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Bovine viral diarrhoea virus infection and fetal twinning as possible causes of bovine fetal mummification and their successful medical intervention

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Abstract

A study was conducted to find out the etiologies of 4 fetal mummifications in jersey crossbred cows, where 2 cases (case No. 1 and 2) belonged to one farms and rest 2 (case No. 3 and 4) were from two different farms of distant places. All the animals were within the 8 to 9 months pregnant without any physical signs of advancement of pregnancy. Based on clinical gynecological and USG findings the cases were provisionally diagnosed as mummification of fetus. The BVDV infection was confirmed in all the four cows housed under same roof with RT-PCR analysis. The two cows from two different farms were free from infections. Out of all the 6 cases 4 cows were treated successfully with IM injections of 500 mcg Cloprostenol and 4mg of Estradiol benzoate simultaneously. In case of other two cows Epidosin injection (Valethamate Bromide) @ 1ml/ 20 kg body weight, IM as single dose along with feathering at the os of the cervix were required in addition to the Cloprostenol and Estradiol. In case number 1 and 2 complete cervical dilatation was observed around 72 hours post treatment and mummies were presented partially in the vaginal cavity and cervical canal. However, in long standing case No.3 and 4, additionally Valethamate Bromide injection was required for complete dilatation of cervix where manual traction was required for expulsion of the mummies. All the four mummies were expelled out manually with gentle traction without any complications to the cows.

Keywords: Bovine viral diarrhoea virus, fetal mummification, fetal twinning, cow

1. Introduction

Fetal mummification in cattle is a rare pathology of pregnancy mostly occurring in between 3 to 8th months of gestation [1]. The condition is characterized by presence of dead, dry, shriveled and fetal membrane wrapped fetus along with persistence of a functional corpus luteum, closed cervix and cessation of estrous cycle. Usually, the condition remains undetected until examined on extension of date of parturition or in the absence of external signs of advancement of pregnancy. The incidence may be as high as 5% and as low as 0.13-1.8% [2]. The mummification of fetus in cattle has been attributed to many etiological factors, such as, autosomal recessive lethal gene [3], torsion or compression of the umbilical cord, placental defects [4], poor nutrition and genetic problems [1], infectious agents like Leptospira, Campylobacter and BVD virus infections, [5] etc. However, it is still remained difficult to fix the exact cause against a particular fetal mummification. The present communication reports successful medical intervention of two cases of mummification possibly with BVDV infection and two other with fetal twinning in jersey cross bred cows.

2. Case History

All the four cases under the study recorded were in multipara jersey crossbred cows belong to private dairy farms in the Kamrup District of Assam, India. Out of the total, case number 1 and 2 were found in one dairy farm only (Fig. 1) and were at 8 months of pregnancy. In the absence of any signs of advanced pregnancy such as udder development, relaxation of sacrosciatic ligament, enlargement of abdomen, etc the owner speculated some abnormalities and presented for examination. Moreover, the case No. 3 and 4 (Fig. 2) were from two different farms and presented for examination on exceeding the expected calving date by 30 and 37 days, respectively. On rectal examination irregular shaped dry and hard fetal mass could be palpated in uterus and bony fragments could be easily visualized in Ultrasonography findings (Fig. 3) of all the cows. The masses were located just at the level of pelvic brim with

corpus luteum in one of the ovaries in all the cows. Moreover, in all the 4 cows no fetal fluid, placentome and fremitus could be detected. On transvaginal examination cervix was found closed in all the cases.



Fig 1: Mummies from cow no. 1 and 2



Fig 2: Twin mummies from cow no. 3 and 4



Fig 3: Ultrasonography imaging of mummified mass

2.1 Tentative Diagnosis: Based on clinical findings the cases were provisionally diagnosed as mummification of fetus. Blood samples from all the four cows were subjected to for RT-PCR test to examine the possible involvement of BVDV in the occurrence of mummification following the below mentioned procedure.

2.2 Molecular detection

The total RNA was isolated from blood using the QIAamp® Viral RNA mini kit as per manufacturer's protocol. Once the RNA has been eluted, quantified and cDNA synthesis was done by standard kit protocol (Invitrogen). BVDV infections were detected using a Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay targeting the 5'-UTR region. In this study 324/5'-ATGCCCWTAGTAGGACTAGCA-3' and 326/5' TCAACTCCATGTGCCATGTAC-3' oligonucleotide sequences of PCR primers were used [6]. For conducting RT-PCR amplification a total volume of 45 µl of reaction mixture was used. To perform the PCR amplification, following thermo cycling conditions are used: initial denaturation was done at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 1 minute, annealing at 56

°C for 1 minute, extension at 72 °C for 1 minute, and a final synthesis done at 72 °C for 10 minutes (Fisher Scientific, Applied Biosystems, ProFlex PCR System). The conformation of RT-PCR amplicons was carried out by agarose gel electrophoresis in 1.7% agarose gel containing ethidium bromide in 0.5X tris borate – EDTA. Electrophoresis was done at 78 V for 1 h 30 min. It was visualized on a UV transilluminator (Kodak, biostep, Germany) and compared with 100 bp DNA markers.

2.3 Interpretation

The amplified product visualized as a single compact fluorescent band of 288bp under UV light and documented by gel documentation system is considered positive for BVDV. (Fig. 4)

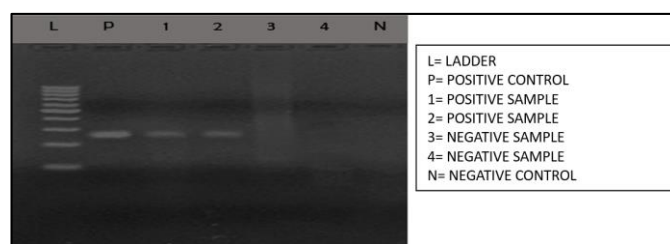


Fig 4: Gel electrophoresis of amplified amplicons

3. Results and Discussion

The RT-PCR revealed BVDV infection in case No. 1 and 2. All the four cows were injected with 500mcg Cloprostenol and 4 mg of Estradiol benzoate simultaneously intramuscularly at two different sites and monitored per vagina for cervical relaxation from 48 hours post injection and verified subsequently at 12 hours interval. In all the cows cervico-vaginal mucus discharge was observed on an average 72 hrs post treatment with remarkable sacrosciatic ligament relaxation. On per vaginal examination in cow No. 1 and 2 the mummies were found in the anterior vaginal cavity and expelled out smoothly manually. In cow No. 3 and 4 transvaginal exploration revealed good relaxation of cervix with 4-5 fingers dilatation of the posterior os. However, the internal os were dilated up to the 2-3 fingers only. Then Epidosin was injected IM @ 1 ml/20 kg body weight, as single dose. After 45 minutes of Epidosin injection mechanical stimulation was applied to the internal os with fingers to create a ferguson reflex like situation (feathering). Interestingly, at 60 minutes post epidosin (Valethamate bromide) injection the cervix of the cows relaxed and wide opened and hand could be introduced into the uterus. The mummies were found just anterior to the pelvic brim and were relatively bigger in size than that of the mummies of case No. 1 and 2, and were expelled out with gentle traction. All the four mummies were rapped with dark brown colour shriveled fetal membranes. On removal of membranes twin fetuses were revealed in cow No 3 and 4.

Out of the four blood samples, cows of case No. 1 and 2 were found positive for BVDV infection. Noteworthy that, both these cows were from one private dairy farm and became pregnant nearly at the same time. So, the BVDV infection perhaps may be attributed to the cause of mummification in these two particular cows. Done *et al.* (1980) recorded 17% mummification from maiden Jersey heifers after induction of BVDV infection experimentally [7]. Although not a single report could be traced from North Eastern part of India on

mummification due to BVDV infection but the BVDV prevalence (1.33%) is reported by Nath^[8] from this zone. Moreover, in the cow No. 3 and 4 the twinning may be the reason behind the mummification. Twinning in cattle said to be a very rare condition with 1% incidence in beef and 5.02% in dairy cows^[9]. Further, Barth (1986) mentioned the incidence of mummification in bovine as much rarer pathology of pregnancy that ranges from 0.13 to 1.8%. From this it can be easily understood why the reports on incidences of fetal mummification associated with twinning is scant^[2]. However, only one report of mummified bovine fetus detected in a cow with twin gestation could be traced^[10].

All the four cases had been successfully treated with Cloprostenol 500 mcg and Estradiol Benzoate 4mg in combination. In the case No. 3 and 4, additionally valethamate bromide injection was required for complete and effective relaxation of the cervix. The Valethamate bromide (Epidosin®, TTK, Healthcare Ltd., India) through its neurotropic (anticholinergic) and papaverine-like action on the smooth muscles might cause dilation of the cervical muscle facilitating easy expulsion^[11]. Singh and his coworkers used a combination of PGF₂, Valethamate bromide and Diethylstilbestrol for successful expulsion of a mummified fetus^[12]. In long standing cases the mummified foetus become drier, firmer, and more leathery causing expulsion a more difficult job^[13-15]. Hence, in the case No. 3 and 4 mild traction was seemed to be necessary as the cases were long standing and mummies were relatively larger in size due to twinning. Kumaresan also reported similar findings with Cloprostenol and Estradiol Benzoate combination^[16]. Roberts also mentioned about the necessity of mild and gradual traction for expulsion of mummified fetus^[1].

4. Conclusion

The present therapeutic regimes were found effective in expelling the mummified fetus partially up to the vaginal cavity. In all the four cases cervical dilation started after 48 hours of treatment and around 72 hours post treatment complete dilatation was observed in cow No. 1 and 2. However, in long standing cases like cow No. 3 and 4 additional Valethamate Bromide injection was required for complete dilatation of cervix. Nevertheless, none of the mummies was found to be expelled out by the cow herself.

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