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Udder health, bacterial isolation and antimicrobial sensitivity of *Staphylococcus* species from non-dairy goats on smallholder farms in Hong Kong

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Research Article

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Abstract

This research article describes an investigation into the udder health, bacterial isolation and antimicrobial sensitivity of three staphylococcal species isolated from the milk of non-dairy goats, suckling their kids, on two smallholder farms in the Hong Kong Special Administrative Region. Udder lesions were visually noted in 21 of 34 goats and two goats had palpable abnormalities. Collected milk samples grew a total of 11 bacterial organisms and the most frequently isolated organism was *Staphylococcus chromogenes*. Selected isolates of *S. aureus, caprae* and *simulans* from both farms were tested by antimicrobial sensitivity testing for 23 antimicrobials and all isolates showed antimicrobial resistance to doxycycline and tetracycline. Less common resistance was shown to ampicillin, chloramphenicol, penicillin and rifampicin. This preliminary study confirms the presence of udder lesions and mastitis bacteria in non-dairy goats in Hong Kong, along with the first information on the antimicrobial profile of three common *Staphylococcus* species bacteria affecting goats.

Goats are an important livestock species for many countries as they provide essential sources of nutrition and income, particularly in lower socioeconomic nations. However, goat udder health is less well studied than in cattle globally, and there is no data available regarding udder health in goats in the Hong Kong Special Administrative Region. Udder health is a broad term that can refer to external abnormalities, palpable defects, subclinical or clinical mastitis or bacterial infection of the udder (Zeleke *et al*., 2021). In goats, external abnormalities include udder asymmetry, pendulous udder, supernumerary teats, nodules, ulceration and crusting. Palpable abnormalities include lumps and hard udder glands, whilst mastitis is common (Persson *et al*., 2014; Menzies, 2021). Mastitis occurs due to mammary gland inflammation in response to bacterial infection, trauma or systemic disease (Menzies, 2021). Sub-clinical mastitis is asymptomatic and tools such as the California mastitis test, somatic cell count or milk bacteriological culture are used for mastitis detection (Persson *et al*., 2014; Bezerra *et al*., 2021; Menzies, 2021; Tvarožková *et al*., 2023). Bacteria commonly associated with mastitis are *Staphylococcus aureus* and coagulase-negative staphylococci (CNS), while environmental pathogens include species of *Streptococcus* and coliforms (Menzies, 2021; Tvarožková *et al*., 2023).

There are most probably a considerable number of smallholder goat farms in Hong Kong, however, the exact number of farms and goats is unknown due to the lack of a formal ruminant agriculture industry. Goats on these farms are kept primarily for meat production, mainly *via* private sale for traditional festival events, or for educational purposes and leisure, such as local tourist attractions. At present, there is no data available regarding the udder health of these goats. Overseas data led us to hypothesise that there are likely to be udder abnormalities in these local Hong Kong goats and that we would be able to isolate bacterial species from their milk, similar to does in other countries. Our first objective was to determine the prevalence and types of udder defects in mixed-age non-dairy does from Hong Kong smallholder farms. Our second objective was to identify the bacterial species present in their milk, and to measure the minimum inhibitory concentration of the major isolates to determine their antimicrobial susceptibility, and thereby guide treatment choices for Hong Kong goat farmers.

Materials and methods

Farms and animals

We visited two smallholder goat farms in Hong Kong located in Cheung Chau (Farm A) and Yuen Long (Farm B) in January 2022 for this cross-sectional study. The goats were pure and

mixed breeds of Saanen, Alpine, Black Bengal and Anglo-Nubian, with herd sizes of 60 and 350 individuals. These goats are kept for meat production and public education and entertainment (local tourists and students can visit and interact with the goats), and lactating does raise their offspring. As we were interested in udder health and bacteriological milk sampling we targeted does with visible udders and those that were vocalising at their kids. We were only able to visit each farm once, so we sampled as many does as possible during this time ($n = 34$ does; Farm A = 13, Farm B = 21). Each sampled doe was restrained in a standing position for udder inspection, palpation and milk collection. We used chalk to mark each doe after sampling to ensure they were only sampled once. All procedures in this study were approved by the City University Animal Research Ethics Sub-Committee (internal reference number: A-0700).

Udder assessment and milk sample collection

We visually inspected each doe for external abnormalities and then palpated their udders for any lumps or hardness (Griffiths *et al*., 2019). Using aseptic technique, we collected a milk sample from both udder halves of the lactating does. Teats were first disinfected using 70% isopropyl alcohol wipes and the foremilk collected was discarded. Then, 3–10 ml of milk from each udder half was collected into a sterile vial. Samples were chilled in cooler bags with ice, before submission to CityU Veterinary Diagnostic Laboratory Co Ltd (VDL) on the day of collection. We collected milk samples from four and 18 does from Farm A and B respectively. For three does we were only able to collect a milk sample from one udder half, as the other half did not express an adequate volume of milk.

Milk microbiological culture

To identify the bacteria present we performed cultures in accordance with standard protocol on aerobic plates. We used the quadrant streaking method using inoculation loops to spread milk samples over 5% sheep blood agar plates and MacConkey agar plates, which were then incubated at 37°C for 24–48 h before examining for any bacterial colonies present. Matrix-assisted laser desorption ionisation-time of flight mass spectrometry was used for bacterial species identification (MALDI-ToF system, Biotyper, Bruker Daltronics, Hong Kong).

Minimum inhibitory concentration (MIC)

MIC is the lowest concentration of an antimicrobial that can inhibit growth of a bacteria. Isolates of *Staphylococcus aureus, caprae* and *simulans* were used for this test, since this bacterial genus was the most frequently isolated, and is an important and common causative agent of goat mastitis (Menzies, 2021). We used 96-well Sensititre™ companion animal Gram-positive COMPGP1F Vet AST Plates for antimicrobial susceptibility testing (AST) by the broth microdilution technique (Sensititre™ Thermo Fisher Diagnostics, The Netherlands). This plate is indicated for non-fastidious Gram-positive isolates of veterinary origin, which includes 23 antimicrobials. The microtitre plates were inoculated and incubated for 18–24 h at 34–36°C by the automated system, then Sensititre™ OptiRead™ fluorescence technology and Sensititre™ SWIN™ software were used to generate the readings. Sensitive (S) means the bacteria is susceptible to and inhibited by the drug serum concentration achieved at the usual recommended dosage. Intermediate (I) means the bacteria can only be inhibited if a dosage higher than the usual is used. Resistant (R) means the bacteria is not susceptible to the usual serum drug level achieved. These categories are based on the breakpoints for each antibiotic determined by the Clinical and Laboratory Standards Institute.

Statistical analyses

We present descriptive statistics for udder appearance and palpation for the 34 does included in our study. The milk microbiological culture results for the four does (7 udder half samples) and 18 does (34 udder half samples) from Farm A and B, respectively are described. Finally, we report the MIC results for the staphylococcus species isolates.

Results

Udder assessment

Of the 34 does examined, 38.2% ($n = 13$) had normal udder appearance, while 52.9% ($n = 18$) had supernumerary teats, 5.8% ($n = 2$) had excoriations or abrasions covered by inflammatory crusts, 2.9% ($n = 1$) had udder asymmetry and 2.9% ($n = 1$) had a pendulous udder. Based on palpation, most does (94%, $n = 32$) had normal udders. One doe had a firm palpable lesion within one udder half (lump), while another doe had a firm, palpable lesion (lump) in one udder half and the other udder half was diffusely hard.

Table 1. Bacterial isolates from milk samples collected from does on two smallholder goats farms (Farm A = 4 does; Farm B = 18 does) in the Hong Kong Special Administrative Region

	Number of isolates		
	Farm A[a]	Farm B[b]	Overall
Gram-positive cocci		3	3
Micrococcus luteus		1	1
Staphylococcus aureus	2	1	3
Staphylococcus caprae	1	2	3
Staphylococcus chromogenes		11	11
Staphylococcus simulans	2	1	3
Streptococcus pluranimalium		1	1
Streptococcus sp.		1	1
Mixed: *B. arsenicus and S. simulans*	1		1
Mixed: *Brevibacterium* sp. *and M. luteus*		1	1
Mixed: *S. chromogenes and Staphylococcus* sp.		2	2
Mixed: *S. simulans and S. chromogenes*		2	2
Mixed: *E. coli, Streptococcus* sp., *and Corynebacterium* sp.		1	1
No organisms grown	1	7	8

[a]Four does (7 udder halves) were sampled on Farm A.
[b]18 does (34 udder halves) were sampled on Farm B.

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Microbiological culture

We isolated *S. aureus, S. capae* and *S. simulans* from samples from both farms (Table 1). Overall, the most frequently isolated bacteria were *S. chromogenes*, but this was only isolated from does on Farm B. The most frequent isolate for Farm A was *S. simulans*. Most samples had only one species isolated, with only 7 samples (17%) having mixed cultures. Eight samples (19.5%) had no organisms grown (Table 1). Both does with palpable udder defects (lump and hard udder) had *S. chromogenes* isolated.

Minimum inhibitory concentration (MIC)

The three species of *Staphylococcus* tested for MIC showed antimicrobial susceptibility to most of the antibiotics (Table 2), but resistance was present for some (Table 2). Specifically, all three species demonstrated resistance to doxycycline and tetracycline (Table 2).

Discussion

Our study is the first to describe udder defects and to isolate bacteria in milk from non-dairy goats on smallholder farms in the Hong Kong Special Administrative Region. Over 50% of the does had udder defects including supernumerary teats, skin lesions, udder asymmetry, pendulous udder and two does had palpable udder defects, including one with a diffusely hard udder. Although only small numbers of does were affected by palpable udder defects (5.8%), this is a comparable frequency to meat-breed extensively managed ewes (Griffiths *et al.*, 2019). In this study, we have only sampled does from two smallholder farms, in a cross-sectional study design, so care should be taken when extrapolating these results to the wider goat population. However, the farms included are considered representative of typical husbandry conditions for smallholder farms in the Hong Kong Special Administrative Region. Given the consequences of the types of palpable udder defects we identified, specifically reduced offspring survival, reduced and poorer quality milk production and compromised animal welfare (Moroni *et al.*, 2005; Griffiths *et al.*, 2019; Zeleke *et al.*, 2021), we recommend further investigation into udder defects, their changes over time, and their impact in the Hong Kong smallholder goat farming context.

We isolated a high frequency of coagulase-negative staphylococci (CNS) *via* microbiological culture, particularly *S. chromogenes,*

Table 2. Antimicrobial susceptibility for bacterial isolates of *Staphylococcus* species from goat milk from does on Hong Kong smallholder farms

	Number of isolates								
	Staphylococcus aureus (n = 3)			*Staphylococcus caprae (n = 3)*			*Staphylococcus simulans (n = 5)*		
Antimicrobial agents	S	I	R	S	I	R	S	I	R
Amikacin	3			3			5		
Amoxicillin/Clavulanate	3			3			5		
Ampicillin	1		2	3			5		
Cefazolin	3			2			5		
Cefovecin	3			3			5		
Cefpodoxime	3			3			5		
Chloramphenicol		3			3		1	3	1
Clindamycin	3			3			4		1
Doxycycline		1	2		1	2	2	1	2
Enrofloxacin	3			3			5		
Erythromycin	3			2	1		2	3	
Gentamicin	3			2		1	5		
Imipenem	3			3			5		
Marbofloxacin	3			2		1	5		
Minocycline	3			3			5		
Nitrofurantoin	3			3			5		
Oxacillin	2			3			5		
Penicillin	1		2	3			5		
Pradofloxacin	3			3			5		
Rifampicin	3			2		1	5		
Tetracycline		1	2		1	2		3	2
Trimethroprim-Sulphamethoxazole	3			3			5		
Vancomycin	3			3			5		

S, sensitive; I, intermediate; R, resistant based on minimum inhibitory concentration (MIC) testing.

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S. simulans and *S. caprae*. This is compatible with overseas results where CNS are commonly detected in goat milk (Moroni *et al*., 2005; Bezerra *et al*., 2021; Menzies, 2021), and are often associated with subclinical or chronic infections (Bergonier *et al*., 2003). In agreement with goats overseas (Moroni *et al*., 2005), we isolated CNS from goats that showed no evidence of clinical mastitis (only two goats in our study had palpable udder defects). The significance of CNS as goat udder pathogens is variable (Bezerra *et al*., 2021), as there can be a number of different species involved and their pathogenicity differs (Bergonier *et al*., 2003; Moroni *et al*., 2005; Menzies, 2021). Importantly, CNS are contagious and can spread between goats (Menzies, 2021), as well as persisting from one lactation to the next (Moroni *et al*., 2005). Therefore, early identification of goats with sub-clinical intramammary infection is advocated to prevent spread within the herd and to reduce the negative effects associated (Persson *et al*., 2014). Going forward, somatic cell counting (SCC) combined with culture (Tvarožková *et al*., 2023) could be investigated as a tool for monitoring sub-clinical infection and its consequences in the Hong Kong goats.

One of the key challenges associated with treating intramammary infections in goats is the limited medications approved for use and the lack of data regarding their efficacy in goats (Menzies, 2021). MIC is recommended to evaluate the efficacy of antimicrobials, allowing for targeted treatment (Kowalska-Krochmal and Dudek-Wicher, 2021). Due to financial limitations we were unable to perform MIC testing on all bacterial isolates. Therefore, we conducted MIC for three species of *Staphylococcus*, selected as they are common causative agents of clinical and sub-clinical mastitis (*S. aureus*) and sub-clinical mastitis (*S. caprae, S. simulans*) overseas, and were isolated from both farms. Our results indicate there are a range of options for Hong Kong goat farmers that would likely lead to therapeutic success if antimicrobials were indicated, as the *Staphylococcus* species showed sensitivity towards most of the antibiotics. However, we would advise farmers to avoid selecting doxycycline or tetracycline, since resistance was detected across all three species, so therapeutic failure would be likely.

Multidrug resistance, defined as acquired non-susceptibility to ≥1 agent in ≥3 antimicrobial categories (Magiorakos *et al*., 2012), was found in the two CNS we tested *via* MIC. *S. caprae* was resistant to five antibiotics of four antimicrobial categories, including gentamicin (aminoglycosides category), marbofloxacin (fluoroquinolones category), rifampicin (ansamycins category), doxycycline and tetracycline (both in the tetracycline category). *S. simulans* showed multidrug resistance against four antibiotics of three antimicrobial categories, including chloramphenicol (phenicols category), clindamycin (lincosamides category), doxycycline and tetracycline (both in tetracycline category). Additionally, *S. aureus* demonstrated resistance against four antibiotics of two antimicrobial categories, including doxycycline and tetracycline (both in tetracycline category), as well as ampicillin and penicillin (both in penicillins category). Multidrug resistant pathogens are challenging to treat, and there is also potential for risk to human health, with pathogens such as *S. aureus* also isolated in human healthcare settings (Magiorakos *et al*., 2012). Prudent use of antimicrobials is essential, and in cases of mastitis where cure is unlikely or infection is likely to persist, culling (i.e., removal from the herd) may be indicated (Bergonier *et al*., 2003; Moroni *et al*., 2005; Menzies, 2021).

In conclusion, our study provides novel data about udder defects and bacterial infection in non-dairy goats in Hong Kong at a time when the goat population is growing in this geographic region. We identified palpable udder defects and isolated common bacterial species from milk samples from these goats that were suckling their kids. CNS, which are often associated with subclinical or chronic infections, were frequently isolated and antimicrobial resistance was identified. It is important for farmers to be aware of this information so they can monitor their does for udder defects and intramammary infection that may reduce productivity and/or compromise animal welfare, and take appropriate treatment and preventative measures.

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