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Diagnosis of urinary tract infection by urinalysis, ultrasonography and quantitative urine culture

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Abstract
This study was conducted to diagnose urinary tract infection from the dogs presented to the Veterinary College Hospital during the period of 6 months by urinalysis, ultrasonography and cultural examination and to determine the antimicrobial resistance profile of the urine samples. 72 dogs were presented to Veterinary College Hospital, Bengaluru with clinical sign suggestive of UTI among that 65 urine samples having more than 10^3 cfu/ml on quantitative urine culture were considered for study. Urinalysis and sonographic changes were evaluated. Urine culture was carried out to isolate bacteria from urine samples and to know quantitative bacterial count in the sample. Increased frequency of urination, straining while passing urine, hematuria and oliguria were the common signs observed in dogs. Change in the colour, turbidity, proteinuria, pyuria, haematuria and bacteriuria were noticed on urine analysis. Thickening of the bladder (> 3 mm), hyperechoic calculi in lumen and sludge in the lumen were noticed in ultrasonography.

Keywords: Urinalysis, ultrasonography, urine culture, Clinical signs, urinary tract infections

1. Introduction
Urinary tract infection (UTI) is commonly diagnosed in dogs (Weese *et al.*, 2011) [1]. UTI refers to microbial colonization of the urine or of any urinary tract organ, except the distal urethra, which has a normal bacterial flora (Somu *et al.*, 2015) [2]. Diagnosis of a UTI includes findings from the history, physical examination, complete urinalysis, and urine culture (Smee *et al.*, 2013) [3]. Clinical signs of UTI are not pathognomonic, and although urinalysis and microscopic examination increase the index of suspicion, quantitative bacteriological culture remains the reference standard for confirming bacteriuria (Bartges, 2004; Weese *et al.*, 2019; Smee *et al.*, 2013) [4, 1, 5]. Studies regarding the canine urinary tract infections are very scanty in India when compared to other countries. This might be due to the misinterpretation of diseases due to overlapping nature of clinical signs of this disease as well as inappropriate diagnostic tools and methods. Urinary tract infections are one of the major etiologies for prostatic diseases in male dogs and this disease can further lead to renal failure, if untreated. Hence, the present study was undertaken to rule out the dogs with urinary tract infection and physiological, chemical and microscopical changes of urine in UTIs. The primary objective of this study was to diagnose and confirm urinary tract infection among the dogs presented to veterinary college hospital and interpret the major changes in ultrasonography, urinalysis and to detect quantity of bacteria in the samples.

2. Materials and Methods
Dogs aged above 1 year of any gender and breed presented to the veterinary College Hospital, Hebbal with suspected UTI and having more than 10^3 colony forming unit per milliliter were enrolled. To enable the easy application of human interpretive criteria, inclusion criteria were as follows

- Dogs with clinical signs or history of urinary tract infection (Dribbling of urine, straining while passing urine, dysuria, oliguria, cloudy urine, increased frequency of urination, constant licking of urinary area, haematuria, fever and lethargy, anorexia, smelly urine and arching of back).
- Specimens were excluded from the study if dog has been treated with antimicrobials within 48 hours prior to the collection of urine.

2.1 Urine collection and handling: Urine collected by ante pubic cystocentesis (Scott *et al.*, 2017) [6] and specimen were stored at 4 degree celsius and cultured within 24 hour.

2.2 Urinalysis: Physical and chemical examination of urine such as colour, odour, pH, transparency, specific gravity, total leukocyte count, proteins, nitrates, glucose, bile pigments, ketones and occult blood were recorded by urine dipsticks (Rose, 2014) [7].

2.3 Urine Sediment Examination; A 5 ml urine sample was placed in to a sterile tube and centrifuged at 1000 rpm for 5 minutes to perform a microscopic examination. The sediment was transferred to a slide and examined under the microscope at 10X and 40X power after the supernatant was discarded. Transferred the sediment to a glass slide, and then checked for the presence of erythrocytes, leucocytes, epithelial cells, casts, crystals, and bacteria (Somu *et al.*, 2015) [7].

2.4 Abdominal ultrasonography: The hair over the abdomen was clipped and shaved. Ultrasonographic examination was performed by placing the animals in dorsal/lateral recumbency. A liberal amount of coupling gel was applied to provide sufficient contact (Mannion, 2008) [8].
The dog placed in lateral recumbency, with the kidney to be examined uppermost, the hair patch ventral to the sub lumbar muscles, just behind the last rib on the left and over the last two intercostals spaces on the right was removed and acoustic coupling gel was applied. The kidney would be located ultrasonographically, lying superficially in each case, just beneath the abdominal wall. Ultrasonographic examination was carried out with either 3.5 or 5.0 MHz transducers according to the body weight of dog's transducers (Barr, 1990) [9].
Urinary bladder was located by placing the transducer in the flank area ventral to the tuber coxae and was easily found in long axis by orienting the transducer longitudinally and sliding it dorsally or ventrally included vertex and trigone (Leveille *et al.*, 1992) [10].

2.5 Quantitative urine culture: All urine samples were analysed at the veterinary college laboratory, samples were cultured quantitatively on plate count agar by inoculating 0.1 ml of inoculum of dilution (10^{-1} to 10^{-6}) each in duplicate. All the agar plates were incubated at 37 degree celsius for 24 hours.
A serial dilution of urine sample from 10-1 to 10-6 was made, and a minimum of 0.1 ml of inoculum of each dilution in duplicate was placed on the surface of each plate count agar and it was then spread uniformly with the help of a sterilized L shaped nichrome wire. All the agar plates were incubated at 37°C for 24 hours. Average number of Colony forming unit /plate was calculated from these dilution, and after applying the dilution factor, the number of bacteria was expressed as CFU/ml (Gatoria *et al.*, 2006) [11]. Descriptive statistics such as simple frequency, percentage, mean, standard deviation were used and the data was presented by tables and figures.

3. Results and Discussion

3.1 Clinical profile of dogs affected with UTI: Among the 65 dogs examined, 19(29.23%) dogs had dribbling of urine, 35 (53.84%) dogs had straining while passing urine. It was observed that 13 (20.00%) dogs suffered from dysuria, 35 (53.84%) dogs had oliguria, 30 (46.15%) dogs had cloudy urine, 46 (70.76%) dogs were having increased frequency of urination, 16 (24.61%) dogs had symptoms of constant licking of urinary area, 40 (61.53%) dogs had haematuria, 19 (29.23%) dogs have fever and lethargy, 9 (13.84%) dogs had anorexia, 14 (21.53%) dogs exhibited smelly urination and 13 (20.00%) dogs had arching of back.
This study is in accordance with Kumar and Kamran (2016) [12], Sorensen *et al.* (2018) [13] and Weese *et al.* (2019) [5] who also reported increased frequency of urination, haematuria and straining while passing urine as the predominant sign of urinary tract infections.
In contrast to the present investigation, Yogespriya *et al.* (2018) [14] and Lippi *et al.* (2019) [15] reported low incidence of pollakiuria among dogs. The presence of pollakiuria in the current study may be due to the presence of uroliths, weak sphincter muscle tone, damage to the mucosal epithelial barrier of urinary tract, chronic use of medicines which cause irritation or may be due to bacterial urinary tract infection (Madavi *et al.*, 2021) [16].
In the current investigation dogs with UTI exhibited dribbling of urine (20%) which was in agreement with Merkel *et al.* (2017) [17] and Lippi *et al.* (2019) [15] who reported 11.12 per cent and 10.5 percent of dribbling of urine respectively.
The current study agreed with the Yogespriya *et al.* (2018) [14] and Peli *et al.* (2003) [18], in the presence of haematuria, smelly urine, arching of back due to abdominal pain and stranguria in dogs with UTI. Manifestation of the clinical signs in UTI can be due to the abnormal urine composition due to variation in the pH, presence of bacteria, pus cells and desquamated cells which leads to the irritation of the bladder mucosa and further cause vascular damage, hemorrhages and mucosal erosions in urinary tract of dogs.
Major clinical symptoms observed in the present study can be due to lower UTIs and which were in concurrence with the studies of Bartges (2004) [4].

3.2 Urinalysis profile of dogs affected with UTI: In the present study, one of the parameter for the preliminary diagnosis of bacterial urinary tract infections was based on urine analysis of freshly collected urine sample which was according to the study of Somu *et al.* (2015) [2] who stated urinalysis is a method for diagnosing UTI.
The present study revealed the colour of the urine varied from pale to dark yellow and sometimes dark red in case of haematuria and the transparency ranged from clear to severely turbid. In this study 12.30 percent had dark yellow urine and 15.38 percent urine sample was severely turbid. These findings were similar to the Blango and Bartges (2001) [19] who observed cloudy urine in dog with UTI. Present investigation detected the presence of turbidity of urine in the infected dogs which may be due to large concentrations of bacteria, fat, crystals or mucus in urine which leads to the cloudy appearance of urine (Meyer *et al.*, 1992; Cetin *et al.*, 2003) [20, 21].
The specific gravity ranged between 1.010 and 1.040 with a mean specific gravity of 1.018±0.0009115. Present study also agrees with Aizenberg and Aroch (2003) [22] and Merkel *et al.* (2017) [17] who revealed mean specific gravity in the range of 1.040 and 1.021 respectively. Low specific gravity with high growth of bacteria on culture indicate UTI (Swenson *et al.*, 2004) [23]. Bailiff *et al.* (2008) [24] opinied that there was no relation between reduction in urine specific gravity and positive urine culture.
Urine specific gravity primarily reflects renal tubular ability to conservation of water. Factors affecting urine specific gravity includes the hydration status of the animal, urine

glucose and protein concentration, concurrent diseases and recent administration of therapeUTIc agents (Elliott and Grauer, 2007) [25].

The pH ranged from 5.0 to 8.0 with a mean value of 6.877± 0.0974. The present study was in accordance with the study of Cetin *et al.* (2003) [21] who reported urine pH between 6 and 9 in dogs infected with UTI. The present study is in contrast with the study of Aizenberg and Aroch (2003) [22] who reported low urine pH in urine samples of dogs affected with UTI.

High pH is caused by urease producing bacteria (eg: Staphylococcus, Proteus) that produce ammonia, high vegetable diet, metabolic alkalosis. Whereas low PH is caused by acid producing bacteria, high meat diet, increased protein catabolism, metabolic acidosis and severe diarrhea (Elliott and Grauer, 2007) [25].

In our investigation, protein concentration ranged from 15 to 300 mg/dl. Proteinuria observed in 30.76 percent. The present study agrees with the previous studies of Aizenberg and Aroch (2003) [22], Cetin *et al.* (2003) [21], Chigozie (2015) [26] and Somu *et al.* (2015) [2] who reported the presence of proteinuria in dogs with urinary tract infections. Proteinuria can result from inflammation or infection of urinary tract (Archer, 2005) [27]. Proteinuria was not only an indicator of renal diseases but can also associated with progression of diseases and inflammation of urinary tract infection (Raila *et al.*, 2011) [28].

Occult blood examination was positive for 61.53 per cent of the cases. This study was in agreement with Somu *et al.* (2015) [2] who observed 28.57 percent cases positive for occult blood examination. Occult blood in the present study can be due to infection and inflammation of urinary tract leading to the presence of blood in dogs affected with UTI.

The urobilinogen content in all animal in the study was in the normal range (0.2-1 mg/dl). This report is in conjunction with the previous study of Parrah *et al.* (2013) [29] in urine samples with UTI.

In the present case study none of the urinary sample was positive for glucosuria. This was similar with the findings of Forrester *et al.* (1999) [30].

Microscopic examination of urine sediment revealed the presence of red blood cells, white blood cells, bacteria, cast, epithelial cells and crystals. Clinically relevant haematuria (> 5 cells per high-power field) was present in 61.53 per cent of cases during urinalysis. This finding was in agreement with that of Somu *et al.*, (2015) [2] and Merkel *et al.* (2017) [17] who detected increased numbers of erythrocytes which probably seen in samples collected by cystocentesis. Blood in urine collected by cystocentesis can cause latrogenic haematuria and also may be due to bleeding from ureter, urinary bladder, proximal urethra or prostate gland (Forrester, 2004) [31]. Urinary tract infection causing bacteria may also cause damage to urinary epithelium and vascular damage which results in leakage of RBC to the urine.

Clinically relevant pyuria was observed in 44.61% of dogs with UTIs in the current study. The observations were similar to the report of Blanco and Bartges (2001) [19] and Seguin *et al.*, (2003) [32]. Similarly, Forrester *et al.*, (1999) [30] documended pyuria in 60 per cent of dogs with UTIs. In this present study microbes were detected in the urine with concurrent increase in WBCs and this can be due to variations in the severity of infection.

Bacteriuria was reported in 100 per cent of dogs, this was in accordance with the result of Somu *et al.* (2015) [2] and Merkel *et al.* (2017) [17] who detected 78.55 percent and 100 percent of bacteriuria while urinalysis of dogs affected with urinary tract infections.

Presence of increased epithelial cells in this study can be due to the urinary tract infection or inflammation similar to the reports of Seguin *et al.* (2003) [32].

Present study reported presence of epithelial cells and struvite crystals as well as the absence of sugars, ketones, and nitrate. The present study agrees with the previous studies of Cetin *et al.* (2003) [21] and Grimes *et al.* (2019) [33] who reported the presence of pyuria, bacteriuria, epithelial cast and struvite crystals in UTI of dogs.

This study concurs with Somu *et al.* (2015) [2] because there was no appreciable change in specific gravity or pH in their study but shows the presence of occult blood and bacteriuria of dogs affected by urinary tract infection. Whereas, the current study contradicts the Guire *et al.* (2002) [34] who detected no significant change of white blood cells, epithelial cells, crystals in urine from the urine sediment examination compared to the control group. Urease producing bacteria, vegetable diet, increases the PH of urine which predispose to the formation of struvite crystals. Acid urine produce by meat diet and hypercalciuria increases the chance of calcium oxalate crystals (Elliott and Grauer, 2007) [25].

The variation in the parameters examined by urinalysis in our study compared to other research workers may be due to nature of infection, season, locality and variation in the severity of infection.

3.3 Ultrasonographic examination of dogs diagnosed with UTI: In the present work ultra-sonographic findings in dogs affected with urinary tract infections revealed thickened bladder wall (> 3 mm) in 44.61 per cent (29) of dogs similar to the previous studies of Dinesh *et al.* (2015) [35], Kumar and Kamran (2016) [12], Yogeshpriya *et al.* (2018) [14] and Lippi *et al.* (2019) [15] who recorded thickening of bladder wall as the predominant change on performing ultrasonography in dogs affected with urinary tract infections.

The presence of hyperechoic calculi in lumen with acoustic shadow in the present study was noticed in 26.15 (17) per cent of dogs. This was in accordance with Dinesh *et al.* (2015) [35] and Kumar and Kamran (2016) [12] who recorded the ultrasonography findings with calculi in lumen of bladder showing acoustic shadow in dogs suffering from urinary tract infections.

The presence of sludge in lumen of bladder in the current study was noticed in (13) 20 per cent of dogs which was similar to the study of Kumar and Kamran (2016) [12]. The reason for sludge in the present study in dogs affected with UTI can be due to concentrated urine resulting in the deposition of urine sediments in lumen of bladder.

3.4 Quantitative urine culture: The 72 urine sample collected from dogs for CFU analysis 65 samples were $>10^3$ CFU/ml. More than 10^3 CFU/ml, more than 10^4 CFU/ml, more than 10^5 CFU/ml, more than 10^6 CFU/ml were observed in 27.69% (18), 36.92% (24), 20.00% (13) and 15.38% (10) cases respectively. The present study agrees that of Chang *et al.* (2015) [36], who consider all the urine sample above 10^3 CFU/ml for further analysis. In this study among the results of dog urine collected by cystocentesis, most dogs yielded > 10^3 CFU/ ml which is similar to the previous studies by Ling *et al.* (2001) [37], Gatoria *et al.* (2006) [11] and Papini *et al.* (2006) [38] who observed > 10^5 CFU/ml in urine cultures with UTIs.

Table 1: History/Clinical signs of dog with UTI

Clinical sign / history	No. of cases (N=65)	Percentage of dogs affected (%)
Increased frequency of urination	46	70.76
Haematuria	40	61.53
Straining while passing urine	35	53.84
Oliguria	35	53.83
Cloudy urine	30	46.15
Dribbling of urine	19	29.23
Fever and lethargy	19	29.23
Constant licking of urinary area	16	24.61
Smelly urine	14	21.53
Dysuria	13	20.00
Arching of back	13	20.00
Anorexia	9	13.84

Table 2: Physical examination of urine (colour)

Urine colour	No of samples from Infected dog with UTI (N=65)	No of samples from control group (N=10)
Pale yellow	7 (10.76%)	5
Yellow	10 (15.38%)	4
Dark yellow	8 (12.30%)	1
Green colour	0 (0.00%)	0
Red colour	29 (44.61%)	0
Amber colour	11 (16.92%)	0

Table 3: Physical examination of urine (Transparency)

Transparency	No of samples from Infected dog with UTI (N=65)	No of samples from control group (N=10)
Clear	16 (24.61)	8
Mildly turbid	25 (38.46)	2
Moderately turbid	14 (21.53)	0
Severely turbid	10 (15.38)	0

Table 4: Chemical examination of urine (Protein)

Urine total protein	No of urine sample from Infected dog with UTI (N=65)	No of urine sample from control group (N=10)
Trace 15-30 mg/dl	45(69.23%)	10
+ (30-100 mg/dl)	18(27.69%)	0
2+ (100-300 mg/dl)	2(3.07%)	0
3+ (300-1000 mg/dl)	0	0

Table 5: Microscopical examination of urine (Red blood cells)

RBC (HPF)	No of samples from Infected dog with UTI (N=65)	No. of urine samples from control group (N=10)
0-5/ HPF	25 (46.15%)	20
5-50/HPF	28 (43.07%)	9
> 50/ HPF	12 (18.46%)	0

Table 6: Microscopical examination of urine (WBC)

WBC	No of samples from Infected dog with UTI (N=65)	No of urine samples from control group (N=10)
0-5 WBC/HPF	31 (47.69%)	10
5-50WBC/HPF	34 (44.61%)	19
> 50WBC/HPF	0 (0.00%)	0

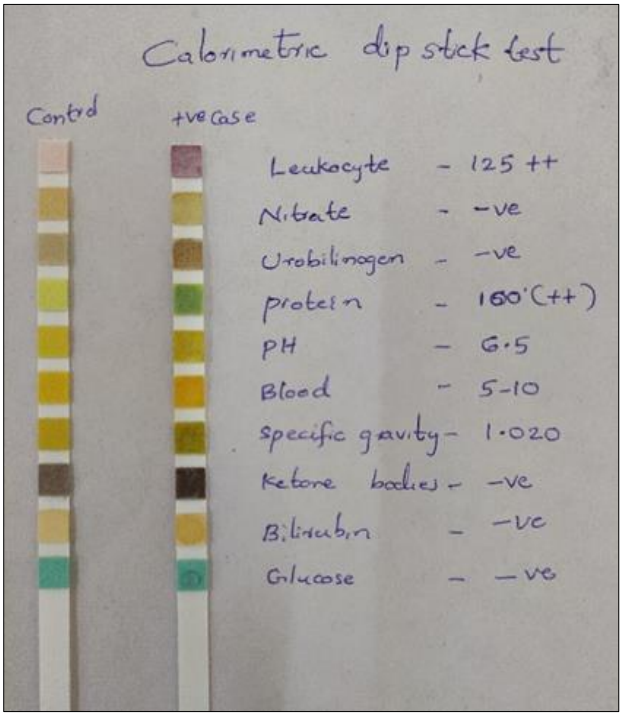

Fig 1: Calorimetric dipstick test

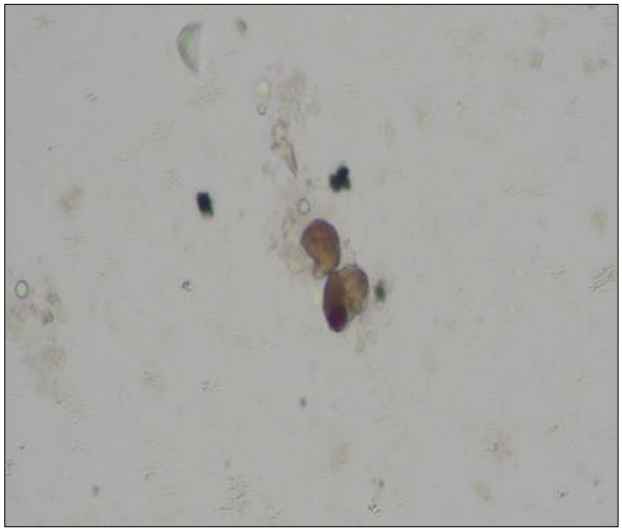

Fig 2: Epithelial cells in urine sediment examination (45x)

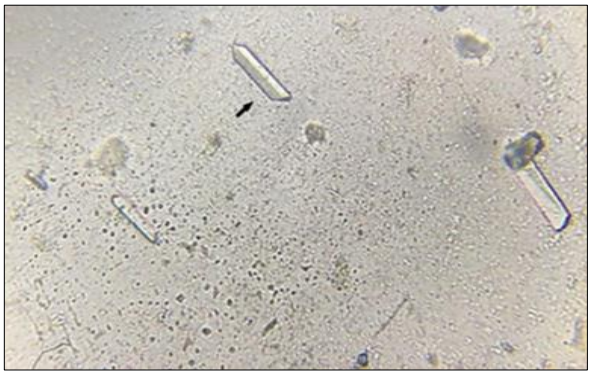

Fig 3: Microscopic examination of calcuim oxalate monohydrate crystals in urine sediment

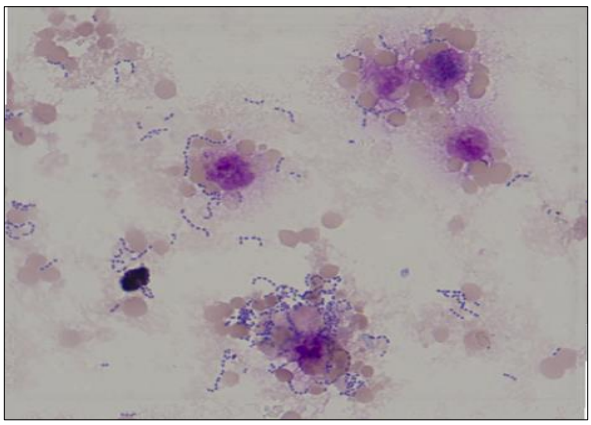

Fig 4: Microscopic examination of a giemsa stained urine sediment from a dog with cystitis showing white blood cells, bacteria and RBC (100x)

Fig 5: Ultrasonograph of normal urinary bladder

Fig 6: Ultrasonograph showing hypoechoic thickening of bladder wall

Fig 7: Ultrasonograph showing bladder wall and hyperechoic calculi in lumen of bladder with thickening

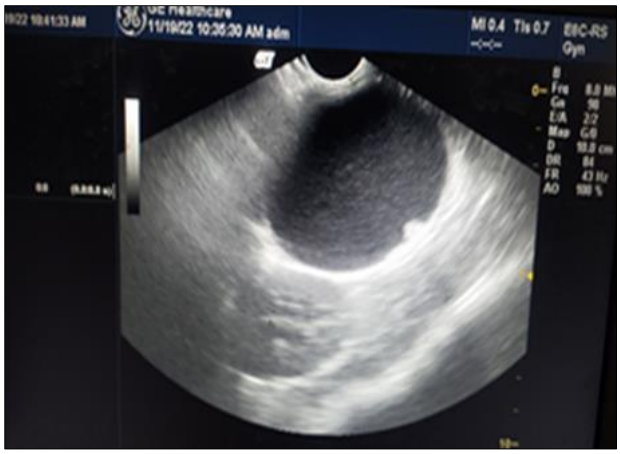

Fig 8: Ultrasonograph showing bladder thickness and sediments in lumen

4. Conclusions

Straining while passing urine, increased frequency of urination, haematuria, cloudy urine, dysuria, loss of bladder control, dribbling of urine, inability to urinate or only passing a small amount of urine, inappetance/anorexia, arching of back (Cystitis), fever and lethargy were the main clinical signs observed in the dogs affected with urinary tract infections. The signs exhibited by the affected dogs varies depending upon age of the dogs and the clinical state at which the dog was presented to clinics for treatment. Relevant changes noted in urine analysis were haematuria, pyuria, proteinuria, slightly alkaline pH and bacteria in urine sediment examination. On ultrasonographical examination major changes noticed were hypoechoic thickening of the bladder (> 3 mm) and hyperechoic calculi in lumen of bladder. All dogs considered for the study yielded > 10^3 CFU/ml on Quantitative urine culture

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