

Review Article

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## Buffaloe Mammillitis: A Systematic Review

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### ABSTRACT

Mammillitis is a condition that causes sudden teat inflammation, enlargement, difficulty milking, difficulty suckle, and the formation of black necrotic patches with circumscribed necrotic areas on the teat base in she buffaloes. It is a disease of buffaloes associated with bovine herpes mammillitis virus 2 (BHV2). Vesicle formation, a characteristic feature of early stages of the disease in cow is either absent or occurs rarely in dairy buffaloes. Stress of calving and hormonal changes close to calving are important predisposing factors. The condition is usually sporadic although outbreaks have also been documented. It is more prevalent in buffaloes in their first lactation. Therapeutic interventions be used for the treatment of topical and parenteral administration of antibiotics, topical administration of lysine/propolis, topical and oral administration of acyclovir (An anti-herpes virus drug), oral administration of zinc sulphate and other immunity enhancing agents and parenteral administration of antihistamine, lithium antimony thiomalate and anti-inflammatory drugs. Future research proposals include investigating the oral use of zinc sulphate, local infiltration of acyclovir into the lesions, and the use of an infrared lamp to raise the temperature of the lesion. There is currently no vaccine available. Biosecurity, prevention and proper treatment, insect control, and milking time hygiene are all recommended control and prevention measures.

#### Keywords

Buffalo, mammillitis, Bovine Herpes Virus, Therapy, Drugs

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### Introduction

Bovine herpesvirus 2 (BHV-2) causes mammillitis of teat in cows. There will be sudden onset of inflammation, papules, skin sloughing, vesicular

growth, scab forming, and ulceration. In early lactating primiparous buffaloes, there will be an acute inflammation of one or more teats leading to necrotic ring formation, gangrene and fibrosis (Mouli, 1992). The condition is less successfully

treated with antibiotics, NSAIDs and glucocorticoids. The therapy by external application with herbal was moderately effective in early stage and administration of herbal capsules with external application of herbal cream (Shridhar, 2005; Sundaresh and Janaki, 1997). Lithium antimony thiomalate was used for the condition with better cure rate in early conditions. The probable mechanism of lithium antimony thiomalate in curing the condition might be due to its anti-fibrosis activity (Shridhar *et al.*, 2014; Wadhwa *et al.*, 1992; Turner *et al.*, 1976; Weaver *et al.*, 1972; Kachawa *et al.*, 2017) who had done intramammary infusions of amoxicillin and sulbactam, ketoprofen, ascorbic acid, and cefoparazone were given once only for 5 days in mastitis-affected quarters, with early recovery. Buffaloes with ulcerative thelitis thought to be caused by bovine herpes mammillitis (BHV-2) infections were given acyclovir orally and externally for 5 days, with a 62.5 percent cure rate. However, the treatment is ineffective at this time. The mammillitis was treated for 5 days with a combination of lithium antimony thiomalate, chlorpheniramine maleate, meloxicam, and acyclovir oral and topical therapy, with a fair success rate (Shridhar, 2020).

### **Epidemiology**

Bovine herpes mammillitis (BHM) is caused by bovine herpesvirus 2 (BHV2), a virus of the genus *Simplexvirus*, subfamily *Alpha herpesvirinae* and the family *Herpesviridae*. BHV-2 also causes the rare pseudo-lumpy skin disease of cows characterized by appearance of edematous painful skin lesions (nodules) all over the body which later on become necrotic with exudation and are eventually covered by scabs (Bitsch, 2011). Although, BHM has been reported from many countries of the world (Bitsch, 2011), the condition has not as yet grabbed the desired attention of the veterinary practitioners, veterinary academicians and animal health researchers in India. The indolent ulcer at the junction of teat and udder colloquially known as *Gulwaddee* in Pakistan (Sehrish Firyall *et al.*, 2019).

The condition is usually sporadic although outbreaks have also been reported (Bitsch, 2011). It is more prevalent in temperate regions of the world. BHM virus is transmitted through milker's hand, milking equipment, biting insects, semen and air (Kemp *et al.*, 2008). Heifers become infected before calving. The stress of calving particularly with udder edema and hormonal changes lead to activation of BHM virus and development of signs (Gibbs, 1984; Kemp *et al.*, 2008; Martin, 1973). Only one teat or at the most two teats are affected in buffalo (Sankaram and Kotayya, 1977). The clinical condition is usually sporadic in occurrence (Sharma *et al.*, 1998) and mostly affects lactating animals particularly the first calvers. Most cases occur within the first two months of lactation (Sharma *et al.*, 1998). Hind teats are more frequently involved than the front teats (Mouli, 1992). Skin lesions of BHM display typical features of inflammation resulting from the activation of complement by the classical pathway as a consequence of production of immune complexes of virus with specific IgM and IgG (Bitsch, 2011). The condition is more prevalent in winter season than in other seasons of the year (Bitsch, 2011; Sharma *et al.*, 2005). Rabbit is a good lab animal for confirmation of BHM. Bacteria-free filtrate of whey obtained from the milk of the affected teat when injected intradermally in rabbits produces mild erythematous lesions 48 h after inoculation that persist for 48 h. Histopathologic examination of the skin of inoculated rabbits may show formation of syncytia (multinucleated cells resulting from fusion of single cells) and intranuclear inclusions in epidermal cells (Lokanadhamu *et al.*, 2005). Mice do not show any such reaction on inoculation (Rao *et al.*, 2003).

Many earlier workers had also reported the incidences of mammillitis due to herpes virus in cattle and buffaloes as reported in the present study (Gourreau *et al.*, 1989; Nauriyal and Randhawa, 1992; Sehrish Firyall *et al.*, 2019).

### **Examination of the Milk Samples**

The milk from the affected quarter was subjected to

California Mastitis Test (CMT) and further culture and sensitivity test (Christie and Koegh, 1940; Shridhar, 2014, 2020). The pH of the milk samples was also recorded. The somatic cell count of milk samples was done as per the method of Schalm *et al.*, (1971). Electrical conductivity was measured using conductivity meter (Sneath Peter *et al.*, 1986). Ultimately, none of the parameters indicated that the disease is mastitis.

### **Clinical Signs**

In most of infected cows (Janett *et al.*, 2000) and buffaloes, the disease is latent and sub-clinical. Only a few infected animals develop clinical signs. The clinical condition has a wide range of symptoms. Without any physical abnormalities, some affected animals may become hard milkers. Others may have minor teat lesions, while a small percentage of those infected suffer severe teat lesions. (Abd-El-Hady, 2015; Sandrucci *et al.*, 2014). According to (Sandrucci *et al.*, 2014; Shearer *et al.*, 2008), BHM in cows starts with painful raised edematous swellings of teats followed by appearance of vesicles (0.5 to 5 cm in diameter) which are irregular in shape. Within 24 h, these vesicles rupture leading to formation of ulcers that exude copious serum. Upon drying of this exudate, thick dark reddish-brown scabs appear on the teats. Healing usually occurs in 3 weeks, although ulcerated lesions may persist for months (Sandrucci *et al.*, 2014). Teat and udder lesions may fuse together and may extend to the perineum resulting in vulvovaginitis. Milking causes pain to the affected animal and sometimes the entire teat becomes necrotic. Calves suckling affected cows may develop lesions in mouth (George *et al.*, 2008).

According to (Sharma *et al.*, 1998), in dairy buffaloes, the lesions of BHM mostly occurred at the udder-teat junction of hind teats. The Punjabi term *Gulwaddee* (literally translated as 'neck cutter') for BHM derives its colloquial name from the occurrence of a deep non healing ulcerative lesion at the junction of teat with the udder i.e. neck of the teat. Raw ulcer or ulcer covered with thick blue-black scab was the most common presenting

lesion. The initial phase of the disease was characterized by the formation of localized plaques (2 to 5 mm) within the thickness of teat wall. These plaques were surrounded by inflammatory zones and ruptured within 48 h leaving very deep ulcers. Vesicle formation was not observed. As the healing progressed, a very thick blue-black scab developed and covered the healing lesion. Systemic signs of illness (e.g. rise in body temperature, pulse and respiration rates) were not present in the affected buffaloes. Milk of affected teats is usually negative for mastitis (Purohit *et al.*, 2014).

According to (Rao *et al.*, 2003), in buffaloes, the size of the affected teat increases 2 to 3 times its normal size with a glossy appearance. Severe inflammation of teat causes tenderness and loss of flexibility. Out of 101 cases examined, 28% cases developed ulcers on the affected teat whereas in 4% cases, there was local necrosis at the base of the teat. Partial sloughing of the teat was noticed in 12% of the cases. Because mastitis was not present, the milk's appearance remained unchanged. Sloughing of the whole teat followed, leaving only the tissue around the teat canal. When the calves suckled the unaffected teat(s), milk flowed spontaneously via the denuded teat canal from the damaged teat. In 9% of the animals, spontaneous recovery was found.

Sharma *et al.*, (2005) had described an unusual form of the disease in a few dairy buffaloes. This form of the disease started with sudden appearance of multiple flattened nodules (1 to 4 cm in diameter) all over the body. In the majority of affected buffaloes, these nodules appeared first on the perineum and later on extended to teats and udder. Some of these nodules became necrotic and sloughed off. Some affected buffaloes also showed systemic signs such as salivation, nasal and lacrimal discharge. BHM affected buffaloes sustained 31.54% reduction in milk yield. In agreement with the findings of Abd-El-Hady (2015), the condition affected buffaloes only and cattle dwelling in the same areas were spared. These signs are quite similar to those of pseudo-lumpy skin disease of cows which is also caused by the bovine herpesvirus 2 (Bitsch, 2011). Thickening of the wall of the affected teat may

result in narrowing of the teat canal and thus pain and difficulty in milking (Abd-El-Hady, 2015).

In dairy buffalo, vesicle formation (a characteristics feature of early stages of BHM in cow) is either absent (Sharma *et al.*, 1998; Sharma *et al.*, 2005) or occurs rarely (Lokanadhamu *et al.*, 2005). Annapurna *et al.*, (2013) briefed about a case of fibroadenoma of the mammary gland in a buffalo heifer where they observed the fibropapilloma of the teat of the buffaloes. Mammary gland neoplasms are commonest in dogs whereas they are relatively infrequent in mares, ewes, sows, cows and buffaloes. It is paradoxical that cattle with a highly developed mammary gland have the lowest incidence of mammary neoplasms. Cows rarely develop mammary tumours although many dairy cows are allowed to survive to cancer age. Perusal of the literature revealed reports of few cases of neoplasms in the mammary glands of buffaloes. Singh *et al.*, (1991) noticed the occurrence of papilloma and fibro adenoma of udder tissue in buffaloes in a survey of tumors in animals. Joshi *et al.*, (1994) recorded a case of fibropapilloma of the mammary gland in a buffalo calf.

### **Milk Sample Status**

Most of the workers investigated that the milk samples will be normal as Shridhar (2020) also reported that the milk drawn from the affected teat revealed negativity to presence of any pathological bacteria. Similar observation was also made by many researchers who also informed that the milk will be free from pathogenic bacteria from the milk of mammillitis affected teat (Thilagar *et al.*, 2000; Kachawa *et al.*, 2017; Purohit *et al.*, 2014).

### **Occurrence**

The reason for the occurrence in heifers is still unanswered but stated that it might be because of stress of calving particularly with udder edema and hormonal changes lead to activation of BHM virus and development of signs (Gibbs, 1984 ; Kemp *et al.*, 2008 ; Martin, 1973; Sankaram and Kotayya, 1977). The clinical condition was usually sporadic

in occurrence within first two months of lactation in the heifers of first calving preferably in winter season with hind quarter infection. Similar observation also made by other researchers (Mouli, 1992; Sharma *et al.*, 1998 ; Sharma and Singh, 2006). Seven out of ten animals that showed early signs of illness recovered normally once treatment was completed. Tose animals with necrotic rings on the teat, on the other hand, did not respond well to the treatment. After 7 days of therapy, teat edoema and hyperiamia gradually returned to normal. *Breyania patens* and *Sclerophyllum wallichianum* pastes moderately cure bovine mammillitis. Teat edema, hyperiamia slowly resolved to normal after 7 days of therapy. Paste of *Breyania patens* and *Sclerophyllum wallichianum* moderately cure the mammillitis in buffaloes (Sundaresh and Janaki, 1997; Misra, 1991). Different parts of these plants, which are known to have anti-inflammatory and anti-histaminic properties, may have played an important role in overcoming mammillitis and preventing further damage to the affected teat.

### **Diagnosis**

Diagnosis of BHM can be based on the composite of clinical signs, epidemiologic features, electron microscopy and serologic tests like agar gel precipitation test and neutralization test in rabbit (Janett *et al.*, 2000; Lokanadhamu *et al.*, 2005; Rao *et al.*, 2003). Histopathologic examination of biopsy samples taken from the lesions may reveal intranuclear inclusion bodies. In fact, the occurrence of multiple syncytia in the stratum spinosum layer containing basophilic or eosinophilic intranuclear inclusions is the characteristic diagnostic histologic feature of naturally occurring BHM-2 infection in buffaloes (Sharma *et al.*, 1998), cows (Rweyemamu *et al.*, 1969) and experimentally inoculated rabbits (Rao *et al.*, 2003). The condition may resemble pseudocowpox but in pseudocowpox, inclusion bodies are seen inside the cytoplasm and not in the nucleus (George *et al.*, 2008). Moreover, lesions of pseudocowpox are ring shaped (Sandrucci *et al.*, 2014). Other conditions that require consideration in differential diagnosis include stephanofilariasis (a parasitic disease of connective and subcutaneous



tissue caused by several nematode species of the genus *Stephanofilaria* e.g. *Stephanofilaria assamensis* that causes dermatitis in buffalo, goat and cattle called humpsore and *Stephanofilaria zeheeri* which is found in the inner side of the ear pinna of buffalo and causes earsore), nonspecific skin infections, photosensitivity, allergic reactions, chemical irritation and trauma (Kathirvel and Dharmaceelan, 2016), feedstuff exanthemata (Heidrich and Renk, 1967).

### **Treatment**

A combination of Whisprec ointment application and Teburb capsule oral administration resulted in a good cure (Shridhar, 2005). Therapy with various drugs and auto immune therapy had little success. (Rao, 2009). Bovine herpes mammillitis, in particular its ulcer at the junction of teat and udder does not respond to routine treatment (antibiotic injections, antibiotic creams/ointments, antiseptics etc. (Lokanadhamu *et al.*, 2005). Many cases in buffaloes are refractory to routine treatment for upto 3 months.

Because BHM is contagious (Bitsch, 2011), the affected animal(s) should be isolated immediately and milked after healthy animals. Boiling or disinfectants should be used to sterilise milking equipment used on affected animals (Kemp *et al.*, 2008). All other biosecurity measures should be implemented as well. In severe cases of thelitis (teat inflammation), a sterile teat syphon or Larson's teat tube (Abd-El-Hady, 2015) may be used to milk the affected teat (s). Post-milking antiseptic teat dipping with iodophore teat dip should be performed on all animals in the herd to prevent disease spread and aid in the healing of affected animals' lesions. In addition, insect control measures should be implemented right away.

Chronic, large sized indolent ulcer with scabs when present at the junction of teat and udder should first be properly debrided using a sterile scalpel blade. If the lesions are small, they can be cauterized by using silver nitrate stick held in hand with thick rubber gloves. For proper removal of the scab, the

use of water should be avoided when cauterization is attempted with silver nitrate stick. Some practitioner use lump of copper sulphate for debridement. Debridement with sterile scalpel blade or chemical cauterization with silver nitrate stick or copper sulphate lump should be continued until a light oozing of blood starts. Since the virus of bovine herpes mammillitis is sensitive to chloroform, after debridement, the affected area may be rinsed with chloroform followed by local application of an antibiotic cream or an insect repellent wound spray. Debridement or cauterization will have to be repeated 2 to 3 times at weekly intervals or as the situation warrants (Sharma and Singh, 2006).

Syed *et al.*, (2009) successfully treated a BHM affected cow with a 5 day treatment consisting of ceftriaxone (3 g daily IM), chlorpheniramine maleate (10 ml IM) and meloxicam (10 ml IM). In addition, the ulcerated area was treated with povidone-iodine ointment twice daily. Buffaloe mammalitis can also be treated with similar line (Shridhar, 2020).

### **Immunopotential**

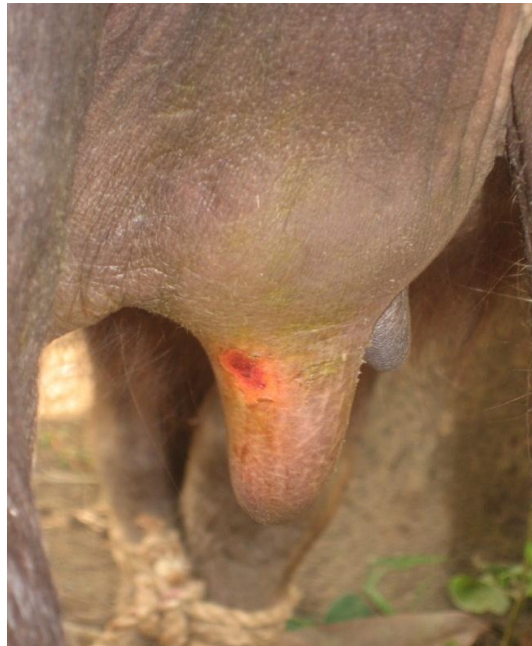
Since the cows and buffaloes that suffer from BHM are in a state of immunosuppression (Bitsch, 2011), immunity enhancing agents such as levamisole about 80 ml per cow or buffalo per day for 4 days PO), Inj. vitamin E-selenium, and zinc sulphate 2 to 3 g per cow or buffalo per day for 10 days can be used for improving the immunity against the herpes virus.

Lokanadhamu *et al.*, (2005) compared 3 treatment protocols for the treatment of herpes mammillitis in 24 buffaloes. Protocol A consisted of enrofloxacin (15 ml of 10% solution IM), diclofenic sodium (20 ml IM), pheniramine maleate (10 ml IM), acyclovir (800 mg; 3 tablets daily), topical application of tablet lysine and topical application of ointment acyclovir (5%). Protocol B was the same as protocol A except that oral administration of tab. where as acyclovir and topical application of lysine was omitted. Protocol C was the conventional treatment consisting of enrofloxacin, diclofenic sodium and

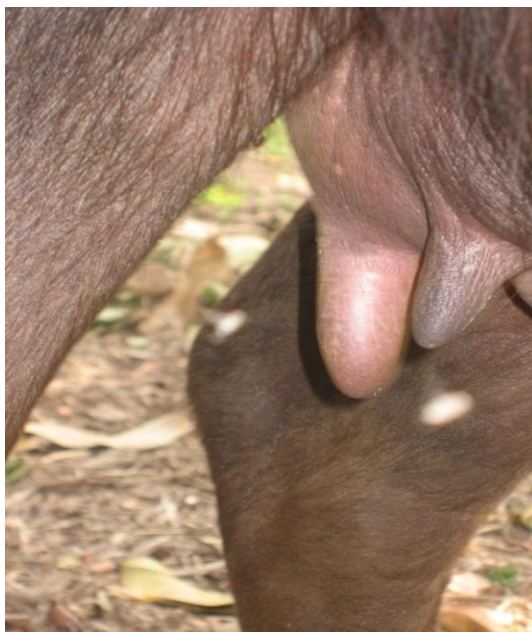
chlorpheniramine melete injections. The cure rate with protocol A was 62.5% in 12 days whereas the cure rate with protocol B was 37.5% within 24 days. The conventional treatment (protocol C) had the cure rate of only 12.5% after 35 days treatment. Acyclovir is an antiviral drug specifically effective against herpes infections. Lysine has been shown to reduce the severity of herpes infections in herpes

simplex infections of human (Griffith *et al.*, 1987). Other workers (Abd- El-Hady, 2015; Bentz *et al.*, 2006; Lokanadhamu *et al.*, 2005; Purohit *et al.*, 2014; Purohit and Markandeya, 2015) have also suggested the combined use of antibiotics, antihistamines and anti-inflammatory drug to hasten the recovery and to prevent secondary bacterial complications like mastitis.

**Fig.1** Hyperemic, edematous udder, ulceration and vesicles on the surface of the affected teats



**Fig.2** Recovery stage after treatment



**Fig.3** Formation of black necrotic ring at the base of the teat



**Fig.4** Fallen off teat with remnant



### **Control and Prevention**

One should not purchase BHM affected cows or buffaloes. Usually the entire herd will be affected and primiparous buffaloes are most susceptible

compared to the multiparous buffaloes (Shridhar, 2005, 2014, 2020). Check the udders and teats of animals at the time of their purchase. Healing of BHM may leave depigmented skin area on the teat (Gibbs *et al.*, 1970; Yager and Scott, 1992) which

may be used as an indication of an exposure of the cows and buffaloes to BHM virus. It is recommended that such animals should never be purchased. Currently, no commercial vaccine is available for immunization against BHM.

Since, edema of udder is a very important predisposing factor to BHM (Bitsch, 2011), all measures should be taken to prevent the occurrence of parturient udder edema and if it still occurs, appropriate therapy should be instituted in a timely manner.

Insect should be controlled as they have documented to transmit BHM virus (Bitsch, 2011). Milking time hygiene and post milking antiseptic teat dipping are obviously important particularly in the face of an outbreak of bovine herpes mammalities.

In some buffaloes which did not respond positively to the treatment and developed necrosis of the affected teat and later fallen off from the udder as per Shridhar (2020). When necrosis develops, the tissue will no longer have active cells to respond. The efficacy of lithium antimony thiomalate will reduce fibrosis through an unknown mechanism. Chlorpheniramine maleate is an antihistamine with antiallergenic properties. Meloxicam is an NSAID with anti-inflammatory properties (Shridhar *et al.*, 2014; Syed *et al.*, 2009). Lokanadhamu *et al.*, (2005) investigated the efficacy of oral and topical acyclovir, a well-known anti-herpes drug, in buffalo mammillitis. It inhibits viral DNA polymerase by converting to its triphosphate form, acyclovir triphosphate (ACV-TP), which incorporates into and terminates the growing viral DNA chain, and inactivates the viral DNA polymerase.

Watson (1989) explained the anti-herpes activity of the bovine herpes mammillitis (BHV-2) virus. The oral kinetics of acyclovir in ruminants are unknown. In horses with herpes virus infection, oral acyclovir did not achieve the required plasma concentration, whereas its pro-drug valacyclovir did after IV administration (EHV-1). Valacyclovir is an acyclovir prodrug that may be an appealing and

valuable candidate for treating EHV-1 infections in horses.

Acyclovir (20 mg/kg) given orally to horses did not eradicate EHV-1 plasma concentrations, which could not be achieved at the site of action.

As a result, oral acyclovir treatment in horses was found to be ineffective for EHV1 infection (Bentz Garré *et al.*, 2006). However, in cell culture studies, acyclovir was able to eliminate BHV-1 virus titre and the progression of cytopathic effect (Enan *et al.*, 2012).

Acyclovir was found to be effective against BHV-2 infection. Based on previous observations, parenteral administration of acyclovir was preferred to ensure good plasma concentration for better antiviral activity (Watson, 1989; Rollinson, 1992; Sehrish Firyall *et al.*, 2019). Chlorpheniramine maleate is an antihistamine with an H1 receptor antagonist that is used to treat a variety of conditions in cattle and buffaloes. It is used to treat cattle and buffalo allergies. It is used as an anti-allergic drug to reduce allergic reactions, which has been shown to be effective in previous studies (Lokanadhamu *et al.*, 2005; Vardanyan and Hruby, 2006; Neelesh Sharma *et al.*, 2006). Shridhar (2020) used meloxicam as an analgesic and anti-inflammatory agent to alleviate pain and inflammation. It is a well-known that NSAIDs are commonly used in the treatment of bovine mastitis. It has a low ulcerogenic property because it is a preferential COX-2 inhibitor with a 17 h half life in cattle (Mathews, 2002; Breen, 2017).

According to Shridhar (2014), calves were not allowed to suckle the affected teat because suckling and forced milking could cause further damage to the teat (Sehrish Firyall *et al.*, 2019). More likely primiparous buffaloes in first calving do suffer from a condition known as mammalitis which in which there will be characteristic teat inflammation, enlargement, difficulty in milking, difficult for the calf to suckle followed by black necrotic ring formation and finally the teat will slough off from udder. The disease is caused by bovine herpes virus



(BHV-2). The buffaloes suffering from the mammalitis were treated with different regimens.

### Conflict of Interest

No conflict of interest.

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