Evaluation of urine cultures obtained by cystocentesis from cats with urethral obstruction at the time of hospital admission and after urethral catheterization

[*Avaliação das uroculturas obtidas por cistocentese de gatos com obstrução uretral no momento da admissão hospitalar e após o cateterismo uretral*]

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ABSTRACT

The aims of this study were to search for the presence of bacterial and fungal growth in urine samples from cats with urethral obstruction, to identify the causative infectious agents, and to compare select clinical-laboratory parameters with urine culture results after urethral catheterization. Twenty-eight male cats were enrolled in this prospective study. Urethral catheterization was performed aseptically, and the catheter was maintained for 24-48 hours with a sterile closed collection system. No antibiotics were used during this period. The first urine sample was obtained at the time of presentation, and the second, 24-48 hours after catheter removal, both by cystocentesis. Two cats (7.1%) presented a positive bacterial urine culture on the first sample. The frequency of positive urine cultures in the second sample was 52.4% (11/21 cats). One third of bacterial pathogens were Gram-positive, one third were Gram-negative, and one third were mixed isolates. Fungal cultures were all negative. There was significant association of positive urine culture results with pyuria (P=0.0128). A high frequency of positive urine cultures after urinary catheter removal was observed, despite respecting the standards of care for urethral catheters. Still, these results should be interpreted with caution, since intermittent bladder flushing was performed disconnecting the collecting system, which may have represented a critical point for bacterial contamination. It is emphasized that urine cultures should be considered as follow up in cats with urethral obstruction, after urethral catheter removal.
Keywords: feline lower urinary tract disease, indwelling urinary catheter, subclinical bacteriuria, urinary tract infection

RESUMO

Os objetivos deste estudo foram pesquisar a presença de crescimento bacteriano e fúngico em amostras de urina de gatos com obstrução uretral, identificar os agentes infecciosos causadores e comparar parâmetros clínico-laboratoriais selecionados com os resultados da urocultura após a cateterização uretral. Vinte e oito gatos machos foram incluídos neste estudo prospectivo. A sondagem uretral foi realizada de forma asséptica, e o cateter foi mantido por 24-48 horas, com um sistema de coleta fechado e estéril. Não foram utilizados antibióticos durante esse período. A primeira amostra de urina foi obtida no momento da admissão do felino, e a segunda 24-48 horas após a retirada do cateter, ambas por cistocentese. Dois gatos (7,1%) apresentaram urocultura bacteriana positiva na primeira amostra de urina. A frequência de culturas de urina positivas na segunda amostra foi de 52,4% (11/21 gatos). Um terço das bactérias isoladas eram Gram-positivas, um terço eram Gram-negativas e um terço eram infecções mistas. As culturas fúngicas foram todas negativas. Houve associação significativa das uroculturas positivas com presença de piúria (P=0,0128). Observou-se alta frequência de uroculturas positivas após a retirada do cateter urinário, apesar de se respeitarem as diretrizes de cuidados com os cateteres uretrais. Ainda assim, esses resultados devem ser interpretados com cautela, pois a lavagem

vesical intermitente foi realizada desconectando o sistema coletor, o que pode ter representado um ponto crítico para contaminação bacteriana. Ressalta-se que as uroculturas devem ser consideradas como acompanhamento em gatos com obstrução uretral, após a retirada do cateter uretral.

Palavras-chave: doença do trato urinário inferior dos felinos, sonda uretral de espera, bacteriúria subclínica, infecção do trato urinário

INTRODUCTION

Urethral obstruction (UO) is a common condition in male cats and there are controversies about post-obstructive care, including urethral catheter maintenance and the use of antibiotics (Cooper, 2015; Cosford and Koo, 2020).

Underlying causes associated with this disease include urolithiases, urethral plugs, strictures and neoplasia, extra luminal compression, bacterial urinary tract infection, and idiopathic conditions (Swalec *et al.*, 1989; Reche Jr *et al.*, 1998; Gerber *et al.*, 2005, 2008; Eggertsdóttir *et al.*, 2007; Sævik *et al.*, 2011; Young *et al.,* 2021). Previous studies reported different positive bacterial urine culture frequencies in cats with UO at presentation, considering different populations and different methods of urine collection (Table 1).

Table 1. Description of the number and frequency of positive urine cultures in cats with urethral obstruction at presentation, according to geographic location and method of urine collection, in different studies

Urine culture positive, N (%)	Location	Sample obtained by	Study (year)
0/45 (0.0%)	Europe	cystocentesis and catheter urine samples	Gerber *et al.* (2005)
0/18 (0.0%)	Europe	catheter urine samples	Hugonnard *et al.* (2013)
0/34 (0.0%)	North America	catheter urine samples	Cooper *et al.* (2019)
3/34 (8.8%)	Europe	only cystocentesis	Sævik *et al.* (2011)
4/36 (11.1%)	Brazil	only cystocentesis	Reche Jr *et al.* (1998)
9/49 (18.4%)	Europe	cystocentesis and catheter urine samples	Eggertsdóttir *et al.* (2007)

The use of indwelling urinary catheters after urethral deobstruction may help clear off debris, clots, and crystals (Cooper, 2015), however, it may cause irritation to urethral epithelium and contribute to inflammation (Lees *et al.*, 1980). Furthermore, a urethral catheter acts as a direct communication between the external environment and the bladder, which creates an inherent risk of bacteriuria or bacterial cystitis (Weese *et al.*, 2019).

Catheter-associated urinary tract infections (CAUTI) are among the most prevalent healthcare-associated infections in humans (Umscheid *et al.*, 2011) as well as in small animal veterinary hospitals (Ruple-Czerniak *et al.*, 2013; Stull and Weese, 2015). On the other hand, there is great concern about overuse and improper antimicrobial therapy, especially regarding the risk of development of antimicrobial resistance (Weese *et al.*, 2019). In addition, standards of care for urethral catheters, such as aseptic catheter placement, the use of closed urinary collection systems and the non-use of prophylactic antimicrobial therapy during urinary catheterization (Weese *et al.*, 2019), are often not respected (Holroyd and Humm, 2016; Beeston *et al.,* 2022).

Previous studies aimed to investigate the presence of bacteria in the urine of cats with UO after urethral catheterization through urinary catheter samples (Cooper *et al.*, 2019) or through urinary catheter samples and catheter tips (Hugonnard *et al.*, 2013). On the other hand, cystocentesis is the preferred method for urine collection due to lower risk of sample contamination (Weese *et al.*, 2019). The primary aim of this study was to search for the presence of bacterial and/or fungal growth in urine samples collected by cystocentesis in cats with UO before and after urethral catheterization, and to identify the causative infectious agents. The secondary purpose was to compare select clinical

and laboratory parameters in cats with positive and negative urine cultures after urethral catheterization.

MATERIAL AND METHODS

A prospective study was conducted to evaluate urine cultures from cats with naturally occurring UO before and after urethral catheterization at the Feline Medicine Service of the Veterinary Teaching Hospital of Rio Grande do Sul Federal University (Porto Alegre, Brazil). The inclusion criteria were male cats with a diagnosis of UO, presenting with a history of lower urinary tract disease and a distended non-expressible urinary bladder. Cats with history of previous urethrostomy or urethral catheterization within the past two months were excluded, as well as those receiving antibiotics within the past two months. Approval from the Ethics Committee on the Use of Animals of this University (no. 29039) was obtained, and owners signed and informed consent.

Cats were treated accordingly to a previously standard established protocol at this institution, which included analgesic therapy, balanced electrolyte intravenous fluid therapy, decompressive cystocentesis and correction of acid-base and electrolyte disorders, as needed. After initial stabilization, cats were anesthetized for retrohydropulsion and urethral catheterization. This procedure was performed aseptically. Initially, the cats were placed in dorsal recumbency with the perineal region clipped and antisepsis was performed with 0.12% chlorhexidine. The procedure was performed with sterile surgical gloves and the placement of sterile surgical drapes. Retrohydropulsion was performed using a 22-24-gauge intravenous catheter (minus the stylet) lubricated with lidocaine gel, that was replaced by a flexible 3.5 French polyvinyl urethral catheter. The bladder was flushed gently with sterile normal saline solution, and the urethral catheter was sutured in the perineal region and maintained for 24-48 hours with sterile closed collection system. The exact duration of catheterization within this time frame was at the discretion of the clinician. No antibiotics were used during this time. Bladder lavage was performed by disconnecting the collecting system and flushing sterile isotonic solution through the catheter (the amount of fluid was at the discretion of the clinician), every four hours. The catheter was handled with disposable non-sterile gloves. No disinfection of the collecting line was performed prior to bladder flushing.

The first urine samples were obtained at time of presentation, during decompressive cystocentesis, prior to urethral catheterization. For this procedure, the abdomen was previously clipped, and antisepsis was performed with 70% alcohol. After that, a needle was connected to extension tubing, to a 3-way stop-cock, and a 20mL syringe was used to withdraw the urine. The second urine samples were obtained by ultrasound guided cystocentesis 24-48 hours after the catheter was removed. The cats remained hospitalized throughout the study period. The urine samples were stored at 4°C in sterile tube pending culture for a maximum time of 24 hours.

Urine bacterial cultures were performed from the first and second samples by plating urine with Blood agar, MacConkey's agar and Nutrient agar at 37°C for 24-48 hours. Bacterial identification was achieved using conventional biochemical tests. Susceptibility analysis was performed using the disc diffusion technique. The second urine samples were also submitted to fungal culture by plating the urine on Sabouraud dextrose agar with chloramphenicol at 30°C and Sabouraud dextrose agar with chloramphenicol and cycloheximide at 37°C for 72 hours.

Urinalysis were performed with first and second samples through chemical evaluation using a urine dipstick (Combur Test®, Roche Diagnostics) and urinary sediment analysis evaluated by optic microscopy (400x) after urine centrifugation. Hematuria was considered when urinary sediment analysis revealed more than five red blood cells per high power field, and pyuria when more than five white blood cells per high power field were observed (Fry, 2011). The presence of any number of bacteria in sediment analysis was considered bacteriuria (Fry, 2011). Complete blood count was also performed using routine techniques at time of presentation, and 24-48 hours after the catheter was withdrawn (the same moments of urine collection).

Rectal temperature was measured daily during hospitalization. Fever was considered when the temperature was above 39.2°C and hypothermia

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was considered when the temperature was below 37.8°C.

The relative frequencies were calculated for categorical variables. The Shapiro-Wilk test was used to evaluate data for normal distribution. Median was calculated for quantitative variables with asymmetric distribution. The Fisher's exact test was applied to compare categorical variables from urine cultures results after catheterization. The variables compared were urine culture results versus pyuria/bacteriuria/hematuria/fever or hypothermia/leukocytosis or leukopenia. The analysis was performed with GradePad Prism 8 (v.8.3.0) software. A P value < 0.05 was considered significant.

RESULTS

Twenty-eight male cats were enrolled in the study, of which fifteen (53.6%) were neutered and all of them (100%) were mixed breed. The median age was two years (Interquartile range: 0.8-4.7) and ranged from three months to 11 years old.

After admission, the first urine sample was collected from all 28 cats. The second urine sample was collected from 21 cats, since there was loss of follow-up of patients due to: death from urethral obstruction complications; and failure to fulfill the urinary bladder before discharge of the patient, probably due to dysuria, making it impossible to obtain a proper sample by cystocentesis. Also, the second sample was not collected from the cats that had a positive first sample.

Only two cats from the 28 (7.1%) presented a positive bacterial urine culture on the first sample, and the bacteria isolated included *Escherichia coli* and coagulase-negative *Staphylococcus* sp. The first isolate showed resistance to doxycycline, trimethoprim-sulfamethoxazole, gentamicin, and amoxicillin and was susceptible to enrofloxacin, amoxicillin-clavulanate, amikacin, cephalexin, and imipenem. The second mentioned bacteria exhibited resistance to amoxicillin, trimethoprim-sulfamethoxazole, enrofloxacin, doxycycline and cefovecin and was susceptible

to amoxicillin-clavulanate, cephalexin, amikacin, and gentamicin. A second urine culture was not performed for these two positive cats, since the first culture was already positive.

Considering that a total of 21 cats were enrolled for the second urine bacterial culture evaluation, eleven (52.4%) came up with a positive result on the second sample. Approximately one third of bacterial pathogens were Gram-positive: *Enterococcus* sp. (n=3) and coagulase-negative *Staphylococcus* sp. (n=1). One third were Gram-negative: *Klebsiella* sp. (n=2), *Proteus* sp. (n=1) and *Pseudomona*s sp. (n=1); and one third were mixed isolates: *E. coli* + *Proteus* sp. (n=1), *Streptococcus* sp. + *Klebsiella* sp. (n=1) and *Proteus* sp. + *Klebsiella* sp. (n=1). All Gram-positive pathogens (100%) after catheterization showed resistance to amoxicillin and doxycycline. All *Enterococcus* sp. isolates showed resistance to enrofloxacin and two isolates to imipenem. In contrast, 3/4 (75%) of Gram-positive species were susceptible to amoxicillin-clavulanate. Regarding Gram-negative bacteria after catheterization, all isolates (100%) showed resistance to doxycycline, 6/7 (85.7%) to trimethoprim-sulfamethoxazole, 5/7 (71.4%) to amoxicillin-clavulanate, 4/7 (57.1%) to enrofloxacin and gentamicin. As opposed to that, 5/7 (71.4%) were susceptible to amikacin. Resistance to two or more drugs was observed in 100% of the isolates. Fungal cultures were negative for all cats.

The select clinical and laboratory parameters in cats with positive and negative urine cultures after urethral catheterization are shown in Tab 2. There was a significant association of positive bacterial urine cultures results after catheterization and pyuria (P=0.0047). The other variables tested were not significantly associated with urine culture results (P>0.05).

The cats that had positive cultures in the first or the second urine sample were discharged home with antibiotics, according to their culture sensitivity analysis. All cats were followed for 30 days and no episodes of reobstruction were documented. Urine cultures were not performed at this time.

Table 2. Select clinical and laboratory parameters in cats with urethral obstruction after catheterization and its association with urine culture results

	Urine culture result, N (%)		*P* value*
	Positive	Negative	
Hematuria	10/11 (90.9%)	5/9 (55.5%)	0.1273
Pyuria	7/11 (63.6%)	0/9 (0.0%)	0.0047
Bacteriuria	8/11 (72.7%)	2/9 (22.2%)	0.0698
Leukocytosis or leukopenia	3/11 (27.3%)	3/9 (33.3%)	>0.9999
Fever or hypothermia	6/11 (54.5%)	6/10 (60.0%)	>0.9999

*Fisher's exact test was applied to compare categorical variables with results of urine culture after urethral catheterization

DISCUSSION

This study evaluated urine samples collected by cystocentesis, before and after urethral catheterization, in male cats presented with UO, and revealed a frequency of positive urine cultures at presentation [2/28 (7.1%)] very similar to previous studies in Brazil (Reche Jr *et al.*, 1998) and in Europe (Sævik *et al.*, 2011).

Regarding urine cultures obtained after urethral catheterization, a high frequency of positive bacterial cultures after catheterization [11/21 (52.4%)] was observed, compared to the same group of animals before the procedure. A previous study with 18 cats with UO showed that one-third of them developed significant bacteriuria during catheterization (Hugonnard *et al.*, 2013), while a more recent study enrolling 34 cats showed a frequency of 13% (Cooper *et al.*, 2019). In the first study, urine samples after catheterization were obtained through the urinary catheter and catheter tips (Hugonnard *et al.*, 2013). In the second study, first samples were obtained by cystocenteses and subsequent samples through urinary catheter (Cooper *et al.*, 2019). The 2019 ISCAID guidelines provides specific recommendation about this topic, suggesting that culture of the catheter tip at the time of removal is not recommended because of the potential for colonization of the catheter with various bacteria (Weese *et al.*, 2019). Furthermore, it is suggested that, if there is a need for culture, urine should be collected by cystocentesis, whenever possible (Weese *et al.*, 2019). Cooper (2015) suggests that the animal should return in three days for a cystocentesis to determine if a urinary tract infection (UTI) has been introduced through catheterization, similar to what was done in this research, in which the urine culture was collected by cystocentesis within 24-48h after catheter removal.

Nevertheless, the frequency of positive cultures after urethral catheterization obtained in the present study was higher compared to the two previously mentioned studies. Some local factors could be implicated, like geographic localization, clinic infection-control practices and adherence to these practices by staff (Stull and Weese, 2015). As the catheter placement was performed aseptically and a closed urine collection system was maintained, the authors suggest that factors associated with handling of the indwelling urinary catheter may have been implicated in the high frequency of positive urine cultures after catheterization, since hospitalized cats are treated by different staff during their hospitalization.

Also, unlike similar studies (Hugonnard *et al.*, 2013; Cooper *et al.*, 2019), this research performed bladder lavage every four hours, which may have contributed to contamination of the urine collection system with hospital environment bacteria. Two recent studies failed to show benefit from bladder lavage (Dorsey *et al.*, 2019; Tsuruta *et al.*, 2022), despite one of them revealed that the incidence of bacteriuria following catheterization was not affected by intermittent bladder flushing (Tsuruta *et al.*, 2022). However, the bladder flushing protocol from the mentioned research differs from the present study. Tsuruta *et al.* (2022) performed bladder lavage with the collection system closed and flush injected through a port. In the present study, the system was disconnected and flushed through the catheter, what may have contributed to its contamination. Cooper *et al.* (2019)

describes additional care with the urine collection system, which includes swabbing the collection bag port with alcohol, in addition to disinfection of the collection line every 8 hours.

Catheter-associated urinary tract infection or bacteriuria may be a result of contamination during catheter placement, ascending migration of bacteria into the bladder (either within the catheter lumen or along the outer surface of the catheter) or, less commonly, from bacteremia (Weese *et al.*, 2019). The causative bacteria commonly originate from the host's own enteric or distal urogenital microbiota (Dorsch *et al.*, 2019), from the hospital environment or people (Stull and Weese, 2015). Regarding the organisms isolated, no fungal growth was observed. Although rare, it is important to search for these agents in patients with risk factors, like those with indwelling urinary catheters (Lulich and Osborne, 1996). A previous study showed fungal growth in one cat with UO maintained with indwelling urinary catheter without concomitant antibiotic treatment (Hugonnard *et al.*, 2013).

Several studies show that *E. coli* is the most isolated bacteria causing UTI in cats. Other commonly isolated bacteria genera include *Staphylococcus* spp., *Streptococcus* spp. and *Enterococcus* spp. (Litster *et al.*, 2007; Dorsch *et al.*, 2015; Marques *et al.*, 2016). On the other hand, in cats with indwelling urinary catheters, *Streptococcus* spp. was the most common isolated species, followed by *E. coli* and *Staphylococcus* spp. (Dorsch *et al.*, 2016). In the present study, beyond those common bacterial genera, less frequent bacteria were isolated, like *Proteus* sp., *Klebsiell*a sp. and *Pseudomonas* sp. The latter may be of concern, as many isolates frequently show multidrug resistance patterns and persist in the hospital environment and equipment (Stull and Weese, 2015). The *Pseudomonas* sp. isolated was susceptible only to ciprofloxacin, norfloxacin and amikacin, which corroborates with a bacterial resistance profile. In respect to *Klebsiella* spp., the isolates presented broad antimicrobial resistance, especially against cephalotin, fluorquinolones, trimethoprim-sulfamethoxazole and, in one case, against third generation-cephalosporin (cefovecin), which was similar to previous data (Marques *et al.*, 2018).

Mixed isolates were found in approximately one third of the samples in this study. Infections with multiple bacterial species are more common in cats with indwelling urinary catheters (Dorsch *et al.*, 2016).

A second urine culture was not performed for the two cats that had a positive culture at admission, as they were placed on antibiotic therapy after catheter removal. It might have been of interest to still perform a urine culture from these two individuals, to evaluate whether the same bacteria profile remained after urethral catheterization or not.

In view of the similar distribution of Gram-positive and Gram-negative bacteria observed in this study after urethral catheterization, there is a clear need for sending urine to bacterial culture before instituting any treatment, in order to guide the antibiotic choice in cats with a suspicious UTI in this hospital.

Subclinical bacteriuria is defined as the presence of bacteria in urine as determined by positive bacterial culture from a properly collected urine specimen, in the absence of clinical evidence of infectious urinary tract disease (Weese *et al.*, 2019). It might be very challenging to differentiate subclinical bacteriuria from UTI in cats with UO, as it is difficult to assess whether the cat presents clinical signs due to UTI or to other causes of lower urinary tract disease, or if these signs are caused by recent catheterization due to inflammation and trauma (Dorsch *et al.*, 2019). Clinical signs like stranguria, pollakiuria, dysuria, periuria and hematuria may also be caused by inflammation, pain or urethral spasm associated with underlying cause of UO and the maintenance of indwelling catheter (Cooper, 2015). This differentiation is particularly important, since current recommendations do not support antimicrobial treatment of subclinical bacteriuria (Weese *et al.*, 2019). In an attempt to differentiate these conditions, other parameters that could be related to UTI were evaluated.

The presence of pyuria was associated with positive urine cultures in this study (P=0.0047), differently from what was found by Hugonnard *et al.* (2013). Although bacteriuria (assessed through urinary sediment analysis) showed no association with urine culture results (P=0.0698), which was demonstrated by Hugonnard *et al.*

(2013), the *P* value was very close to the significance level. Hematuria showed no association with urine culture results, as most positive and negative samples showed hematuria, even after bladder lavage. Hematuria may be justified by prolonged bladder distention in cats with UO and bladder wall injuries (Beer and Drobatz, 2016). Although urinalysis findings do not confirm bacterial adhesion to the bladder mucosa, they show persistence of bacteria and inflammation in positive urine culture samples 24-48 hours after catheter removal. Weese *et al.,* (2019) declare that cytological data are useful adjunctive data to assess animals with potential urinary tract disease, but may not be highly predictive of culture results, infectious disease or correlate well with clinical signs.

Urinary tract infection should be suspected in all cases of fever or bacteremia of an unknow origin (Weese *et al.,* 2019). The frequency of systemic signs like fever or hypothermia and alterations in WBC total count were very similar between the two groups (positive versus negative urine culture) and, therefore, were not significantly correlated with urine culture results. This may be explained, in part, by the fact that these are signs of systemic disease in general, and not necessarily of UTI.

A study conducted with cats with experimentally induced bladder wall injury demonstrated that the use of indwelling urinary catheters with a closed collection system for three days can predispose cats to persistent UTI/bacteriuria, since many cats developed UTI/bacteriuria that persisted for seven days after catheter removal (Barsanti *et al.*, 1992). In the study mentioned, bladder injury was induced by intravesical administration of irritants. In cats with naturally occurring UO, the bladder mucosa is often injured, due to prolonged time of distention, which may contribute to adhesion of bacteria to the bladder mucosa, predisposing to infection. As the differentiation between UTI and subclinical bacteriuria is challenging and directly impacts decision-making about antibiotic therapy, the authors suggest that new studies should be conducted to follow up cats after catheter removal for longer periods of time, to evaluate the presence of a true UTI versus a transient bacterial population.

Despite the knowledge that the treatment of CAUTI is antibiotic therapy, the surface of the catheter is subject to biofilm formation and, therefore, is often resistant to antibiotic penetration. Antibiotic treatment while the catheter is in place, consequently, can select for resistant bacterial strains and alter the individual microbiota, predisposing to additional niches for colonization by resistant organisms (Werneburg, 2022). In this context, and according to the 2019 ISCAID guidelines, prophylactic antimicrobial therapy for prevention of cystitis in catheterized animals is not indicated either during or after catheter removal (Weese *et al.*, 2019). Considering that antimicrobial resistance is a public health problem of great importance (Werneburg, 2022), and that the bladder can be a source of systemic infection for the patient (Weese *et al.*, 2019), CAUTI prevention strategies become critically necessary in veterinary hospitals settings. Therefore, it is emphasized the need for aseptic catheter placement and maintenance, the use of closed collection system, periodic inspection for catheter contamination and short-term duration of catheterization (Weese *et al.*, 2019).

CONCLUSION

A high frequency of positive urine cultures after urinary catheter removal was observed, despite respecting the standards of care for urethral catheters, including aseptic catheter placement, the use of closed urinary collection systems and the non-use of prophylactic antimicrobial therapy during urinary catheterization. Still, these results should be interpreted with caution, since intermittent bladder flushing was performed disconnecting the collecting system, which may have represented a critical point for bacterial contamination. The isolated agents included both Gram-positive and Gram-negative bacteria, emphasizing the need to consider a urine culture as follow up in cats with urethral obstruction, after catheter removal. The presence of pyuria was associated with positive urine cultures in this study.

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