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ORIGINAL ARTICLE

Microbial Contaminants in Fresh and Extended Turkey Semen and their Sensitivity to Antibiotics

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SUMMARY

Microorganisms that inhabit the avian cloaca usually contaminate poultry semen which could easily spread throughout an entire flock. This study was conducted to determine the presence of microbial contaminants in turkey semen and evaluate their antibiotic sensitivity. Semen was collected from each tom, pooled and then divided into two aliquots A and B. Aliquot A was immediately evaluated for microbial contaminants and antibiotic sensitivity while aliquot B, was extended and preserved for 24 hours at 4^0 C and thereafter microbial culture, identification and antibiotic sensitivity were conducted. *Escherichia coli, Enterococcus faecalis, Bacillus subtilis, Corynebacteria species* and a fungal organism *Candida albican* were isolated and identified in both aliquots. All the identified organisms were sensitive to pefloxacin, gentamicin and ciprofloxacin, while *Enterococcus faecalis*, *Bacillus subtilis* and *Corynebacteria species* were resistant to Ampicillin-cloxacillin, cefuroxime, amoxicillin and ceftriaxone. *Escherichia coli* was only resistant to co-trimoxazole, ofloxacin and nalidixic acid. The study concludes that, *Escherichia coli, Enterococcus foecalis, Bacillus subtilis, Corynebacteria species* and *Candida albican* were found to be turkey semen contaminants and were resistant to penicillin and streptomycin combination in turkey semen extender but sensitive to pefloxacin, gentamicin and ciprofloxacin.

Key words: Microbial contaminants, turkey semen, extender, antibiotic sensitivity.

INTRODUCTION

The cloaca is a common outlet for the alimentary, urinary and genital tracts of birds and some lower vertebrates (Boden, 2005). Microorganisms that inhabit the avian cloaca include coliforms, non-lactose fermenters, *Campylobacter*, *Clostridium*, *Lactobacillus* and yeast such as *Candida albicans* (Cox *et al.*, 2002a; Cox *et al.*, 2002b; Donoghue *et al.*, 2004; Haines *et al.*, 2013) Previous studies have

demonstrated these organisms in ejaculated chicken semen and was found to have deleterious effects on semen quality parameters (Reiber *et al.*, 1995; Haines *et al.*, 2013). Turkey semen collected for artificial insemination is often pooled and then used to inseminate multiple hens, thus contaminated semen could easily spread these organisms throughout an entire flock. Commercially available poultry semen extenders are fortified with antibiotics to reduce or eliminate contaminating microorganisms (Hafez and Hafez, 2000). However, studies have shown that poultry semen extenders containing antibiotics were not consistent in reducing microbial concentrations in turkey semen (Donoghue *et al.*, 2004). Although some studies have been published on semen contaminants in various poultry species, little had been reported in turkey semen. Thus, the objectives of this study were to demonstrate the presence of microbial contaminants in turkey semen and evaluate their antibiotic sensitivity.

MATERIALS AND METHODS

Semen was collected using the abdominal massage method described by Burrows and Quinn (1937). Single ejaculates were collected from each tom between 7:00 and 9:00 AM twice weekly as described by (Noirault *et al.*, 2006). Five replicates were collected from each tom, pooled and then divided into two aliquots A and B. Aliquot A was immediately evaluated for microbial contaminants and antibiotic sensitivity while aliquot B, was extended in egg yolk based extender containing egg yolk 5%, sodium chloride 120mM, potassium chloride 5mM, potassium dihydrogen phosphate 10mM, magnesium phosphate heptahydrate 5mM, tris hydrochloride 1mM, glucose 1%, penicillin G and streptomycin 1g/l. the extended aliquot B was stored for 24 hours at 4^0 C and thereafter sent for microbial culture and sensitivity. Sensitivity test was done using the conventional disc inhibition test. Organism was classified as sensitive to the test antibiotic when the zone of inhibition was greater or equal to the reference value for the organism. Observations were presented in tables.

Laboratory isolation and identification of microbial contaminants

The semen samples were inoculated onto Blood and MacConkey agar and incubated aerobically and anaerobically at 37°C for 24 hours to obtain bacterial colonies. Colonial characteristics including size, shape, colour and character were observed and recorded. Bacterial colonies were stained with Gram's staining techniques and examined for staining properties and cellular morphology under x100 objective of light microscope. Single isolated colony from both blood and MacConkey agar, were subsequently sub cultured onto selective/differential media and subsequently nutrient broth to obtain pure culture. The pure cultures were transferred on to nutrient agar slants for biochemical test using standard bacteriological procedures according to Cowan and Steel (1974).

Disc diffusion antibiotic susceptibility test

Disc diffusion was tested using fifteen (15), common commercially prepared antibiotic impregnated discs (Oxoid Ltd., Basingstoke, United Kingdom): Pefloxacin (5 µg), Gentamicin (30 µg), Ampicillin-cloxacillin (10 µg), Cefuroxime (30 µg), Amoxycillin (10 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Streptomycin (25 µg), Co-trimoxazole (1.25/23.75 µg), Erythromycin (30 µg),

Ofloxacin (5 µg), Cefalexin (30 µg), Nalidixic acid, Ampicillin (25 µg) and Amoxicillin Clavulanate (30 µg). Disc diffusion or Kirby Bauer antibiotic susceptibility test was carried out according to method described by Clinical Laboratories Standards Institute (CLSI) C.L.S.I (2007). An overnight culture of the identified, grown on nutrient agar was used, to prepare the inoculum. One (1) isolated colony (1 to 2 mm in diameter) was taken with a sterile wire inoculating loop with the bacterial material visible on the edge of the wire loop and inoculated into tube containing 2 mL sterile isotonic saline gently mixed. The mixture was further agitated at least 10 times using a Pasteur pipette and compared with of McFarlan turbidity standard 0.5. If necessary, the sterile isotonic saline was added until the mixture matched the McFarlan turbidity standard. Each bacterial inoculum was swabbed onto the surface of 150 mm diameter Mueller-Hinton agar (MHA) plate using a sterile swab. Up to six commercially prepared, antibiotic impregnated paper discs were placed on each plate of the inoculated MHA surface and gently pressed down with sterile thumb forceps to ensure adequate contact with the MHA. Plates were incubated for 16-24 h at 37 ^{o}C (Jorgensen and Ferraro, 1998).

RESULTS

Microorganisms isolated and identified from turkey semen

A total of five microorganisms were isolated and identified as *Escherichia coli, Enterococcus faecalis, Bacillus subtilis, Corynebacteria species* and a fungal organism *Candida albican.* These organisms were found in both freshly collected semen as well as extended and chilled preserved semen. *Escherichia coli* was found to be predominant isolate followed by *Enterococcus faecalis*. The summary is presented in table I.

Antibiotic sensitivity

Table II presents the summary of the antibiotic sensitivity test with some selected antibiotics for all the organisms identified except *Candida albican*. All the organisms tested were sensitive to pefloxacin, gentamicin and ciprofloxacin. Similarly, all the strains of *Enterococcus faecalis, Bacillus subtilis* and *Corynebacteria species* were resistant to Ampicillin-cloxacillin, cefuroxime, amoxicillin and ceftriaxone while *Escherichia coli* was only resistant to co-trimoxazole, ofloxacin and nalidixic acid.

Table I. Detection of microorganisms in pooled turkey semen

Organism	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
	A	B	A	B	A	B	A	B	A	B
Escherichia coli	+	+	+	+	+	-	+	+	+	-
Enterococcus faecalis	+	+	-	-	+	+	+	+	+	+
Bacillus subtilis	+	-	+	+	+	-	+	+	+	+
Corynebacteria species	-	-	+	-	+	+	+	-	+	-
Candida albican	+	+	-	-	-	-	+	+	+	+

Table II: Antibiotic sensitivity of bacterial strains isolated from turkey semen

Antibiotic	*Escherichia coli*	*Enterococcus faecalis*	*Bacillus subtilis*	*Corynebacteria species*
Pefloxacin	Sensitive	Sensitive	Sensitive	Sensitive
Gentamicin	Sensitive	Sensitive	Sensitive	Sensitive
Ampicillin-cloxacillin	-	Resistant	Resistant	Resistant
Cefuroxime	-	Resistant	Resistant	Resistant
Amoxicillin	-	Resistant	Resistant	Resistant
Ceftriaxone	-	Resistant	Resistant	Resistant
Ciprofloxacin	Sensitive	Sensitive	Sensitive	Sensitive
Streptomycin	Sensitive	Resistant	Sensitive	Resistant
Co-trimoxazole	Resistant	Resistant	Sensitive	Resistant
Erythromycin	-	Resistant	Sensitive	Resistant
Ofloxacin	Resistant	-	-	-
Cefalexin	Sensitive	-	-	-
Nalidixic acid	Resistant	-	-	-
Ampicillin	Sensitive	-	-	-
Amoxicillin Clavulanate	Sensitive	-	-	-

DISCUSSION

Microorganisms that often contaminate avian semen were known to be resident in the cloaca and usually found in faeces of birds (Haines *et al.*, 2013). In the present study, *Escherichia coli* have been isolated which is among the coliforms earlier reported in fowl semen (Reiber *et al.*, 1995; Haines *et al.*, 2013). Similarly, Gram positive bacteria including *Enterococcus faecalis, Bacillus subtilis* and *Corynebacteria species* were also isolated in the present study. This is similar to previous studies on cock semen by Reiber *et al.* (1995). *Enterococci* and *Bacillus* species have been classified as natural intestinal flora of most mammals and birds (Barbosa *et al.*, 2005; Tam *et al.*, 2006), likewise *Candida albicans* (Burrello *et al.*, 2004). Thus the presence of these organisms in semen indicates faecal contamination of the semen. Effect of these contaminants on semen quality is still not fully elucidated (Haines *et al.*, 2013). However, semen quality parameters including sperm motility and morphology have been affected by the presence of microbial contaminants perhaps due to release of endotoxins from dying microorganisms (Haines *et al.*, 2013). Moreover, *Escherichia coli* has been shown to reduce sperm motility in ram semen (Zan Bar *et al.*, 2008; Yaniz *et al.*, 2010) as well as in boar semen (Bussalleu *et al.*, 2011). Therefore, the presence of bacteria in turkey semen and their multiplication could result in deterioration of semen samples *in vitro.*

In the present study, preserving turkey semen in an extender fortified with penicillin and streptomycin did not completely inhibit bacterial growth. This finding is supported by an earlier report which shows that, poultry semen extenders containing antibiotics were not consistent in reducing microbial concentrations in poultry semen (Sexton *et al.*, 1980). Similarly, recent studies using commercially available semen extenders containing various combinations of antibiotics have failed to significantly reduce or eliminate bacterial growth in turkey semen (Donoghue *et al.*, 2004).

The antibiotic susceptibility test in the current study had indicated that all the identified organisms tested were sensitive to pefloxacin, gentamicin and ciprofloxacin. This is similar to reports by (Donoghue *et al.*, 2004). In addition, gentamicin is among the most widely used

antibiotic in poultry semen extenders sold commercially (Sexton *et al.*, 1980). However, there is need for antibiotic sensitivity testing to determine the most suitable antibiotic to be incorporated in turkey semen extenders.

CONCLUSION AND RECOMMENDATION

From the data presented in this study, we conclude that microorganisms including *Escherichia coli, Enterococcus foecalis, Bacillus subtilis, Corynebacteria species* and *Candida albican* were found contaminating turkey semen and were resistant to penicillin and streptomycin combination which are the conventional antibiotic supplement in extenders. However, they were sensitive to pefloxacin, gentamicin and ciprofloxacin.

In light of our observations, we recommend that turkey semen should be evaluated for microbial contaminants and antibiotic sensitivity test be conducted to determine a suitable antibiotic for extender preparation. Similarly, antimycotic agents could be used to supplement turkey semen

Studies should be conducted to devise strategies of eliminating microbial contaminants in turkey semen. Similarly, more studies should also be conducted to determine the influence of antibiotics and microbial contaminants on turkey semen quality.

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