

Short Communication



Bacteria Associated with Conjunctivitis in *Bubalus arnee fulvus* (Swamp Buffalo) in Nagaland

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Abstract | Bacteriology of conjunctivitis cases in swamp buffaloes indicated that *Enterobacter agglomerans* in association of other bacteria including *Escherichia coli* and *Pseudomonas* may be an important eye pathogen causing conjunctivitis in male swamp buffaloes. However in female swamp buffaloes only one kind of bacteria (no mixed infection) was detected and conjunctivitis might be associated with *E. coli*, *Aeromonas media* or *Enterococcus faecalis* infection. The study indicated that ciprofloxacin, gentamicin and tetracycline may be effective antimicrobials in therapy of the conjunctivitis in swamp buffaloes.

Keywords | *Enterobacter agglomerans*, *Aeromonas media*, *Vibrio anguillarum*, *Pseudomonas aeruginosa*, Pink-eye

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Bubalus arnee fulvus (swamp buffalo) is extant in North Eastern parts of India including Nagaland (Groves, 1996). However, only little is understood about its diseases (Singh et al., 2012) probably because of its wild/ semi-wild nature. Eye infections are quite irritating and may alter the mood of animals; they are commonly reported in domestic buffaloes and cattle (Ali et al., 2011) but rarely diagnosed in swamp buffaloes (Singh et al., 2012). We report here bacteriological analysis of seven cases (three in female and four in male buffaloes) of conjunctivitis (pink-eye) in swamp buffaloes (Table 1). In February 2013, we came across seven cases of conjunctivitis in swamp buffaloes in different parts of Medziphema subdivision of Nagaland while organizing veterinary health camps. All cases brought into notice were from animals grazed on banks of Cathe (Pugla) River. From December onward till March end swamp buffaloes are allowed to roam free in empty field for grazing and intermixing of buffaloes from different households take place. This period is time of scarcity of water and fodder in the area. Swamp buffaloes are not reared on organized farms but reared in number of one or two by farmers for using as draught animal for ploughing the fields. There is no specific breed or animal density description.

The disease was evident by weeping eye discharges with or

without white/ creamy discharges, sticking on eye lashes or canthi. Affected buffaloes were properly restrained in cattle crush and swabs were collected from conjunctivae of affected eyes in one ml of sterile buffered peptone water (BPW, Hi-Media, Mumbai). Swabs were brought to laboratory for bacteriological examination using standard bacteriological culture technique for mesophilic aerobic and microaerobic bacteria to determine the causal organism (Singh, 2009; Quinn et al., 1994). Briefly, 2µL of the BPW from sample container was plated onto blood agar (BA, 5% defibrinated sheep blood in blood agar base), and MacConkey agar (MA, Hi-Media) plates each, incubated at 37°C for 24-48 h. All different types of colonies were counted if countable. Three to five colonies of each type appearing on the MA or BA or both were picked up and re-streaked on BA for purity. All isolates were characterized using growth, staining and biochemical characteristics (Singh, 2009; Quinn et al., 1994). All the isolates were tested for sensitivity to antimicrobial drugs through disk diffusion method (CLSI, 2006) against amoxicillin 30µg (Am), amoxicillin+ clavulanic acid 50+10µg (Amc), amoxicillin+ sulbactam 30+15µg (Ams), ampicillin 10µg (A), azithromycin 30µg (Azm), aztreonam 50µg (Azm), cefotaxime 10µg (Cft), cefoxitin 30µg (Cxt), ceftriaxone 10µg (Ctr), chloramphenicol 25µg (C), ciprofloxacin 10µg

Table 1: Details of cases of conjunctivitis in swamp buffaloes and isolated pathogens

Case number and description of swamp buffaloes with conjunctivitis*, place	Bacteria isolated	CFU/ 2 µl	Resistance to antimicrobials
Adult male (~3 years), Peren	<i>Pseudomonas aeruginosa</i> **	87	A, P, Nit, C, Cd, Pb, Cx, At, L
	<i>Enterobacter agglomerans</i> **	UC	A, P, Cd, Cl, Pb, Cx, At, L
	<i>Enterobacter agglomerans</i> **	UC	A, P, Nit, Cd, Pb, Cx, At, L
She buffalo (>7 years) calved twice, Peren	<i>Escherichia coli</i> **	UC	A, P, Cd, Pb, Cx, At, L
Adult male (~5 years), Peren	<i>Pseudomonas aeruginosa</i>	76	A, P, Cd, AT, L
	<i>Enterobacter agglomerans</i> **	UC	A, P, Nit, Cd, Cl, Pb, Cx, L
She buffalo (~5 years) calved once, Kukidolong	<i>Enterococcus faecalis</i> **	UC	A, P, Na, Cl, Pb, Cx, At, L
She buffalo (~3 years) not calved, Kukidolong	<i>Aeromonas media</i>	UC	P, Cx, At, L
Adult male (~5 years), Jharnapani	<i>Vibrio anguillarum</i> **	117	A, P, Nit, Amx, Ams, Amc, Cd, Cl, Pb, Cx, At, L
	<i>Escherichia coli</i> **	17	A, P, Cd, Pb, Cx, At, L
	<i>Enterobacter agglomerans</i>	UC	P, C, cd, Cx, At, L
	<i>Ps. pseudoalcaligenes</i>	9	P, Cd, Cx, At, L
Adult male (~5 years), Jharnapani	<i>Enterobacter agglomerans</i> **	UC	A, P, Imp, Cd, Pb, Cx, At, L
	<i>Escherichia coli</i> **	UC	A, P, Cd, Pb, Cx, At, L
	<i>Escherichia coli</i>	UC	A, P, Cd, Pb, Cx, L

*All were adult buffaloes of >2.5 years of age. All cases were brought to notice in first and second week of February 2013 in Meziphema region of Nagland in vicinity of Cathe (Pugla) River. From December onward till March end (period of water and fodder scarcity in the region) swamp buffaloes were allowed to roam free in empty field for grazing and intermixing of buffaloes from different households takes place. Swamp buffaloes were not reared on organized farms but reared in number of one or two by farmers for using as draught animal for ploughing the fields. There is no specific breed or animal density description available. Case numbers 1, 2, 3 were from Peren camp, 4, 5 from Kukidolong camp and 6, 7 from Jharnapani camp.

**MDR strain, resistant to three or more commonly used drugs (for counting MDR, resistance to one or more antibiotics of a group was counted as one, such as A, Amx, Ams, Amc belongs to one group; Cx, Ctx, Ctr belongs to one group; resistance to P, Cd, Cl, Pb, and L was not counted for MDR; CFU, colony forming units on blood agar and or MacConkey agar; UC, uncountable (>200); Amc: amoxicillin+ clavulanic acid; Ams: amoxicillin+ sublactam; Amx: amoxicillin; A: ampicillin; At: aztreonam; Azm: azithromycin; Ctx: cefotaxime; Cx: cefoxitin; Ctr: ceftriaxone; C: chloramphenicol; Cf: ciprofloxacin; Cd: clindamycin; Cl: colistin; Co: cotrimoxazole; G: gentamicin; Imp: imipenem; L: lincomycin; Na: nalidixic acid; Nit: nitrofurantoin; P: penicillin; Pb: polymyxin B; T: tetracycline.

(Cf), clindamycin 10µg (Cd), colistin 25µg (Cl), cotrimoxazole 25µg (Co), gentamicin 30µg (G), imipenem 10µg (Imp), lincomycin 10µg (L) nalidixic acid 30µg (Na), nitrofurantoin 300µg (Nf), penicillin 10 IU (P), polymyxin B 50IU (Pb), and tetracycline 30µg (T) discs (Hi-Media, Mumbai) on Mueller Hinton agar (High-Media) plates. Isolates resistant to three or more antimicrobial groups were considered multiple-drug resistant (MDR) type. All isolates resistant to imipenem were tested using Ezy MIC strips (Hi-Media) to determine production of extended spectrum β-lactamase (triple ESBL detection strip) or metallo-β-lactamase (meropenem with and without EDTA) as per manufacturer's guidelines. Multiple isolates of a species of bacteria from any case but having similar growth, staining, biochemical and antimicrobial sensitivity characters were considered as single isolate.

A total of 15 isolates of bacteria were detected from swabs in the study. Conjunctival swabs from all seven cases were positive for one (3) or more (4) types of bacteria (Table 1). Isolates of *Enterobacter agglomerans* (5) were the most common bacteria followed by *Escherichia coli* (4) and *Pseu-*

domonas aeruginosa (2) detected from swabs of 4, 3 and 2 of the affected animals, respectively. From three cases of she-buffaloes, only one type of bacteria was detected from both of the eyes of the affected animals, one animal each was positive for *A. media*, *E. coli* and *Ec. faecalis*. Though the most commonly isolated from conjunctival swabs of male buffaloes, *E. agglomerans* were not detected from any of the three she-buffaloes with conjunctivitis. However, multiplicity of bacteria was evident in conjunctival swabs of male buffaloes. All the four he-buffaloes with conjunctivitis had *E. agglomerans* common in all cases while other bacteria were present in only one or two cases including *P. aeruginosa* (2), *E. coli* (2), *Pseudomonas pseudoalcaligenes* (1) and *Vibrio anguillarum* (1) only. All the bacterial isolates were resistant to penicillin and lincomycin, but sensitive to gentamicin, tetracycline, cotrimoxazole, ciprofloxacin, cefotaxime, ceftriaxone and azithromycin. Of the total 15 bacterial isolates tested seven had MDR (Table 1). The MDR strains belonged to *Enterobacter agglomerans* (from all four animals), *Ec. faecalis* (1), *P. aeruginosa* (1) and *V. anguillarum* (1) species only. None of the four isolates of

E. coli and one each of *A. media* and *P. pseudoalcaligenes* was resistant to more than two types of drugs. None of the 15 except an *Ec. faecalis* isolate was resistant to nalidixic acid. Similarly, only one isolate of *V. anguillarum* was resistant to amoxicillin and its combinations with sulbactam and clavulanic acid. Imipenem resistance was detected only in an isolate of *En. agglomerans*. However, it did not have either extended spectrum β -lactamase or metallo- β -lactamase activity and its MIC for meropenem and ceftazidime+cefotaxime+ cefepime was 16 $\mu\text{g}/\text{ml}$ and 0.38 $\mu\text{g}/\text{ml}$, respectively.

In the study most of the isolates were of Gram negative bacteria (GNB) and an *Ec. faecalis* isolated in pure culture was the only Gram positive bacterial (GPB) isolate identified in the study. The results are in agreement to earlier observations (Tantivanich et al., 1988) showing GPBs as the common bacteria in healthy eyes of swamp buffaloes where GNBs were of rare occurrence. The study made it evident that *En. agglomerans* in association with one or more types of potentially pathogenic bacteria might be an important cause of conjunctivitis in male swamp buffaloes in Nagaland. Results are in concurrence to earlier study (Singh et al., 2012) reporting *En. agglomerans* and *P. aeruginosa* as cause of conjunctivitis in swamp buffaloes. In human beings, *En. agglomerans* with other pathogens has been reported to be an important cause of conjunctivitis (Bottone and Schneierson, 1972; Ben-Tovim et al., 1974; Sowka et al., 2001). Therefore, *En. agglomerans* and *P. aeruginosa* isolated as cause of conjunctivitis in male buffaloes might be considered an important zoonotic risk to animal handlers, however, need further confirmation. Another important pathogen causing conjunctivitis in swamp buffaloes might be *E. coli*, which was detected either as the sole isolate or along with *En. agglomerans*. *Escherichia coli*, though common in environment has often been reported in association with eye infections (Golshani et al., 2013).

Though isolation of *V. anguillarum* as cause of conjunctivitis is rare its inherent virulence potential (Milton et al., 1996) might be responsible for its association with conjunctivitis in swamp buffaloes. Moreover, it is considered as halophilic organism (Pazos et al., 1993) and its isolation from buffalo might be important; the salty nature of tears in conjunctival sac might be associated with growth of the bacterium in swamp buffalo eyes.

Isolation of *A. media* as cause of conjunctivitis from a she-swamp buffalo appears to be first report but aeromonads have been recognized as potential eye pathogens in humans since long (Sartory, 1999).

Isolation of multiple drug resistant (MDR) bacteria from swamp buffaloes, the animals which are rarely been treated in Nagaland with antibiotics, though looks awkward but

isolation of MDR strains of several bacteria from clinical as well as environmental sources in Nagaland has commonly been reported (Singh, 2012; Singh et al., 2012, 2013, 2014a, 2014b and 2014c) from varied sources including from swamp buffaloes indicating circulation of the antibiotic resistant strains in Nagaland. Emergence of MDR is bacteria may not be always associated with use of antibiotics in the host there may also be several other factor leading to existence of MDR strains even in those places where no earlier antimicrobial exposure was recorded (Kumar and Singh, 2014).

Though study was on limited number of cases, the study is of veterinary clinical significance in little explored field of swamp buffalo diseases. The study provides an insight into microbiology of eye infections of swamp buffaloes.

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CONFLICT OF INTEREST

There exists no conflict of interest.

AUTHORS' CONTRIBUTION

Planning, isolation and identification of bacteria, their testing and manuscript preparation was performed by Bhoj Raj Singh. Vidya singh and Raj Karan Singh did planning, organization of animal health camp, identification of cases, collection of clinical samples, sample transportation and preparation of manuscript.

REFERENCES

- Ali HH, Al-Abidy HF, Mechael NS (2011). Isolation and identification of conjunctival bacteria in cattle in Mosul. Iraq. J. Vet. Sci. 25: 11-14.
- Ben-Tovim T, Eylan E, Romano A, Stein R (1974). Gram-negative bacteria isolated from external eye infections. Infect. 2: 162-165. <http://dx.doi.org/10.1007/BF01642238>
- Bottone E, Schneierson SS (1972). *Erwinia* species: An emerging human pathogen. Am. J. Clin. Pathol. 57: 400-405.
- Clinical and Laboratory Standards Institute. 2006. Performance standards for antimicrobial disk susceptibility tests. Approved Standards, 9th ed. Document M02-A9, and M100-S18-Seventeenth Informational Supplement, Wayne.798p.
- Golshani C, Sachdeva S, Hoffner W, Khan N (2013). *Escherichia coli* conjunctivitis. Cons. Ped. 12: 426.
- Groves CP (1996). The taxonomy of the Asian wild buffalo from the Asian mainland. Int. J. Mammal. Biol. 61: 327-338.

- Kumar S, Singh BR (2013). An overview on mechanisms and emergence of antimicrobials drug resistance. *Adv. Anim. Vet. Sci.* 1(2S): 7 – 14.
- Milton DL, O'Toole R, Ho-Rstedt P, Wolf-Watz H (1996). Flagellin A is essential for the virulence of *Vibrio anguillarum*. *J. Bacteriol.* 178: 1310–1319.
- Pazos F, Santos Y, Magarinos B, Bandin I, Nunez S, Toranzo AE (1993). Phenotypic characteristics and virulence of *Vibrio anguillarum*-related organisms. *Appl. Environ. Microbiol.* 59: 2969-2976.
- Quinn PJ, Carter ME, Markey B, Carter GR (1994). *Clinical Veterinary Microbiology*. Mosby, Elsevier Lts. Spain. ISBN 07234 17113. Pp. 388.
- Sartory DP (1999). *Aeromonas*. World Health Organization. Viewed on 6th April 2014 at http://www.who.int/water_sanitation_health/dwq/admicrob2.pdf
- Singh BR (2009). Lab top for Microbiology Laboratory. Lambert Academic Publishing, AG and Co. KG, Koln, Germany. Pp. 83.
- Singh BR, Singh V, Singh RK (2012). Multiple drug resistant *Enterobacter agglomerans* and *Pseudomonas aeruginosa* causing conjunctivitis in swamp buffaloes in Nagaland. *Noto-are Med.* Viewed on 10th October 2013 at: <https://www.notoare.com/index.php/index/explorer/getPDF/14612376>
- Singh BR (2012). Drug resistance in enteroptahogens of zoonotic significance isolated from diarrhoeic pigs and house sparrow perching in piggery in Jharnapani, Nagaland. *Noto-are Med.* Viewed on 10th October 2013 at: <https://www.notoare.com/17378234>
- Singh BR, Singh RK, Singh V (2014a). Microbial quality and safety of axone -akhuni, a fermented soybean food of Nagaland. *Noto-are Med.* Viewed on 4th April 2014 at: <http://www.notoare.com/15185525>
- Singh BR, Singh V, Ebibeni N, Singh RK (2014b). Maternal transfer of bacteria to eggs of common house gecko (*Hemidactylus frenatus*). *J. Microbiol. Res.* 4: 78-85.
- Singh BR, Singh RK, Singh V (2014c). Carriage of multiple drug resistant bacteria in vagina of apparently healthy swamp buffaloes in Nagaland. *Adv. Anim. Vet. Sci.* 2(5): 292-295. <http://dx.doi.org/10.14737/journal.aavs/2014/2.5.292.295>
- Singh BR, Singh V, Ebibeni N, Singh RK (2013). Antimicrobial and herbal drug resistance in enteric bacteria isolated from faecal droppings of common house lizard/gecko (*Hemidactylus frenatus*). *Int. J. Microbiol.* 8p. <http://dx.doi.org/10.1155/2013/340848>
- Sowka JW, Gurwood AS, Kabat AG (2001). Acute bacterial Conjunctivitis. In: *Handbook of Ocular Disease Management*, Jobson Publishing, L.L.C. Viewed on 10th October 2013 at: <http://cms.revoptom.com/handbook/>
- Tantivanich P, Suttipong P, Naweparp O (1988). Conjunctival flora of normal Thai swamp buffalo. *Thai. Vet. Med.* 18: 159-165.