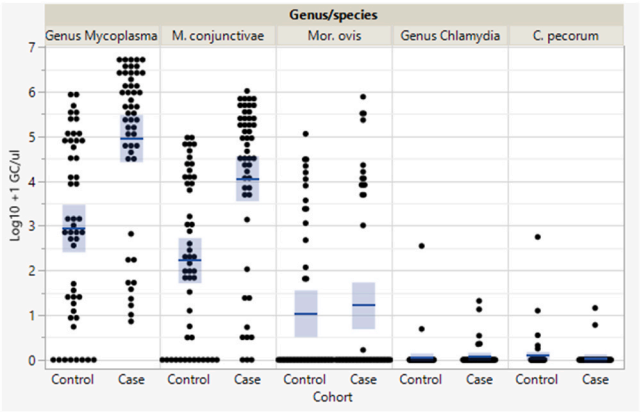

Fig. 2. Dot plots showing the range of $\log_{10}$ gene copy numbers of different pathogens in individual eye swab samples from case and control goats including all samples. Means are indicated by the horizontal blue lines.

The pathogen species with the highest $\log_{10}$ GC value in each goat was considered the dominant bacterial species in that goat eye. Based on this, the dominant pathogen in eyes of case goats were *M. conjunctivae* (82%), *Mor. ovis* (12%) and *C. pecorum* (2%). Only 4% of case goats were negative for each of these pathogens; however, these samples were positive for genus *Mycoplasma*. The dominant pathogen species in the eyes of control goats were *M. conjunctivae* (66%) and *Mor. ovis* (22%). *C. pecorum* was not identified as the dominant species in any control goats. None of the tested pathogens were detected in 12% of goats from the control group.

3.4. Sequence data analysis and phylogenesis

Consensus sequence data were obtained for 26 PCR products from the *Mycoplasma* genus conventional assay targeting 16 S rRNA region. Nucleotide blast results indicated that all of them were *M. conjunctivae* (Query cover = 99 – 100% and % identity = 99.54 – 100%). Nucleotide sequences of *M. conjunctivae* were deposited in GenBank (GenBank accession no. OR018861 to OR018886). Those sequences were used in phylogenetic analysis (Fig. 3) together with the 7 available reference sequences in NCBI GenBank. A total of 21 of the 26 sequences generated in this study were 100% identical to each other and to 3 of the reference sequences from China (OQ983594), India (OM140684) and the United States (Type strain HRC/583; NR_074135). Those 21 sequences were 100% identical to the mentioned reference sequences but slightly different to sequences from Iraq (at 4 bases), Taiwan (at 1 base) and another sequence from India (at 1 base). Cluster A comprised 5 isolates from this study (marked in blue), all of which were isolated from

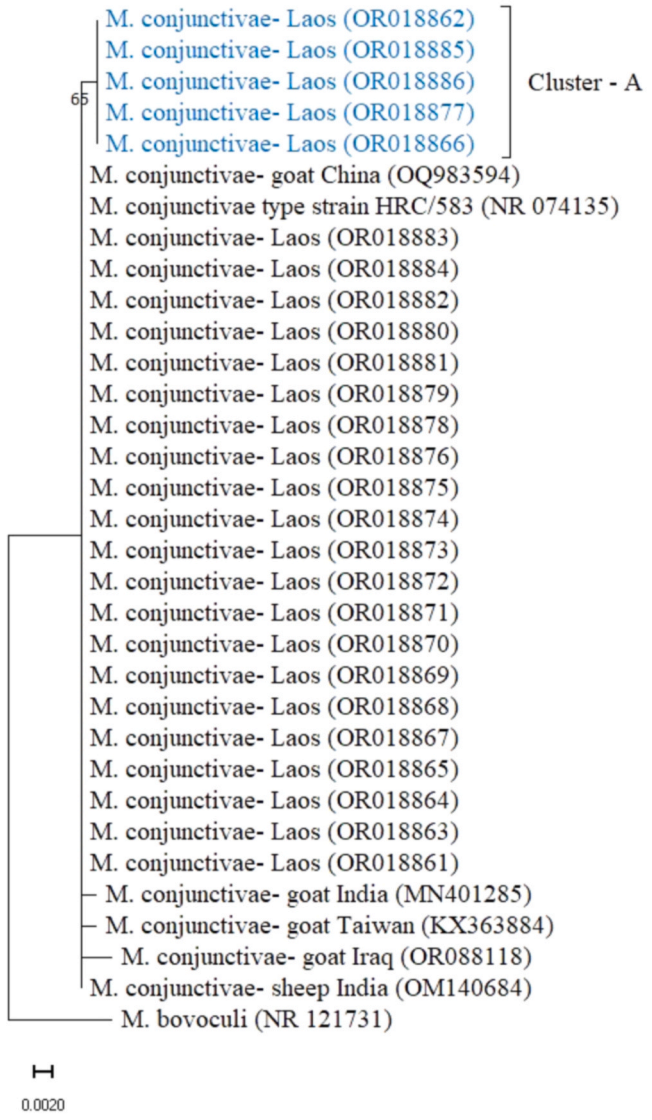

Fig. 3. Phylogenetic tree based on 715 bp consensus sequences of 16 S rRNA gene of 26 *M. conjunctivae* and 7 reference sequences from NCBI GenBank.

Songkhone village, across 5 different holdings. Sequences in cluster A did not have an identical match in the NCBI database and contained a single nucleotide substitution ("A" to "C") at position 562 relative to the type strain of *M. conjunctivae* strain HRC/583.

Only 2 good quality sequences were obtained from *Mor. ovis* specific conventional PCR. NCBI nucleotide blast results identified both of these sequences as *Mor. ovis* with 99.81 and 98.77% identity respectively. Nucleotide sequences of *Mor. ovis* were deposited in GenBank (GenBank accession no. OR088209 and OR088210).

3.5. Treatment

After sample collection, affected goats were treated with 3 different types of eye drops available in Lao PDR in order to prevent further spread of the eye disease within the herd until a definitive diagnosis could be obtained and specific treatment implemented. The 3 eye drop solutions contained antibiotic Neomycin alone, Neomycin plus Dexamethasone or Antazoline (an antihistamine) respectively. The eye drops containing neomycin solutions were reported subjectively by the field veterinarian on the study to provide good efficacy in reducing clinical signs while Antazoline was ineffective.

4. Discussion

All the goats in the case group showed typical clinical signs of pinkeye with the majority (94%) belonging to the < 12 months old age group. This likely reflects a lower level of acquired immunity to infection compared to older goats > 12 months old. More female goats (74%) were found with pinkeye than male goats (26%), likely reflecting the greater number of female goats in the overall population.

With regard to the hypothesis that eye infection in Lao goats is caused predominantly by *M. conjunctivae* and *C. pecorum,* the results of the case-control study provide strong support for the former, but not the latter organism. Both the prevalence of detection of *M. conjunctivae* and the GC level were significantly greater in case than control goats and the odds ratio for presence in case goats was 5.5, strongly indicative of a role in causation. However, it was also the predominant pathogen identified in eye swabs from control goats indicating that healthy goats could be carriers of *M. conjunctivae*. Carrier status of *M. conjunctivae* in the eyes of goats has been previously documented (Smith and Sherman, 2009). Similarly, healthy carriers of *M. conjunctivae* have been reported in goats from Pakistan (Fernández-Aguilar et al., 2017) and China (Yang et al., 2022) and in sheep (Fernández-Aguilar et al., 2013; Janovsky et al., 2001). However, the high odds ratio for presence of *M. conjunctivae* (5.5) in case goats together with its significantly higher GC level in case goats ($P = 0.0001$) indicates a strong association between the eye infection and *M. conjunctivae*. This organism was also identified as the dominant pathogen in 82% of the case animals meaning that it was the organism with the highest GC level among the pathogens tested. Based on these results, *M. conjunctivae* can be considered as the principal cause of eye infection in Lao goats and the syndrome can be definitively diagnosed as pinkeye. The triggers that cause multiplication of *M. conjunctivae* and progression from carrier to clinical status was not investigated in this study.

M. conjunctivae is considered the principal pathogen responsible for pinkeye in goats (Constable et al., 2017). It is the most frequently identified eye pathogen of goats (Chao et al., 2018; Yang et al., 2022) and sheep (Janovsky et al., 2001; Naglić et al., 2000). To the authors' knowledge, there are no studies assessing the risk factors for pinkeye caused by *M. conjunctivae* in goats. However, there are some reports on risk assessments of pinkeye in cattle.

The identified risk factors were ocular trauma from straw, chaff, sand, fights, barbed wire, thorns, hay or dry grass stalks, environmental factors such as low rainfall, direct sunlight, the presence of flies and the location of the farm and the grazing land, and host factors such as animal age and herd size (Alexander, 2010; Kneipp et al., 2021; Lane et al., 2015). In the case of sheep, mixed grazing and introduction of sub clinically infected sheep to existing flocks have been considered as risk factors for infection (Janovsky et al., 2001). As with cattle, flies have been recorded as a possible source of infection in sheep including the possible introduction of secondary bacterial agents, thereby provoking clinical manifestation of eye infection with *M. conjunctivae* (Degiorgis et al., 1999).

Mor. ovis and *C. pecorum* were also identified in Lao goats but a role in clinical pinkeye was not shown in the present study. *Mor. ovis* was detected in 30% of samples but at the same level in both case and control goats with an odds ratio of 1 indicating a lack of association with clinical pinkeye. The level of infection detected also did not differ between infected case and control goats. The role of *Mor. ovis* in eye infection in goats is not yet well established as *Mor. ovis* has been also detected in the eyes of healthy goats (Abdullah et al., 2015). *Mor. bovis* and *Mor. bovoculi* were not detected in any samples in the present study although *Mor. bovoculi* has been identified as an opportunistic eye pathogen of goats (Meekins et al., 2017). *Mor. bovis* has also been found in goats but more rarely compared to cattle (Ojo et al., 2009). *C. pecorum* was only detected in 7% of goats overall, with no difference in prevalence or level of infection between control and case goats. It was the dominant pathogen in only a single goat in the case group. *C. pecorum* has been

identified from eyes of apparently healthy goats (Fernández-Aguilar et al., 2017) as well as from sheep with and without clinical conjunctivitis (Jelocnik et al., 2019).

In general, goats of all ages are susceptible to pinkeye and recurrent infections are common (Underwood et al., 2015). The disease is transmitted directly by carrier animals or infected goats whereas indirect transmission by flies, dust particles and contaminated objects is also possible. Moreover, disease prevalence is high in warm, dry and dusty environments. Flies, concurrent diseases, malnutrition, stress, adverse weather and overcrowding also predispose animals to the infection. Morbidity is typically around 10 – 15% but can be as high as 80% leading to growth retardation and ill-thrift due to poor feed intake (Constable et al., 2017). *M. conjunctivae* is transmitted by direct contact between animals (Underwood et al., 2015) and mild infections resolve in around 10 days where severe infections may persist for up to 12 weeks (Smith and Sherman, 2009).

Whether the introduction of these diagnostic methods to the NAHL in Lao PDR is appropriate or not is debatable given the presence of higher priority farm animal species and diseases and resource constraints at the laboratory. PCR is a widely used diagnostic technique with many advantages. In the present study, probe-based assays were used to identify the probable pathogens in all samples due to their sensitivity and ability to quantify the target. The potential to use the existing probe assays to develop a multiplex assay detecting *M. conjunctivae*, *C. pecorum* and *Mor. ovis* will be an added advantage given that these assays are compatible together.

DNA sequencing of *Mycoplasma* spp. PCR products only identified *M. conjunctivae.* This suggests that there were no mixed infections with multiple species or strains of *Mycoplasma*. However, phylogenetic analysis clearly identified that the *M. conjunctivae* detected belonged to two closely related clusters with geographical clustering within the sampling area. Cluster A comprised of 5 isolates that had a consistent "A" to "C" mutation in these sequences at 562 bp position relative to the reference strains. This may be a novel strain of *M. conjunctivae*. Interestingly, all these came from different herds within one village. *M. conjunctivae* detected in samples from different goats and districts exhibited low genetic diversity providing evidence for herd to herd transmission within the study area. This is not surprising given the free ranging, village-based husbandry goat raising practices used in the study area. Phylogenetic relationships with *M. conjunctivae* from Vietnamese and Thai goats with pinkeye could not be determined due to the unavailability of sequence data at NCBI GenBank.

The present study had certain limitations. Firstly, the clinical signs of case goats were not recorded in detail. Such detail would have been useful to characterise goats based on the severity of clinical signs or type of discharge and to determine relationships between these and the presence or level of the causative pathogens. Secondly, more of the possible farm-level risk factors were not recorded at the time of sample collection due to resource constraints and survey fatigue by the farmers. Thirdly, the efficacy of the eye drops available in Lao PDR was determined on a subjective rather than an objective basis. A more formal evaluation would have more scientifically based guidelines on treatment options in Lao PDR.

5. Conclusion

Eye infection in goats is a common problem in Lao PDR. This study has confirmed that this syndrome is pinkeye (contagious ophthalmia) and that *M. conjunctivae* as the principal causative pathogen. Risk factors for clinical pinkeye caused by *M. conjunctivae* infection have not been determined in Lao goats but are likely to be similar to those documented elsewhere. Subjective field observations from the present study indicate that pinkeye in Lao goats can be effectively treated with readily available eye drops containing Neomycin but not those containing an antihistamine. Such treatment is practical in Lao husbandry systems where small numbers of goats come into a goat house for containment every night, making repeat application possible without undue disturbance to normal management.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetmic.2024.110195.

References

Abdullah, F.F.J., Naidu, N.R.G., Sadiq, M.A., Abba, Y., Tijjani, A., Mohammed, K., Chung, E.L.T., Norsidin, M.J.M., Lila, M.A.M., Haron, A.W., Saharee, A.A., Omar, A. R., 2015. Prevalence of *Moraxella ovis* Infection in goats under the Ladang Angkat Programme. Universiti Putra Malaysia: A cross-sectional study. IOSR-JAVS 8, 99–102.

Alexander, D., 2010. Infectious bovine keratoconjunctivitis: A review of cases in clinical practice. Vet. Clin. North Am.: Food Anim. Pract. 26, 487–503.

Burns, R.J.L., Douangngeun, B., Theppangna, W., Khounsy, S., Mukaka, M., Selleck, P. W., Hansson, E., Wegner, M.D., Windsor, P.A., Blacksell, S.D., 2018. Serosurveillance of coxiellosis (Q-fever) and brucellosis in goats in selected provinces of Lao People's Democratic Republic. PLoS Negl. Trop. Dis. 12, e0006411.

Chao, W.-H., Lai, J.-M., He, X.-X., Lin, Y.-H., Chang, G.-R., 2018. Case Report: an infectious keratoconjunctivitis outbreak caused by *Mycoplasma Conjunctivae* in a dairy goat farm. Taiwan Vet. J. 44, 33–39.

Constable, P.D., Hinchcliff, K.W., Done, S.H., Grünberg, W. 2017. Diseases of the skin, eye, conjunctiva, and external ear, In: Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Ltd, 3251 Riverport Lane, St. Louis, Missouri 63043, US, 1540-1661.

Degiorgis, M.-P., Obrecht, E., Ryser, A., Giacometti, M., 1999. The possible role of eye-frequenting flies in the transmission of *Mycoplasma conjunctivae*. J. SES 72, 189–194.

Fernández-Aguilar, X., Cabezón, Ó., Marco, I., Mentaberre, G., Frey, J., Lavín, S., López-Olvera, J.R., 2013. *Mycoplasma conjunctivae* in domestic small ruminants from high mountain habitats in Northern Spain. BMC Vet. Res. 9, 253.

Fernández-Aguilar, X., Rossi, L., Cabezón, Ó., Giorgino, A., Victoriano, L., I., Frey, J., López-Olvera, J.R., 2017. Infectious keratoconjunctivitis and occurrence of *Mycoplasma conjunctivae* and Chlamydiaceae in small domestic ruminants from Central Karakoram, Pakistan. Vet. Rec. 181, 237.

Gray, D., Walkden-Brown, S., Phengsavanh, P., Patrick, I., Hergenhan, R., Hoang, N., Phengvilaysouk, A., Carnegie, M., Millar, J., Hữu Văn, N. 2019. Final Report-Assessing goat production and marketing systems in Laos and market linkages into Vietnam. ACIAR Project No: LPS/2016/027 (Australian Centre for International Agricultural Research, Canberra), 180.

Janovsky, M., Frey, J., Nicolet, J., Belloy, L., Goldschmidt-Clermont, E., Giacometti, M., 2001. *Mycoplasma conjunctivae* infection is self-maintained in the Swiss domestic sheep population. Vet. Microbiol. 83, 11–22.

Jayasekara, P.P., Jenkins, C., Gerber, P.F., Olmo, L., Xaikhue, T., Theppangna, W., Walkden-Brown, S.W. 2023. Causation of pinkeye in goats from Savannakhet

province, Lao PDR. In: 9th International Conference on Sustainable Animal Agriculture for Developing Countries, Vientiane, Lao PDR, 21-24 November, 67.
Jelocnik, M., Laurence, M., Murdoch, F.R., Polkinghorne, A., 2019. Detection of *Chlamydiaceae* in ocular swabs from Australian pre-export feedlot sheep. Aust. Vet. J. 97, 401–403.
Kneipp, M., Green, A.C., Govendir, M., Laurence, M., Dhand, N.K., 2021. Risk factors associated with pinkeye in Australian cattle. Prev. Vet. Med. 194, 105432.
Lane, J., Jubb, T., Shephard, R., Webb-Ware, J., Fordyce, G., 2015. Final Report-Priority list of endemic diseases for the red meat industries. Project code: B.AHE.0010 (Meat & Livestock Australia Limited. North Sydney NSW 2059, 282.
Meekins, J.M., Apley, M.D., Lubbers, B., Peddireddi, L., Rankin, A.J., 2017. Evaluation of conjunctival bacterial flora in a herd of goats in the Midwestern United States. Vet. Ophthalmol. 20, 40–45.
Naglić, T., Hajsig, D., Frey, J., Šeol, B., Busch, K., Lojkić, M., 2000. Epidemiological and microbiological study of an outbreak of infectious keratoconjunctivitis in sheep. Vet. Rec. 147, 72–75.
Nietfeld, J.C., 2001. Chlamydial infections in small ruminants. Vet. Clin. North Am.: Food Anim. Pract. 17, 301–314.
Ojo, O.E., Oluwole, O.A., Adetosoye, A.I., 2009. Isolation of *Moraxella bovis* from infectious keratoconjunctivitis in a flock of goats. Niger. Vet. J. 30, 56–59.
Olmo, L., Phengvilaysouk, A., Colvin, A.F., Phengsavanh, P., Millar, J., Walkden-Brown, S.W. 2022. Improving Lao goat production when resources are limited. In: 34th Biennial Conference of the Australian Association of Animal Sciences (AAAS), Cairns, Queensland, Australia, 5-7 July, cvi.
Phengvilaysouk, A., Colvin, A.F., Olmo, L., Phengsavanh, P., Millar, J., Walkden-Brown, S., 2022. Smallholder goat herd production characteristics and constraints in Lao PDR. Lao J. Agric. For. 46, 1–17.
Smith, M.C., Sherman, D.M. 2009. Goat Medicine (US, Willey-Blackwell), 870.
Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol. Biol. Evol. 38, 3022–3027.
Thrusfield, M. 2007. Veterinary Epidemiology (UK, Blackwell Science Ltd, a Blackwell Publishing company), 626.
Underwood, W.J., Blauwiekel, R., Delano, M.L., Gillesby, R., Mischler, S.A., Schoell, A., 2015. Biology and Diseases of Ruminants (Sheep, Goats, and Cattle). In: Fox, J.G., Anderson, L.C., Otto, G., Pritchett-Corning, K.R., Whary, M.T. (Eds.), Laboratory Animal Medicine. Academic Press, US, pp. 623–694.
Windsor, P.A., Nampanya, S., Tagger, A., Keonam, K., Gerasimova, M., Putthana, V., Bush, R.D., Khounsy, S., 2017. Is orf infection a risk to expanding goat production in developing countries? A study from Lao PDR. Small Rumin. Res. 154, 123–128.
Yang, Y., Li, Y., Dao, X., Fei, L., Yang, F., 2022. Molecular detection and isolation of *Mycoplasma conjunctivae* from goats with infectious keratoconjunctivitis in Sichuan province, southwest China. Thai J. Vet. Med. 52, 601–606.