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LISTERIOSIS IN AN EGYPTIAN DAIRY COW HERD INSEMINATED WITH INFECTED FROZEN SEMEN WITH *LISTERIA MONOCYTOGENES*

by

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INTRODUCTION

The maintenance of good fertility efficiency in milking cow herds is very important nowadays in order to minimize or shortage the period between two calving and subsequently increase the milk gain. Micto-organisms may gain access to the genital tract from the haemotogenous route or via the vaginal cavity during natural mating or artificial insemination (AI.). *L. monocytogenes* was reported to be associated with bovine abortion, although, it is still not known how it is transmitted to the bovine genital tract (FAO, 1981). Although there is a lack in available literature increminated the role of listeria in causeing abortion and reproductive disturbance in Egypt. Hajtes and Malik (1983) reported that listeriosis in milking cows in usually accompanied with sporadic cases of abortion in the 6th-8th months of gestation with retention of foetal membranes and metritis. Sofija (1977) stated that *L. monocytogenes* was most frequently encountered in the uteri of cows resulting in pyometra, endometritis, abortion and other reproductive disorders. Moreover, clinical and subclinical mastitis due to listeria have been reported by Gitter, 1985. *L. monobytogenes* is widespread in nature as

it has been isolated from soil and from the mammalin intestinal tract (Elisehcrova et al.,1979). Toaff et al. (1962) reported the possibility of sexual transmission of *L. monocytogenes* in human beings.

The farm history:

In the present work, as in Table (1), 577 milking cows in a farm, located in lower Egypt in Sharkia governorate used imported frozen semen in artificial insemination were studies as they showed reproductive disorders, low fertility rate (51.8%), sporadic cases of abortion between 5-7 months of gestation, still births, neonatal deaths. There was no evidence of clinical meningo-encephalitis; but rather, there were cases of common clinical illness (Viz. emaciation and fever), sudden death of calves and cows recurrent corneal opacity in the same cases. Some calves had respiratory affection and were emaciated. The farm was free from leptospirosis, campylobacteriosis and mycoplasmosis, but there was past history of infection with Br. abortus and after applying the regular control measures for brucellosis they became serologically negative for brucella infection. An emergency slaughtered cow for emergency, with a history of abortion in the 7th month of gestation with retained placenta and subsequent pyometra was examined and the P/M picture revealed that the most characteristic macroscopic findings were: enlarged liver with focal hepatic necrosis (Fig. I), accumulation of pus in the uterus, bilateral corneal opacity, opaque straw-coloured synovial fluid and jaundiced flesh. There were many pigeons (about 3000, pigeons) bred in that farm, some of which showed circling movements, weakness, emaciation and others were found dead; the P/M picture of some killed affected and of dead pigeons showed no detectable macroscopic changes.

Table (I): Type and number of examined cows

Type of examined cows	No.of cows.	Percentage %
- Parturated	104	18.7
- Pregnant	148	26.6
- Clinically normal non-pregnant.	29	5.2
- Metritis & endometritis	172	31.4
- Sporadic abortion.	28	5.0
- Pyometra.	21	3.8
- Retained placenta.	24	4.3
- Repeat breeder (over 1 year)	28	5.0
Total	557	100%

Fig (I): Numerous necrotic foci were visible on the surface of the liver of a cow with listeriosis

MATERIAL AND METHODS

Samples:

In this study 30 cervical mucus samples were collected from cows suffering from reproductive disorders, specimens from five aborted foeti (stomach content, brain), two milk samples from mastatic cows and samples from emergency slaughtered cow (uterine content, urine, liver), all those samples were palced in sterile dry MacCarteny bottles and transferred to the laboratory in an ice box. Six bull straws samples from imported frozen semen were supjected for baceriological examiantion and transported to the lab. in liquid nitrogen container. Also in this work samples from animal fooder, intestinal contents of apparently normal animals in different localities of the farm, all together with pigeons showing circling disease examined bacteriologically on the same day.

Isolation and identification:

Specimens from animal food, soil, pigeon droppings, animal faeces were prepared according to the techique of (Dijkstra, 1984). All samples obtained from the cervical mucus, nasal discharge, stomach content, brain of aborted feoti and liver, urine and uterine content of slaughtered animal and brain of pigeons were cultured immediately in broth for the cold enrichment. Frozen semen straws were thawed in a water bath at 37°C and incubated in broth under complete sterile condition. The cold enrichment broth for each samples was streaked every 7 days on to CNA plate medium (Biomeraux) and incubated at 4°C, 37°C under 5-10% Co_2 tension for 24 - 48 hrs, suspected colonies were picked up for identification according to Sneath et al., 1984, pathogenicity of the isolates to mice were studied according to Gray and Killenger, 1966. The isolates were serotyped using specific antisera provided from Difco laboratory (No. 2300-50 & 2301 - 50).

RESULTS

All isolates from animal samples, soil, faeces, brain of pigeons and dropping showed β haemolysis on blood agar, motile at 22°C, positive for catalase and voges-Proskauer reaction. They hydrolysed aesculin and produced acid but no gas with glucose. They failed to produce indole and urease. All strains were pathogenic for mcie I/P. Serotyping showed that all isolates were of the serotype 4b. Aborted cases from positive listeria infected animals usually occurred in the 5-7 month of pregnancy. Necropsy examination of foeti did not reveal specific lesions. Non had advanced uterine foetal autolysis indicating that aborted foeti have been dead in uteri a short time before explusion. *L. monocytogenes* was also isoalted from urine, uterine content and liver from an emergency slaughtered cow. As well as from 4 out of 15 intestinal content and from 3 out of 12 soil samples obtained from different areas of the farm. *Listeria monocytogenes* was recovered from imported frozen semen of 2 out of 6 straws examined and they were of serotyped 4 b. (as shown in the following Table

L. monocytogenes isolates from different speciments examined

Material examined	Number examined	Number positive	%
Endometritis	30	21	70
Aborted foeti (5-7 M)	5	2	40
Organs of slaughtered cow emergency (uterine cont, urine + liver)	1	1	100
Nasal swab from calves	3	2	66.6
Mastatic milk cows	2	1	50
Frozen semen	6 *	2	33.3
Brain of pigeons	4	3	75
Pigeon dropping	1	1	100
Feed stuffs	3	0.0	0.0
Farm soil from differed area.	12	3	25
Apparently healthy animals intestinal content	15	4	26.6

* Each one represents one bull.

DISCUSSION

All the previous studies carried out on bacterial diseases transmitted through semen was attentionly focused on brucellosis, campylobacteriosis, mycoplasmosis and their is lack of evidence of a causal relationship between the presence of potentially pathogenic of non specific micro-organisms in semen and its fertilizing capacity. The present study represents the first work that explains the masked relationship between the presence of pathogenic. *L. monocytogenes*, as a semen born-non specific micro-organism, and the way by which such organism gain access to the genital tract, leading to abortion and other reproductive disorders in cows.
As early as in 1954 Macpherson and Fish demonstrated by laboratory methods that *L. monocytogenes* may survive the conditions of semen freezing in egg yolk citrate extender without antibiotics.

on the other hand, Blood and Henderson, 1974 mentioned that *L. monocytogenes* can possibly transmitted by sexual transmission, moreover Toaff et al., 1962 succeeded in isolating *L. monocytogenes* from semen of 3 husbands out of 60 men, whose wives suffering from repeated abortions. Furthermore Macpherson and Fish, 1954 support the existing and viability of such organism in frozen semen, also agree with what was recorded by (FAO, 1981) that the non specific microflora which exist in semen could play an important role in reproductive disorders.

As it was dictated in our results, the isolation of *L. monocytogenes* was from cows showing reproductive disorders and also from cases exhibiting the septicaemic form of lsiteriosis but the nervous form was demonstrated among infected pigeons. In addition, the organism was successefully isolated from mastitic milk of diseased cow in the farm showed normal parturation and mastitis in 2 left quarter characterised by some firmness and slight suppurative milk secretion. The isolation of *L. monocytogenes* from

the emergency slaughtered cow with typical picture of repoductive form (abortion at 7 month) and septicaemic form (generalized jaudice, bilateral opacity of the eyes, liver necrosis **(Fig. 1)**, pyometra, and general emaciation) confirm the presence of both septicaemic and reproductive forms of the disease in such farm and these findings in agree with what was reported by Sofija (1977), Gitter (1979) and Hajtas and Malik (1983).

Regarding the isolation of such organism *L. monocytogenes* from the nasal discharge of calves showing respiratory manifestation and also from brai.. of sacrified pigeons with nervous manifestations, could be explained on the basis that these cases get infected from the contaminated soil, so this in agreement with Elischerova et al., 1979 who reported that the soil and faeces are the natural habitat of *L. monocytogenes*, also the organism transmitted from animal to animal by faecal to oral route. In this study *L. monocytogenes* was isolated from soil and faeces in different localities in the farm which indicated wide spread of the organism in that farm. In fact, the isoaltion of the organism from the faecal meterial of apparently healthy animals may attributed to the subclinical existence of listeriosis.

As a conclusion, during our study to this farm it seemed to us that imported frozen semen is the only incriminated source of infection with *L. monocytogenes* among animals and birds in that farm as it was primarily transmitted the infection to the artificially insemianted cows via the reproductive tract while other animals and pigeons were get infection from the contaminated soil with the infected material shed from the infected cows.

Further investigations should be carried out on the relationship between the semen meiroflora and the fertilizing capacity of semen used in artificial insemination. Also several efforts must be done in order to clarify the role by which such saprophytic organism gain access to the straws of semen.

Our work revealed that there is a high percentage of reproductive disorders among cattle herds caused by non-specific microorganisms, in particular *L. monocytogenes*, the frequent isolation of such organism from imported frozen bull semen as well as from cows insemianted with such semen and showing reproductive disorders strongly incriminate this semen as the main source of lsiteriosis among our herds and necessitates the examiantion of all imported frozen semen patches for *L. monocytogenes* in order to control the increasing rates of listeriosis among the Egyptian herds El-Ayouby et al. (1991).

SUMMARY

An Egyptian dairy farm (557 cows) located in lwoer Egypt in Sharkia governorate was subjected to clinical and laboratory investigation follwoing a continuous complain, represented mainly in low fertility rate. This farm used infected frozen semen with *L. monocytogenes* in artificial insemination. Listeriosis was diagnosed in different cows showed the typical reproductive and septicaemic form of the disease, also pigeons bred in the farm showed the typical nervous signs of circling disease. *L. monocytogenes* was isoalted from 3 out of 12 soil sampels taken from different areas from the farm. In addition, *L. monocytogenes* was isoalted from the intestinal contents of apparently normal cows. Fourty isoaltes from cows, pigeons and soil, along with imported frozedn semen were serotyped and all of them belonged to serovar 4 b and all isolates were pathogenic for white mcie.

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