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Salmonella induced abortion storm in donkeys at tropical southern agroclimatic zone of Tamil Nadu

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Reproductive disorders comprise a plethora of clinical manifestations spanning from infertility to spontaneous abortion. Abortions in jennies are frequently caused by infectious agents, such as equine herpesvirus-1, equine viral arteritis virus, *Leptospira* spp., *Streptococcus equi* subsp. *zooepidemicus* (Brinsko *et al.* 2011). *Salmonella enterica* subsp. *enterica* serovar *Abortus equi* (*S. Abortus equi*) is a serovar adapted and restricted to equines, associated to abortion in jennies, orchitis in jacks, and septicemia and polyarthritis in foals (Grandolfo *et al.* 2018). Since, 2011, equine paratyphoid abortion has been affecting the global equine industry as a re-emerging cause of abortion storms (Bustos *et al*. 2016). Recent outbreaks have been reported in China (2019), Italy (2018) and Croatia (2016). In India, Equine paratyphoid infertility in stallions was reported in Udgir, Maharashtra in 2019 (Awandkar 2019). The purpose of the current report is to describe the abortion storm caused by *Salmonella* spp. in the donkey farming industry of southern Tamil Nadu and to document the various pathological lesions and diagnostic tools used to identify the causative agent as well as investigate the carrier status of jennies within a herd.

From August to September 2023, an abortion outbreak occurred in a commercial donkey farm located in the Tirunelveli district of Tamil Nadu (Tropical Southern Agroclimatic zone). The farm had 489 indigenous grey donkeys, 323 of which were pregnant. In this abortion storm, 53 jennies were aborted and were characterized with similar features. Therefore, six pilot cases were subjected to detailed investigation.

Owner reported that after the breeding season, jennies had co-grazed along with cattle on roadside and few had suffered automobile accidents and were treated at the nearby veterinary hospital where jennies were in contact with diseased animals. By early August, jennies were stabled and the first abortion occurred between 17-20 August, 2023. Thereafter most of the jennies in the 5-10th month of gestation aborted without any impending signs. Postpartum metritis occurred in most of the cases with copious serosanguinous foul-smelling discharge. Jennies had not been vaccinated against any infectious disease.

Out of many aborted jennies, one was presented with the complaint of inability to expel the aborted foetus per-vaginally. Eight-year-old nulliparous grey pregnant Jenny presented with both fetal forelimbs presented at the vulva. The owner reported that water bag had ruptured before 6 h and thereafter no progression of parturition occurred. On general examination, the jenny had pyrexia (40°C) and congested mucous membrane. Gynaeco-clinical examination revealed an oedematous vulva, congested vaginal mucous membrane, and both foetal forelimbs presented at the vulva without any signs of foetal liveability and no autolytic changes. Per-vaginal examination revealed anterior longitudinal dorso-sacral dead foetus with right lateral deviation of head and neck. Foetal malpresentation was corrected by repelling the extended forelimbs craniolaterally, and contralateral to direction of deviation. Deviated head pulled laterally, towards the pelvic inlet using eye orbits and lower jaw as points of grip. Dead male foetus and foetal membranes were expelled by forced traction.

Necropsy of the foetus was performed and fetal stomach contents along with tissue specimens of placenta suspected for abortions of bacterial or viral origin were submitted to the Veterinary University Disease Diagnostic Laboratory, Tirunelveli.

Necropsy revealed that foetus was well-formed, aborted near term with no foetal anatomic anomalies (Fig. 1A). Necrotic placentitis was seen with chocolate brown coloured cellular detritus on the side of chorionic villi without particular odour. Umbilical cord had diffused haemorrhages (Fig 1B); diffused haemorrhagic inflammation of the spleen, hepatization of the lungs, and liquefactive necrotic lesions of the kidneys and liver were also observed (Fig. 1 C-F). The lymph nodes were swollen and haemorrhagic.

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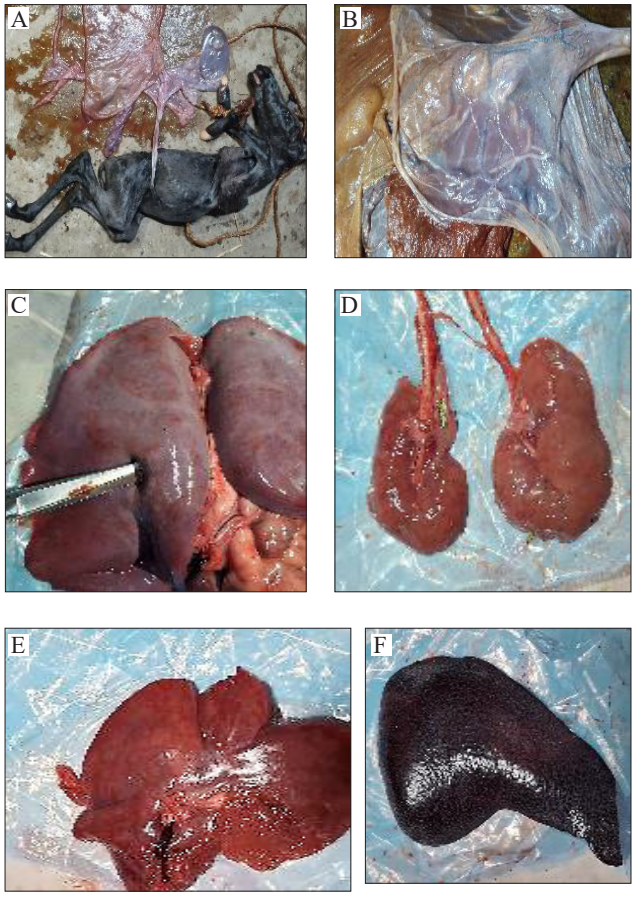

Fig. 1. Gross postmortem examination of foetus revealed: (A) Dead male foetus without any congenital anamolies; (B) Chocolate brown detritus in placenta; (C) Hepatinization of Lungs; (D) Necrosed kidneys; (E) Necrotic changes in liver; (F) Haemorrhagic spots in spleen.

Microbiological culture of foetal stomach fluid revealed the presence of gram-negative rods. Further, samples were aseptically processed and inoculated on suitable selective media and also subjected to serological and biochemical techniques to trace out bacterial etiological agents. These studies revealed the causative agent as Salmonellosis.

Blood samples were collected by jugular venepuncture of the dams and centrifuged at 2000×g for 15 min to separate the serum. Separated serum was analyzed for BUN, Creatinine assay (Biosystems S. a A15C Automatic serum biochemistry analyzer, Costa Brava, Spain). Whole blood collected in EDTA vacutainers was subjected to complete blood analysis (Mindray BC-2800 auto hematology analyzer, Mindray Medicals, India). Whole blood collected in heparin vacutainers was subjected to cortisol and progesterone assay (i Chroma II Immunoassay Reader). Serum biochemical assay revealed elevated blood urea nitrogen (38±1.94 mg/dL) and creatinine (2.7±0.23 mg/dL) indicative of azotemia as a result of sepsis induced acute renal insufficiency. *Salmonella* spp. produce haem-oxygenase I cytotoxin which is responsible for utero-placental ischaemia which in turn causes production of soluble fms-like tyrosine kinase-1 which causes endothelial cell swelling, accumulation of protein and cell debris in the sub-endothelial layer of blood vessels, and splitting of the glomerular basement membrane resulting in acute renal insufficiency which is in accordance with haematobiochemical analysis of present cases.

Haemogram revealed leukocytosis ($20.5\times10^3\pm1056$ per µL) indicating first line defensive phase against abortifacient. Haemoconcentration (39.3±1.5%) revealed osmotic imbalance induced dehydration in aborting dams (Zhu *et al.* 2021).

Elevated cortisol (6.14± 0.13 ng/mL) in spite of normal late gestation progesterone level (5.35±0.28 ng/mL) was indicative of septic shock rather than gestational pathologies as the cause of abortion. According to Bendel *et al.* (2008) sepsis induces adrenal response indicated by high peripheral cortisol level and in pregnant jennies' high peripheral cortisol initiates parturition due to parasympathetic dominance in spite of adequate progesterone level which resulted in expulsion of the foetus. These finding correlated with present study.

Gopinath *et al.* (2012) stated that ShdA and MisL are two fibrinonectins helping *Salmonella* spp. to adhere and survive in the intestinal microbiota of the recovered equids and are constantly eliminated in the faeces of such animals, thus acting as super shedder carriers in the herd. Such carriers are identified using faecal culturing technique which was also employed in present case for carrier isolation.

Faecal pellets from the donkeys (n=6) were subjected to direct microscopic examination which had no substantial findings. Hence, faecal culturing with suitable culture media was performed which revealed presence of *Salmonella* colonies.

All the dams in the herd who aborted suffered from metritis, having copious viscid foul-smelling greyish red vaginal discharge. Post-abortion transrectal ultrasonographic examination (Nero scan B1 PRO, Konica Minolta, India) of jennies was performed to analyze the reproductive status, which revealed presence of regressing corpus luteum (12.18±0.67 mm) and medium-sized follicles (7.13±0.15 mm) on ovaries (Fig. 2A). Uterine musculature showing pronounced hyper echogenicity and accumulation of mixed echogenic fluid in the uterine lumen (Fig. 2B) in abnormally involuting state was indicative of septic metritis, major predisposing factor to systemic inflammatory response syndrome in Equines. Timely treatment secured the fertility of jennies. Treatment protocol involved addressing septicemia, fluid resuscitation and effective control of inflammation associated with endotoxin (Taylor 2015).

Septic metritis was treated with intrauterine douching with 5% Povidone iodine + Metronidazole solution (1:10) @ 500 mL, Ceftriaxone @ 15 mg/kg IV, Chlorpheniramine maleate @ 50 mg IM, Meloxicam @ 0.2 mg/kg IM, Dextrose normal saline @ 10 mL/kg IV for 5 consecutive days after which animals had an uneventful recovery.

To assess the incidence of salmonellosis in pack of 489

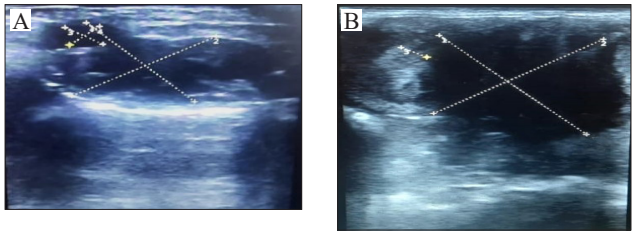

Fig. 2. (A) Right ovary with regressing corpus luteum and medium sized follicle; (B) Abnormally involuting uterus with hyperechoic contents in uterine lumen.

donkeys, faecal samples from 323 pregnant jennies were collected and subjected to microbiological culture which revealed 16.4% (53 jennies) facing abortion were positive for Salmonellosis.

According to Zhu *et al*. (2021) the incidence rate of most recent *Salmonella*-induced abortion storm in China (2019) was 22.5% however, present case report had lower incidence rate of 16.4%. Perry *et al*. (2019) reported that the *Salmonella* spp. has high affinity to the synctioblast like binucleated cells in placenta. In equines, the placental binucleate cells population was highest in mid to late gestation (Schuler *et al.* 2018) which could be the probable reason for abortions in mid gravidity in present study.

Thus, it can be concluded that emerging infectious agent such as Salmonellosis acted as a novel cause of abortion storm and reproductive loss in donkey farm in Southern Tamil Nadu.

SUMMARY

A commercial donkey farm at Tirunelveli with 323 pregnant donkeys was facing an abortion storm affecting 53 (16.4%) pregnant jennies in the last trimester, which were presented to LAC-OP-OG unit of Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli. Clinical examination revealed pyrexia (39.4°C) and congested conjunctival mucous membrane. Gynaecological examination revealed oedematous vulva with serosanguinous vaginal discharge. Ultrasonography examination revealed the presence of corpus luteum, medium-sized follicles, thickened uterine endometrium and fluid accumulation at the tip of both uterine horns. Serum progesterone and cortisol levels were 5.35±0.28 ng/mL and 6.14±0.13 ng/mL, respectively. Microbiological examination of foetal fluids revealed gram-negative bacilli which was confirmed as *Salmonella* spp. Histopathology of aborted foetal parts revealed cellular degenerative changes. All jennies were treated with antibiotics, and antihistamines along with intrauterine douching for five consecutive days. Other pregnant jennies in the farm were isolated and prophylactic antibiotics and immune booster supplements were administered. Thus, this study documented unconventional infectious agent such as Salmonellosis emerging as a leading cause of abortion storm and reproductive loss in donkey farming in Tamil Nadu.

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