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## Prevalence and risk factors of *Klebsiella* mastitis in Beetal goats of Punjab and animal disease prediction using advanced machine learning models

**Moon Moon Satpathy, Narinder Singh Sharma, Paviter Kaur and Anil Kumar Arora**

### Abstract

**Background:** *Klebsiella pneumoniae* is one of the environmental pathogens responsible for causing mastitis in dairy goats. Mastitis caused by *Klebsiella* is particularly important due to its transmission mode, zoonotic significance, clinical outcome, and poor response to antibiotic treatment.

**Objectives:** This study investigates the prevalence, risk factors, and most favourable predisposing conditions of *Klebsiella* mastitis in dairy goats of Punjab.

**Methods:** A total of 125 milk samples were collected from goats affected with clinical mastitis. The samples were processed for bacterial isolation and identification. Data regarding the mastitis status, age, bedding used, faecal contamination, weather condition, mixing with other animals were recorded and their association with *Klebsiella* mastitis was analysed through statistical methods and advanced machine learning models like Random Forest Classification, Gaussian Naïve Bayes Classification, XGBoost Classifier, and K-nearest neighbor Classifier Models.

**Results:** After molecular confirmation, the prevalence recorded was 9.6% (12/125). It was seen that all the four models recorded Sand bedding, cold weather, and age of 3 and  $\geq 3$  as most appropriate conditions predisposing to *Klebsiella* mastitis even if they vary in their mean accuracy. However Random Forest Classification Model was the most accurate model for prediction with a Mean Accuracy of 68%.

**Conclusion:** Prediction modeling or Artificial intelligence are practical tools to predict conditions precisely where basic statistical techniques can fail to provide a complete picture.

**Keywords:** *Klebsiella* mastitis, machine learning models, goat, milk, environmental pathogens

### Introduction

Mastitis is the leading cause of production loss worldwide and is thus investigated from time to time. Well known for its vast livestock population, India ranks first in goat milk production. Goat milk has witnessed increased demand with many benefits like high nutritional and medicinal values. Thus any disease that affects production is considered to be most significant. *Klebsiella* are Gram-negative, thick rods belonging to the family *Enterobacteriaceae*. They are catalase positive and oxidase negative, facultatively anaerobic bacteria with a prominent polysaccharide capsule. They are non-spore-forming, non-motile, occur singly or in pairs. *Klebsiella* spp. are found commonly in soil, surface water, sewage, and plants as saprophytes. They are commonly found in sawdust, beddings. *Klebsiella* spp. act as an opportunistic pathogen responsible for causing many infections like pneumonia, mastitis in cattle, pigs, goats, neonatal septicaemia in kids, dogs, foals, bloodstream infections, surgical site infections, meningitis, urinary tract infections, endometritis, and so on. An environmental pathogen is responsible for causing peracute and chronic mastitis in dairy animals (Ribeiro *et al.* 2008; Cheng *et al.* 2021) [1, 2].

Goat milk and its products have significance in human nutrition. Goat milk is highly nutritious and rich in essential vitamins and minerals. It is used to feed starving and malnourished people in the developing world compared to cow milk; it is also used to treat milk allergies and gastro-intestinal disorders (Haenlein 2004) [3]. Though goat milk has high-quality protein, Alpha S1 casein is present lesser in amount than cow milk, which is probable for its better tolerance by people allergic to cow milk (Tomotake *et al.* 2006) [4]. It is easily digestible due to smaller fat globules and protein composition (Attaie *et al.* 2000) [5]. It is a rich source of crucial vitamins viz. B5 (pantothenic acid), B7 (biotin), vitamin A and essential minerals like phosphorous, iodine, potassium, chloride thus are beneficial for various body systems. Goat milk is widely used in Punjab, particularly during the occurrence season of Dengue, believing

that it confers a certain level of protection against it attributed to the selenium content (Mahendru *et al.* 2011)<sup>[6]</sup>.

Mastitis is a very costly disease for both small-scale and large-scale dairy farmers. It is a significant disease of sheep and goats as it affects the amount and quality of the milk produced, and hence it also reduces weight gain in lambs and kids. Mastitis in dairy goats is caused by various organisms, including bacterial, viral, and fungal organisms. Among the bacterial causes, *Klebsiella pneumoniae* is one among the "other organisms responsible for caprine mastitis (Schukken *et al.* 2012)<sup>[7]</sup>.

Reports suggest that faeces, water, soil, sawdust, and shavings primarily act as a reservoir of coliform pathogens. Reports by various authors suggest faecal shedding of *Klebsiella* spp. as a potent source of exposure and oro-faecal route as a possible means of transmitting the organism from the environment (Zadoks *et al.* 2011)<sup>[8]</sup>. The teat canal is the portal of entry of *Klebsiella* into the mammary gland, primarily due to unsanitary conditions or lack of hygiene during milking. They do not adhere to the epithelial cells of the udder or colonize the udder; instead, they multiply without attachment. *Klebsiella* can enhance their iron uptake in deficiency conditions better than other family members by the secretions of 'enterochelin' and 'aerobactin' siderophores. Mastitis due to *K. pneumoniae* is known for poor response to antibiotic therapy, rapid evolution to toxic shock, high production loss, and death (Langoni *et al.* 2015)<sup>[9]</sup>. Thus *Klebsiella* mastitis is not only responsible for profound loss to dairy farmers due to production loss but also is equally responsible for additional burden due to antibiotic treatment, poor response to antibiotics, extra labour, death, or culling of infected animals, and decreased fertility (Hansen *et al.* 2004)<sup>[11]</sup>. *K. pneumoniae* is zoonotic, and it can be transmitted to humans through contaminated food or water (Hu *et al.* 2021)<sup>[12]</sup>. Hence keeping in view the previous findings, the prevalence and risk factors associated with *Klebsiella* mastitis have been studied in the dairy goats of Punjab. Various intrinsic and extrinsic factors are tested statistically to determine their association. Prediction modeling tools are used to study the most relevant predisposing factors responsible for causing *Klebsiella* mastitis.

## Research methods and design

### Study area, study design, sampling protocol

Punjab is located in the North of India, extending from the latitudes 29.30° North to 32.32° North and longitudes 73.55° East to 76.50° East with an average elevation of 300 meters above sea level. The state experiences three main seasons, Summer (mid-April to the end of June), Monsoon (early July to the end of September), and Winter (early December to the end of February). It is primarily an agriculture-based country of India. For centuries, goat farming has been an integral component of rural livelihood in Punjab. Beetal is the indigenous goat breed of Punjab, and the demand for its milk rises during the outbreak of dengue and other viral diseases. A cross-sectional study was carried out from December 2020-May 2021 in which milk samples of 125 dairy goats suffering from mastitis were collected from various villages of Punjab. The districts covered were The samples were collected from Moga (Kishanpura, Dainiwal Kallan, Talwandi Kallan, Dhanuwal, Daaya, Talwandi-Mallian Kokri-Kallan), Ferozepur (Indgarh, Bahadur-ke), and Ludhiana (Galib-Kallan, Galib-Khurd) districts. The sampling size for the

cross-sectional random sampling method was determined using the methodology suggested by Sergeant 2016. For a population of 300 dairy goats, the sample size calculated was 119. However, a total of 125 goats were included in the study.

### Sample collection

A total of 125 mastitic milk samples were collected aseptically from goats suffering from mastitis. Sample collection was made based on clinical symptoms, history, changes in milk quality, quantity, or both of the animals as obtained from the farmers. Thus a total of 125 pooled mastitic milk samples were collected in sterile test tubes and transported to the laboratory in collection boxes containing ice packs.

### Data collection

Data on mastitic status, age, bedding used, faecal contamination, weather condition, mixing with other animals were noted in an organized way (Figure 3-6).

### Testing for mastitis

Clinical mastitis was evident from the inflammatory changes in the udder like heat, pain, swelling, redness, and visibly abnormal milk. The samples were further subjected to Sodium Lauryl Sulphate (SLS) test to confirm the animal's mastitic status and were immediately processed for bacterial isolation.

### Isolation and identification of *Klebsiella* spp. from milk samples

Each milk sample was initially streaked into BHI agar and incubated overnight at 37 °C. Subsequently, individual colonies were streaked on HiCrome™ Universal Differential Medium and kept for overnight incubation at 37 °C. After that, the colonies showing mucoid appearance with characteristic blue-green colour were suspected to be *Klebsiella* spp. These colonies were selected and restreaked on MLA to check the lactose fermentation. After a period of overnight incubation (18-24 h) at 37 °C, pink, mucoid, and lactose fermenting colonies were suggestive of *Klebsiella* spp.

### Biochemical tests

The pure colonies from MLA plates were selected and subjected to biochemical tests viz. catalase oxidase, indole, Methyl Red, Voges Proskauer, Citrate utilization test, nitrate reduction, Urease, Triple sugar iron (TSI), lysine decarboxylase, and gelatine liquefaction tests (Table 1).

### Morphological characters

The morphological characters like Gram's staining, capsule, and motility were also studied using Gram's staining, Negative staining, and Hanging drop method.

### Molecular detection of *Klebsiella pneumoniae*

Cultural, morphological and biological tests were used to identify *Klebsiella* spp. (Quinn *et al.* 1994). The isolates were further confirmed by molecular test using species-specific primers per reference number CP0248381 (Sekhri 2019) of *Klebsiella pneumoniae* in Polymerase chain reaction.

### Statistical Analysis

Statistical analysis was carried out on various non-parametric factors viz. bedding used, total herd size, the climatic

condition of the area, age, mixed sheep-goat farming, and bedding material used. IBM SPSS (Statistical Package for the Social Sciences) Version 15 was used to analyse the association between the parameters and occurrence of *Klebsiella* mastitis. The Chi-square test is used under the null hypothesis that there is no association between the factors tested and the occurrence of *Klebsiella* mastitis. Logistic regression was used to test the individual risk factors and their association. The odds ratio and 95% CI was calculated for statistical data analysis (Table 2).

### Predicting outcomes through machine learning models

Advanced Machine Learning Models were used to predict favourable conditions predisposing the dairy goats to *Klebsiella* mastitis. The supervised learning models used are Random Forest Classification, Gaussian Naïve Bayes Classification, XGBoost Classifier, and K-nearest neighbor Classifier Models. Random Forest Method for Classification is an ensemble learning technique that functions by forming many decision trees during training. Its outcome is the class selected by most of the trees. Gaussian Naïve Bayes is a variant of Naïve Bayes that follows Gaussian Normal distribution and supports continuous data based on the Bayes Theorem. XGBoost is a decision tree-based ensemble Machine Learning Algorithm that uses a stochastic gradient boosting framework. K Nearest Neighbor algorithm is an Instance-based Supervised Learning algorithm used for Classification and Regression, which considers K Nearest Neighbours to predict the class. After the ML models were constructed, a set of test cases were passed for predicting the most appropriate outcomes, used for disease prediction.

### Ethical considerations

The current study was ethically approved by Institutional Animal Ethical Committee, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana IAEC Approval No (497/GO/Re/SL/02/CPCSEA dated 10-11-2016).

### Results

From a total of 125 mastitic milk samples, 12 isolates were obtained. Thus the prevalence of *Klebsiella pneumoniae* in caprine mastitis was calculated to be 9.6% (12/125) in dairy goats of Punjab. The isolates tested Gram positive, catalase positive and oxidase negative and --++ to indole, Methyl Red, Voges Proskauer, and citrate utilization tests. A prominent polysaccharide capsule is observed for each *K.pneumoniae* isolate (Figure 1-2). The *Klebsiella* isolates were confirmed to be *Klebsiella pneumoniae* molecularly by Polymerase chain reaction (PCR) (Figure 7), suggesting its predominant role in caprine mastitis. The study investigated that 91.66% of the cases have been reported in cold weather conditions. On statistical analysis, weather conditions showed a significant association with the occurrence of *Klebsiella* mastitis ( $p<0.05$ ). Faecal contamination in dairy farms has also been observed in our study. In most cases, the farm where one animal is affected with *Klebsiella* mastitis has also witnessed some other cases. However, no association has been recorded for *Klebsiella* mastitis and the type of bedding used ( $p=0.100$ ). No association has been seen between *Klebsiella* mastitis and the animal's age. No significant association has been observed between housing (grouping) and the occurrence of *Klebsiella* mastitis. No association has been observed between rearing goats and sheep together, and goats rose separately. It was seen that all the four models recorded Sand bedding, cold weather, and age of 3 and  $\geq 3$  as most appropriate conditions predisposing to *Klebsiella* mastitis even if they vary in their mean accuracy. Hence, these combinations are most significant in predisposing the animals to *Klebsiella* mastitis. According to Naïve Bayes Classification Model and XGBoost Model for Classification, animals of age groups 2, 2.5, 3, 3.5 kept in sand bedding can also develop *Klebsiella* mastitis in cold weather conditions. However, the mean accuracies of the models are 62% and 60%, respectively. Hence predictions of the Random Forest Classification Model and KNN Classification Model are more accurate with a mean efficacy of 68% and 66%, respectively. Random Forest Classification Model predicts the conditions most accurately (Figure 9-12).

**Table 1:** Panel of biochemical tests used to identify *Klebsiella* spp.

S. No	Catalase	Oxidase	Urease Production	Lysine decarboxylation	Indole test	Methyl Red test	Voges Proskauer test	Citrate
1	+	-	+	+	-	-	+	+
2	+	-	+	+	-	-	+	+
3	+	-	+	+	-	-	+	+
4	+	-	+	+	-	-	+	+
5	+	-	+	+	-	-	+	+
6	+	-	+	+	-	-	+	+
7	+	-	+	+	-	-	+	+
8	+	-	+	+	-	-	+	+
9	+	-	+	+	-	-	+	+
10	+	-	+	+	-	-	+	+
11	+	-	+	+	-	-	+	+
12	+	-	+	+	-	-	+	+

S. No.	Reaction on SIM Media			Sugar Fermentation				Reduction on TSI slant			
	H <sub>2</sub> S	Indole	Motility	Glu	Lac	Suc	Mal	Slant	Butt	H <sub>2</sub> S	Gas
1	-	-	-	+	+	+	+	Yellow	Yellow	-	+
2	-	-	-	+	+	+	+	Yellow	Yellow	-	+
3	-	-	-	+	+	+	+	Yellow	Yellow	-	+
4	-	-	-	+	+	+	+	Yellow	Yellow	-	+
5	-	-	-	+	+	+	+	Yellow	Yellow	-	+
6	-	-	-	+	+	+	+	Yellow	Yellow	-	+

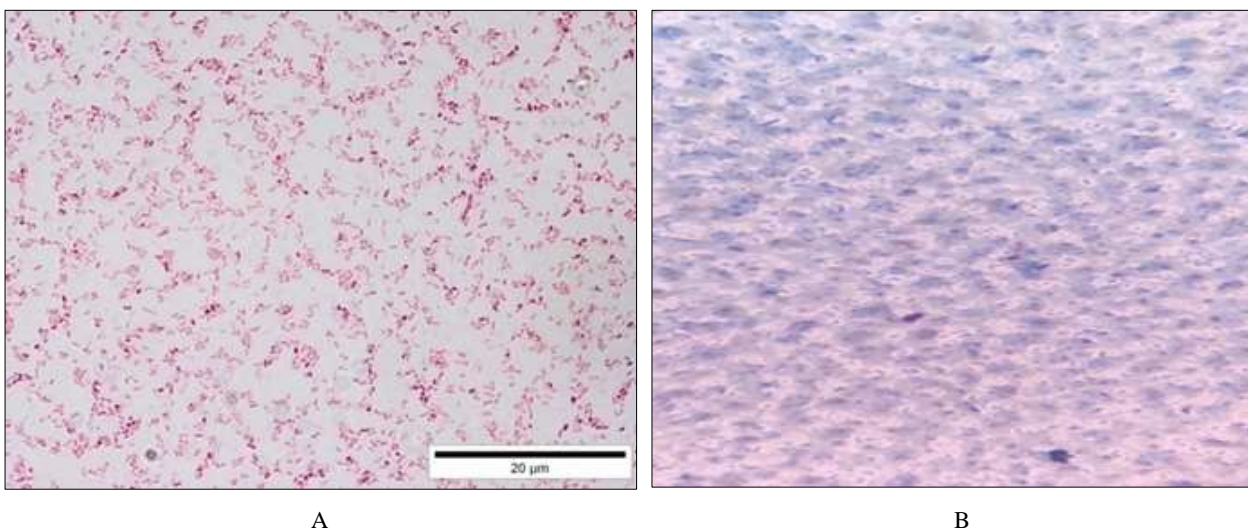


7	-	-	-	+	+	+	+	Yellow	Yellow	-	+
8	-	-	-	+	+	+	+	Yellow	Yellow	-	+
9	-	-	-	+	+	+	+	Yellow	Yellow	-	+
10	-	-	-	+	+	+	+	Yellow	Yellow	-	+
11	-	-	-	+	+	+	+	Yellow	Yellow	-	+
12	-	-	-	+	+	+	+	Yellow	Yellow	-	+

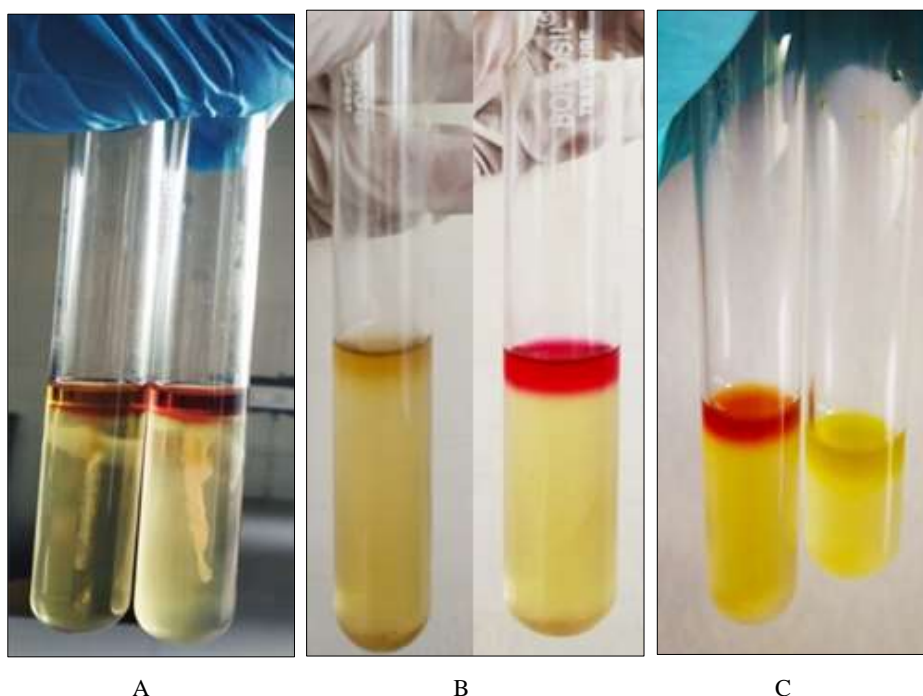
**Table 2:** Univariate Analysis of various factors and risk associated with *Klebsiella* mastitis

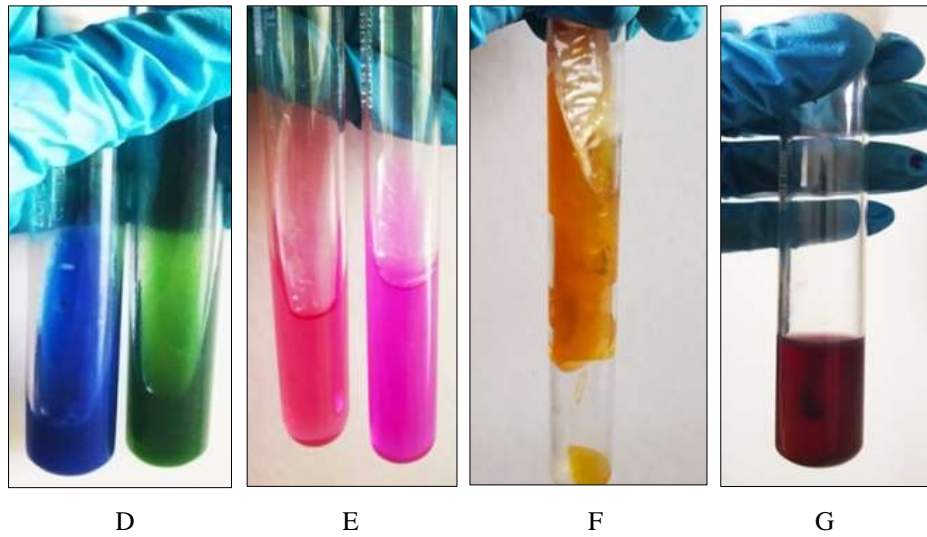
S. No	Variables	Labels	<i>Klebsiella</i>	others	OR	95% CI	$\chi^2$	P value
1	Season	Cold Season *	91.67%	52.2%	10.068	1.25-80.598	6.853	0.009
		Hot Season*	8.33%	47.79%				
2	Bedding	Sand	91.67%	69%	0.203	0.025-1.631	2.712	0.100
		Concrete	8.33%	31%				
3	Housing	Together	100%	91.15%	*		1.154	.283
		Separately	0%	8.84%				
4	Mixed with sheep	Mixed	33.33%	38.05%	0.814	.231-2.866	0.103	0.748
		Not Mixed	66.67%	61.94%				
5	Age	<=2.5 years	0%	0%	*	*	1.945	.378
		3- 4.5years	91.67%	91.67%				
		>=5 years	8.33%	8.33%				

\* No Statistical analysis needed



**Fig 1:** a. Gram negative bacilli b. *Klebsiella pneumoniae* capsule (Negative staining)





**Fig 2:** A. Indole (+), B. Methyl Red(-/+), C. Voges Proskauer(+/-), D. Citrate(+/-), E. Urease(-/+), F. TSI Slant(lactose fermentation +gas) G. Lysine decarboxylase



**Fig 3:** Concrete bedding



**Fig 5:** Sand bedding (housed together)

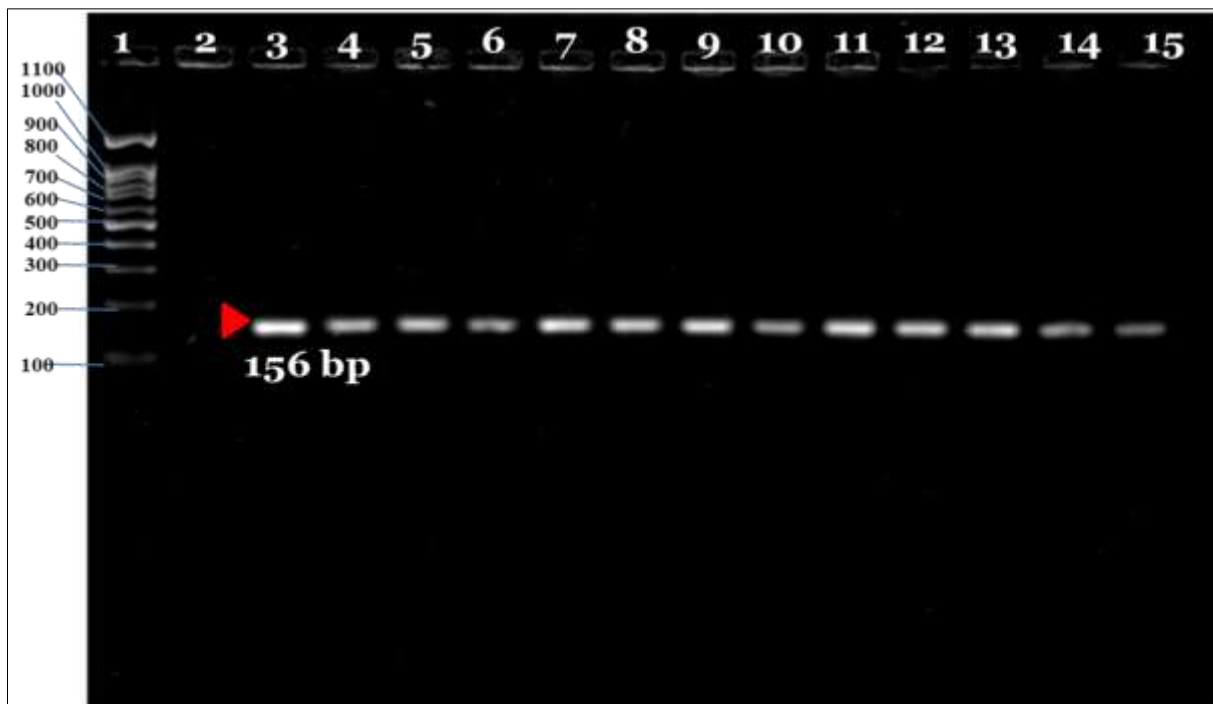


**Fig 4:** Concrete bedding (Housing together)



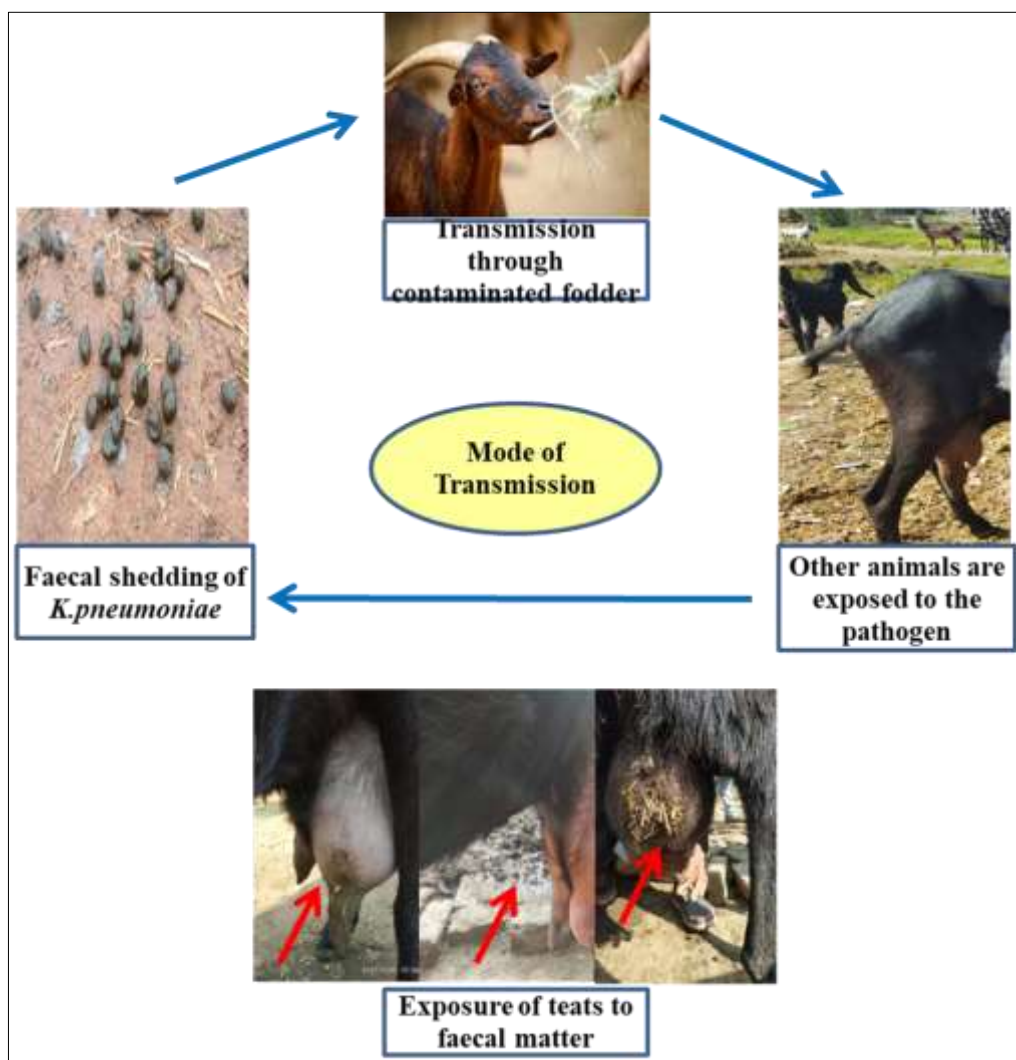
**Fig 6:** Sand bedding (Housed separately)





Lane 1: 100 bp ladder; Lane 2: Negative control; Lane 3: Standard strain (VTCCBAA6) Lane (4-15) *Klebsiella pneumoniae*.

**Fig 7:** Gel Electrophoresis of Amplicon (156 bp) of *Klebsiella pneumoniae* isolates.



**Fig 8:** Schematic representation of Mode of transmission of *K.pneumoniae*.

	Weather	Bedding	Age (years)	Most Suitable
0	Cold	Sand	2.0	0
1	Cold	Sand	2.5	0
2	Cold	Sand	3.0	1
3	Cold	Sand	3.5	1
4	Cold	Concrete	2.0	0
5	Cold	Concrete	2.5	0
6	Cold	Concrete	3.0	0
7	Cold	Concrete	3.5	0
8	warm	Sand	2.0	0
9	warm	Sand	2.5	0
10	warm	Sand	3.0	0
11	warm	Sand	3.5	0
12	warm	Concrete	2.0	0
13	warm	Concrete	2.5	0
14	warm	Concrete	3.0	0
15	warm	Concrete	3.5	0

Fig 9: Random Forest Classification Model Mean Accuracy: 68%

	Weather	Bedding	Age (years)	Most Suitable
0	Cold	Sand	2.0	0
1	Cold	Sand	2.5	0
2	Cold	Sand	3.0	0
3	Cold	Sand	3.5	1
4	Cold	Concrete	2.0	0
5	Cold	Concrete	2.5	0
6	Cold	Concrete	3.0	0
7	Cold	Concrete	3.5	0
8	warm	Sand	2.0	0
9	warm	Sand	2.5	0
10	warm	Sand	3.0	0
11	warm	Sand	3.5	0
12	warm	Concrete	2.0	0
13	warm	Concrete	2.5	0
14	warm	Concrete	3.0	0
15	warm	Concrete	3.5	0

Fig 11: KNN Classification Model Mean Accuracy: 66%

	Weather	Bedding	Age (years)	Most Suitable
0	Cold	Sand	2.0	1
1	Cold	Sand	2.5	1
2	Cold	Sand	3.0	1
3	Cold	Sand	3.5	1
4	Cold	Concrete	2.0	0
5	Cold	Concrete	2.5	0
6	Cold	Concrete	3.0	0
7	Cold	Concrete	3.5	0
8	warm	Sand	2.0	0
9	warm	Sand	2.5	0
10	warm	Sand	3.0	0
11	warm	Sand	3.5	0
12	warm	Concrete	2.0	0
13	warm	Concrete	2.5	0
14	warm	Concrete	3.0	0
15	warm	Concrete	3.5	0

Fig 10: Naïve Bayes Classification Model Mean Accuracy: 62%

	Weather	Bedding	Age (years)	Most Suitable
0	Cold	Sand	2.0	1
1	Cold	Sand	2.5	1
2	Cold	Sand	3.0	1
3	Cold	Sand	3.5	1
4	Cold	Concrete	2.0	0
5	Cold	Concrete	2.5	0
6	Cold	Concrete	3.0	0
7	Cold	Concrete	3.5	0
8	warm	Sand	2.0	0
9	warm	Sand	2.5	0
10	warm	Sand	3.0	0
11	warm	Sand	3.5	0
12	warm	Concrete	2.0	0
13	warm	Concrete	2.5	0
14	warm	Concrete	3.0	0
15	warm	Concrete	3.5	0

Fig 12: XGBoost Model for Classification Mean Accuracy: 60%

## Discussion

Mastitis due to *Klebsiella* species in goats has been reported to be between 3.65%-16% in various studies from the year 2005-2019 by (Ajuwape *et al.* 2005; Ali *et al.* 2010; Mahlangu *et al.* 2018; Ferdous *et al.* 2018; Abdalhamed *et al.* 2018; Danmallam *et al.* 2019) [13, 14, 15, 16, 18, 17]. Among *Klebsiella* spp, *Klebsiella pneumoniae* is the major pathogen of caprine mastitis. *Klebsiella pneumoniae* is considered one of the known environmental pathogens responsible for causing *Klebsiella* mastitis in dairy farms (Ohnishi *et al.* 2013) [19]. Faecal shedding of *K.pneumoniae* is the source of *Klebsiella* in dairy animals. The highest prevalence of *Klebsiella pneumoniae* in faecal samples has been reported previously (Munoz *et al.* 2006) [20], with most infections occurring in the winter season. Highest incidence of *Klebsiella* mastitis in dairy cows has previously been reported in winter season which is similar to this study (Paulin *et al.* 2007) [21]. A possible explanation may be, when the air temperature drops, goats spend less time outdoors than indoors (Bøe & Ehrlenbruch 2013) [22]. During their stay indoors, they get exposed to the pathogens present abundantly in the faecal shedding of the infected goats, resulting in mastitis (Young *et al.* 2019) [23]. Two or more cases from a single farm support the possible faeco-oral transmission of *Klebsiella pneumoniae* in which other goats in the herd get exposure to the faecal shedding of the infected goats (Figure 8). Goats are browsers and they spend at least 8 hours a day nibbling on vegetation. There may be possible exposure to the animals irrespective of bedding used by this time. The other reason is improper sanitation. Sanitation plays a significant role than just bedding itself, and the animals get exposure to faecal matter even when concrete bedding is used. However, many authors reported sand bedding related to *Klebsiella* mastitis in dairy animals. This is due to the high prevalence of *Klebsiella pneumoniae* in soil, where it helps plants with nitrogen fixation. In *Klebsiella* mastitis, two risk factors, weather, and bedding have been widely discussed to be significant by various authors. Still, no statistical significance has been observed between many parameters. Predictive analytics, also known as Prediction modeling, is a mathematical process to predict future events or trends by analysing a current pattern. These tools have wide application in modern-day studies ranging from disease prediction to predicting criminals (Steven 2014) [27]. However, there is not much literature available regarding its use in animal diseases which is very important. In the present era where we witness many EIDs (emerging infectious diseases), supervising animal diseases through prediction modeling will help keep a check on many Zoonoses. In this study, prediction modeling predicts the most favourable combination of parameters predisposing to *Klebsiella* mastitis. Mastitis is a very costly disease; it requires timely screening in dairy herds by various direct and indirect methods like SCC, BTB, CMT, and SLS tests. The etiological agents must be identified to assess their role in disease development and pathogenesis. *Klebsiella pneumoniae* is an environmental pathogen. Hence, animals and human beings are also at risk of getting infected as it is zoonotic. This mastitis is challenging to treat and ultimately results in the animal's death or culling in many instances. This pathogen can acquire drug resistance very fast (Coates *et al.* 2011) [24], thus adding fuel to the fire. *Klebsiella* can enhance their iron uptake in deficiency conditions better than other family members by the secretions of 'enterochelin' and

'aerobactin' siderophores, thus favouring their survival better than the latter many other pathogens. The faeco-oral route of transmission also exposes the other animals in the herd to the pathogen. Segregating the diseased animal can help prevent an outbreak due to the pathogen. As we all know, prevention is better than control, we must take utmost care of the farm, animals, and the milkers to prevent the disease.

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## Competing interests

The authors declare that no competing interests exist.

## Authors' contribution

All the authors contributed significantly to the research. Dr. Moon Moon Satpathy has performed the lab work and prediction modeling, Dr. Narinder Singh Sharma has provided his expert guidance, Dr. Paviter Kaur has helped with the data processing, and Dr. Anil Kumar Arora has contributed in terms of statistical analysis.

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## Data availability

The data is available from corresponding author on reasonable request.

## Disclaimer

The article is my own and not an official position of the institution or funder.

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