

## Short Communication

# Isolation of Antibiotic Resistant *Salmonella* Organisms from Aborted Cows and Emu Birds from District Ludhiana, Punjab, India

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**Abstract** | We investigated 102 samples (faecal swabs of live birds, spleen, caecum, liver and congested intestines of dead birds and uterine discharges, and diarrheal faeces of cattle, buffaloes and horses) for the presence of *Salmonella* organisms. *Salmonella* could be isolated from nine samples (7 from uterine fluid of aborted cows and 2 from fecal swabs of Emu birds). Identification of the isolates was done on the basis of cultural, staining and biochemical characteristics. All the 9 isolates were negative for oxidase and positive for catalase, motility indole lysine, and citrate utilization tests. The isolates were confirmed to be *Salmonella* by PCR using *Salmonella* genus specific oligonucleotide primers which yielded the desired amplicons of 496-bp. This indicates that *Salmonella* may also be associated with bacterial abortion and infertility in cattle. We found resistance to certain antibiotics among *Salmonella* and suggest here some of the most effective antibiotics for use in the field. The isolated organisms were found to be resistant to erythromycin and penicillin and sensitive to gatifloxacin, gentamicin, ciprofloxacin, norfloxacin, doxycycline, cefoparazone, ceftaxime, and oxytetracycline. Salmonellosis being a zoonosis, the findings are of public health significance as well.

**Keywords** | *Salmonella*, Abortion, Antibiotic Resistance, Cow, Emu

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*Salmonella* causes diarrhoea, dehydration, abortion, pneumonia, septic arthritis, meningitis, gangrene of distal extremities, and sudden death in domestic animals. It is a zoonotic disease which manifests as gastroenteritis, enteric fever, and a carrier state in most of the affected species. It decreases productivity and causes mortality in domestic animals, inflicting great economic losses to the dairy farmer, and is of public health significance.

The Centre for Disease Control and Prevention (CDC) reported that 9.4 million food borne illnesses,

55,961 hospitalizations and 1351 deaths occur in the United States each year (Scallan and Hoekstra, 2011). *Salmonella* is a major food borne bacteria which causes infection in humans as well as in food producing animals such as cattle, poultry and swine (Lynne et al., 2009; Guibourdenche et al., 2010). The infection of *Salmonella* is mostly due to contaminated food products from poultry, pigs and ruminants, contaminated drinking water or direct contact with infected animals (Mullner et al., 2009; Abdullah et al., 2010). In case of cattle and buffaloes it may cause abortion and infertility and leads to productive and economic loss (Verma

et al., 1999). The organism may either cause abortion, or be associated with stillbirths or with the birth of normal healthy full-term calves (Hinton, 1971). *Salmonella* Enteritidis is an important cause of human illness with symptoms typically including fever, vomiting, diarrhea and abdominal cramps 12-72 hours after ingestion of the bacterium (CDC, 2010) and is responsible for millions of human cases of gastroenteritis and deaths worldwide (Majowicz et al., 2010).

Due to increase in use of antimicrobial agents in food animals as growth promoters, prophylactic agents and therapeutic remedies, increasing number of bacterial strains are gaining resistance (Zewdu and Cornelius 2009; Alexander et al., 2008). Nonspecific and non-judicious use of antibiotics for the bacterial agents aggravates such conditions. The number of antibiotic-resistant isolates identified in humans is steadily increasing, suggesting that the spread of antibiotic resistant strains is a major threat to public health (Dimarzio et al., 2013). The choice of right antibiotics is crucial to the control of the disease. We, therefore, analysed clinical samples from common domestic animals and poultry for presence of *Salmonella* and assessed the susceptibility of the field isolates of *Salmonella* to commonly used antibiotics to get the current status of antibiotic resistance in organisms in field conditions.

A total of 102 samples (faecal swabs of live birds, spleen, caecum, liver and congested intestines of dead birds and uterine discharges, and diarrheal faeces of cattle, buffaloes and horses) were collected. Samples were transported to laboratory on ice and processed within 1 hour. For isolation of organisms from clinical samples, first pre-enrichment and then enrichment were done in buffered peptone water and selenite cysteine broth at 37°C for 18 hours and 24 hours, respectively. Selective plating on Hektoen Enteric Agar (HEA)/Brilliant Green Agar (BGA) plates was done by overnight incubation at 37°C as per Kaushik et al. (2014).

Identification of *Salmonella* isolates was done on the basis of cultural, staining and biochemical characteristics [ $H_2S$  production, oxidase, catalase, motility indole lysine (MIL), and citrate utilization tests]. The genomic DNA of field isolates of *Salmonella* and the standard culture strain of *Salmonella* Enteritidis were extracted by the standard bacterial DNA extraction

method as described by Wilson (1987). The DNA samples with  $OD_{260/280}$  value of ~1.8-1.9 were considered as pure and used for further analysis. The extracted DNA from *Salmonella* isolates and *Salmonella* Enteritidis were subjected to PCR by using *Salmonella* genus specific oligonucleotide primers of 25 base pairs as per Cohen et al. (1995). The primer sequences for the upper and lower oligonucleotides from 5' to 3', were as follows:

Lower strand- ACT GGC GTT ATC CCT TTC  
TCT GGT G;

Upper strand-ATG TTG TCC TGC CCC TGG  
TAA GAG A.

Antimicrobial agents (Himedia) viz. doxycycline, oxytetracycline, enrofloxacin, penicillin, neomycin, amoxicillin, ampicillin, streptomycin, gentamicin and erythromycin were used for testing the sensitivity of the isolates. The culture plates were incubated micro aerobically at 37°C for 16-24 hours and zone of inhibition was measured for comparing the sensitivity and resistance of each organism.

A total of 102 samples comprising of fecal swabs of live birds, spleen, caecum, liver and congested intestine of dead birds and uterine discharges and diarrheal faeces of cattle, buffaloes and horses suspected of Salmonellosis were analysed (Table 1).

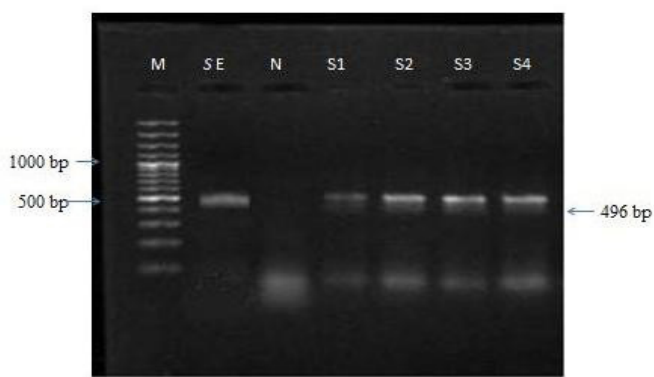
*Salmonella* could be isolated from 9 (7 from uterine fluid of cows and 2 from fecal swabs of Emu birds) out of 102 samples (Table 1). Light pink colonies of organisms on BGA and green colonies with black centre on HEA were observed. The organisms appeared as gram negative rods.

The colonies were subjected to biochemical tests like  $H_2S$  production, catalase, oxidase, MIL and citrate utilization tests. All the 9 isolates were found to be negative for oxidase and positive for catalase, MIL and citrate utilization tests. All the culturally positive samples were further confirmed at molecular level by PCR. On amplification the extracted bacterial DNA yielded the desired amplicons of 496 bp (Figure 1).

*Salmonella* isolates were found to be 100% resistant to erythromycin and penicillin and sensitive to gatifloxacin, gentamicin, ciprofloxacin, norfloxacin, doxycycline, cefoparazone, ceftaxime, oxytetracycline. Sensitivity was 100% for gatifloxacin, gentamicin, cip

**Table 1:** Isolation of *Salmonellas* from clinical samples of common domestic animals/birds

Animal/Bird	Clinical symptom	Type of sample	No. of samples examined	No. of negative samples	No. of positive samples
Poultry birds	Diarrhea	Faecal swabs	44	44	0
	Congested intestine	Liver, caecum, spleen	19	19	0
Emu birds	Congested intestine	Liver, caecum, spleen	2	0	2
Cows	Diarrhoea	Faecal sample	7	7	0
	Abortion	Fetal stomach content, Uterine discharge	18	11	7
	Dystocia	Uterine discharge	3	3	0
Buffaloes	Diarrhoea	Faecal sample	2	2	0
	Abortion	Fetal stomach content, Uterine discharge	2	2	0
Horses	Diarrhoea	Faecal sample	5	5	0



M=Marker, SE = *Salmonella* Enteritidis, S = *Salmonella* isolates

**Figure 1:** PCR confirmation of *Salmonella* isolates

rofloxacilin, and norfloxacilin, 77.78% for doxycycline, cefoparazone, and ceftaxime and 44.44% for oxytetracycline, respectively (Table 2).

Although *Salmonella* is associated with enteric infection and diarrhoea, particularly in calves, it may also cause infection in pregnant cows and may result in abortion which is the only clinical manifestation of infected herd. Colonization in reservoir hosts often occurs in the absence of clinical symptoms; however, *S. enterica* may cause acute enteritis or translocate from the intestines to other organs causing fever, septicemia and abortion (Stevens et al., 2009). Rings (1985) opined that abortion due to *Salmonella* may

be due to development of fever. In our study, 7 out of 18 samples from aborted cows and buffaloes, were positive for *Salmonella*. Thus, *Salmonella* may be a major cause of abortion in cows which leads to a great economic loss to the farmers.

Sanjrani et al. (2013) found that in case of mixed infection, abortions due to *Brucella* along with *Salmonella* were in highest frequency rather than other mixed infections. Verma et al. (1999) investigated the role of salmonellosis in causation of abortions and infertility from 43 buffaloes and 110 cows. They isolated *Salmonella* Typhimurium from single abortion in a buffalo and 2 isolates of *Salmonella* Dublin from 7 aborted cows and 2 isolates of *Salmonella* Typhimurium from uterine discharges of 33 endometritic cows. They also observed that cyclic repeat breeding in buffaloes and cyclic repeat breeding, cervicitis and vaginitis in cows were also related to *Salmonella* infection. There may be incidence of dystocia, still births and retention of the placenta in case of *Salmonella* Dublin infection (Richardson, 1973). These findings are in concurrence with the findings of our present study.

Our results have reconfirmed the earlier observations of widespread prevalence of antibiotic resistance among *Salmonella* and prompt for undertaking such surveillance from time to time in different regions to

**Table 2:** Susceptibility of *Salmonella* isolates to various antimicrobial agents

Antimicrobial agent	Number of isolates analyzed	Number (& percentage) of samples	
		Positive	Negative
Gatifloxacin	9	9 (100) <sup>†</sup>	0
Gentamycin	9	9 (100)	0
Ciprofloxacin	9	9 (100)	0
Norfloxacin	9	9 (100)	0
Doxycycline	9	7 (77.78)	2 (22.22)
Cefoparazone	9	7 (77.78)	2 (22.22)
Ceftaxime	9	7 (77.78)	2 (22.22)
Oxytetracycline	9	4 (44.44)	5 (55.56)
Erythromycin	9	0 (0)	9 (100)
Penicillin	9	0 (0)	9 (100)

<sup>†</sup>Values in the parenthesis indicate percent positivity or negativity.

get a realistic picture of the problem of emergence of resistant pathogens and to enable selection of the most effective antibiotics for use in the field. Murugkar et al. (2005) have also reported varying degrees of resistance of their *Salmonella* isolates against doxycycline, ampicillin, amoxicillin, tetracycline, chlortetracycline, nitrofurantoin, chlortetracycline, kanamycin, cephalixin, nalidixic acid, chloramphenicol, trimethoprim, ciprofloxacin, gentamicin, enrofloxacin and norfloxacin. Irimie et al. (2010) isolated *Salmonella cholerae suis* spp. from liver and intestine which were resistant to all the antibiotics used. Resistance to multiple antibiotics could possibly be due to the misuse of antibiotics in the feed.

Based on the findings of the present study, we opine that *Salmonella enterica* is not only a typical enteric pathogen but it may also be a major cause of bacterial abortion in cattle.

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