

## MYCOTIC ABORTION IN IRAN

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### ABSTRACT

In a review of bovine and ovine mycotic abortion over the past fifty years, reports have been increasing in frequency in recent years. Most of fungi involved are opportunistic. A survey was carried out on 119 samples of bovine and ovine aborted fetuses that were submitted to the microbiology department for detection of mycotic infection from different parts of IRAN. In this study we isolated *Aspergillus* spp, *Mucor* spp, *Absidia* spp, *Candida* spp, and *Allescheria boydii*, from internal organs, while no other infection agents were found on microbiological examination of fetal and stomach contents.

### Introduction

The first case of mycotic abortion in cattle was reported in 1920 by Smitih (17) who observed that some cotyledons were larger than usual and also showed necrosis microscopically, *Mycellia* were observed in the necrotic lesions and in the stomach contents, lung and amniotic fluid of the fetuses.

In 1926 Gilman and Birch (7) isolated *Aspergillus fumigatus*, *Absidia corymbifer*, and *Absidia ramosa* from three fetuses and were able to produce placental infections experimentally by injecting five pregnant cows intravenously with suspension of *mucor* spp. Bendixen and Plum (4) in 1929 examined the placentas from 14 cases of aborted fetuses and found *Aspergillus* spp, in eight of the cases, and *Absidia ramosa* from two. They distinguished between the hyphae of the two fungi by noting that, those of the *Aspergilli* were septate and thin, while those of the *Absidia* spp, were nonseptate thick and swollen. They also succeeded in reproducing abortion experimentally by inoculating pregnant cows intravenously with *Absidia* and *Aspergillus fumigatus*.

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Plum in 1932 (15) continuing these studies, found that among 4133 placental specimens submitted for examination of causes of abortion, 230 (12%) showed microscopic evidence of mycotic abortion and 118 were classified as *Aspergillus fumigatus*, *Aspergillus versicolor*, *Aspergillus flavus*, *Absidia ramosa*, *Absidia lichtheimi*, *Mucor pusillus* and *Rhizopus* spp.

Jungherr (8) in 1933, described five abortions associated with fungi from two Brucella free herds. Rollinson and Hag in 1948 (16) isolated *Absidia* spp from a bull and from the vaginal discharges of a cow. They suggested the possibility of transmission of this fungus through coitus and its pathogenic nature for the bovine genitalia, but failed to reproduce abortion experimentally by intrauterine injection of pregnant cows. In 1952 Wiedlich (20) isolated *Aspergillus fumigatus* from skin lesions of a five month old fetus, then Engel and Vandermass (21) 1955 reported the isolation of *Absidia ramosa* and *Glenspora graphii* from skin Lesions of a seven month old fetus. Fifty one fetal stomach contents were examined by Ainsworth and Austwick 1955 (1) They found that *Aspergillus fumigatus* (63%) *Absidia ramosa* (12%) and *Absidia cormbifer*, *Aspergillus terreus* and *Aspergillus nidulans* could be isolated from these cases. Austwick and Venn 1957 (2) recognized hyphae in necrotic cotyledons and stomach contents from aborted fetuses. The fungi most frequently isolated were *Aspergillus fumigatus*, *Absidia ramosa* as well as *Candida tropicalis* and *Bsidiomyceta*. In 1964 Cordes et al, (5) reported 34 cases of fungal abortion in cows from which they isolated *Aspergillus* SPP, *Mucor* SPP and *Penicillium* SPP. Hyphae were also seen in hematoxylin and eosin stained sections.

In 1965 Mahffey and Rossdale (13) reported an abortion of a six month old equine fetus.

Nodules were removed aseptically from the lung and cultured on blood Agar and sabouraud Dextrose Agar, following, which *Allescheria boydii* was recovered. Tarner (1965) (19) found ten cases of Mycotic abortion in 34 cases of bovine abortion studied. *Mucor*, *Rhizopus*, *Aspergillus fumigatus* and *Candida tropicalis* were isolated from fetal membrans, amniotic fluids, and stomach contents. Examination of the feed and the air for spores was positive for all fungi except *Candida tropicalis*.

Smith (1966) (18) described the association between Mycotic abortion and Mycotic pneumonia. Fungi isolated from these diseases were *Aspergillus fumigatus*, *Absidia ramosa*, *Candida parapselosis* and *Mortierella zchae*.

Kirkbride et al, (9) in 1972 isolated *Torupicalis glabrata* from a six month old aborted bovine fetus, that was negative when cultured for bacteria and viruses. *Allescheria boydii* was isolated in pure culture from placental tissue from 3 cases of bovine mycotic placentitis by Knudtson et al in 1976 (10) and the yeasts were isolated from abomasal contents and lung. Bacteriological and virological examination were negative.

Cephalosporium SPP was isolated from a six month old male fetus by Olson and Olson in 1978 (14), and skin lesions were observed on the neck, head, and face of the fetus.

## Materials and Methods

The materials utilized in this portion of the study were specimens obtained from field cases of bovine and ovine abortions submitted to the Microbiology Department of the Razi Institute for diagnosis from 1979 to 1984. The samples submitted, included fetuses or parts of fetuses, placentas and abomasal contents, although emphasis was placed on the importance of obtaining fresh specimens. A small portion of these materials were placed on a slide and examined as a wet mount, under phase microscopy, for the presence of spore or hyphae the following culture media were inoculated with internal organs

- 1) Cystine heart Agar 51 gm

Distilled water 1000cc

Autoclave in flask, cool and add 10% sterile whole citrate sheep Blood.

Most organisms grow well in this medium.

- 2) Sabourauds Dextrose Agar, this medium gives good growth for most molds. Heat in steamer to dissolve, Autoclave at 15 lbs pressure for 15 minutes. These cultures were checked at intervals of 3 to 4 days until growth was detected, or until a period of two weeks had elapsed and no growth became evident. Each time a mold was cultured from a specimen, the slide culture technique was employed to assist in identification.

Experimental production of Mycotic Abortion. Five 2 year old native sheep were received for the experiment. 12cc of suspension containing approximately  $23 \times 10^6$  *Aspergillus fumigatus* spores were injected intravenously during the two months of gestation. This suspension was prepared from a culture isolated from a field abortion. The colonies were grown on tryptocase Soy Agar, plates incubated for three days at  $27^\circ\text{C}$ . The plates were allowed to stand at room temperature

for two days to enhance sporulation. The plates were then flooded with sterile distilled water and surface gently agitated with a sterile loop to assure maximum suspension of the spores. The resulting suspension was filtered through a sterile gauze pad to remove clumps of mycelia. The suspension was shaken vigorously and concentration was determined by a direct count in a red blood cell count.

The sheeps temperature was recorded daily and weekly, and cultures were made of the cervical mucus. (Table 1).

## Result

As previously noted, 119 specimens were presented consisting of fetus and placenta and abomasal contents. Sixty cases were identified as being mycotic abortion, in each instance these organisms were obtained in pure culture. The fungi isolated from fetuses included 22 *Aspergillus fumigatus*, 7 *Aspergillus niger*, 10 *Mucor pusillus*, II *Rhizopus*, 7 *Candida tropicalis*, 3 *Allescheria boydii*, (table II). *Aspergillus fumigatus* was characterized by narrow, branching, septate hyphae but *Mucor* species by wider frequently branching without septate hyphae. No serological and bacteriological evidence of brucellosis was found.

Abortion produced between 25-32 days after intravenous injection, the organism was isolated from the dams's uterus and placenta as well as the fetal abomasal content. The fetal lung, liver and spleen failed to yield cultures of the fungus.

## Discussion

Laboratory diagnosis of abortion must be done by an experienced practitioner and an adequate history is also important. Classifying bovine and ovine abortions on the basis of epizootiologic data is helpful for differential diagnosis of selection procedures by both clinician and laboratory diagnosticians. The incidence of mycotic abortion among failing specimens during the current 3 year survey period was 50% (table I).

The incidence of mycotic abortion is higher in the winter months when cattle or sheep are stabled, than it is during the pasture season. Mycotic abortion can be produced by intravenous inoculation of *Aspergillus fumigatus* spores. How organism enters the animal is unknown. Bendixen and Plum (4) were of the opinion that the infection was primerily pulmonary and it was carried by the blood to the placenta. They failed to reproduce abortion by feeding cultures of *Aspergillus*

fumigatus and Absidia corymbifer. Experimentally ovine abortion has been produced by intravenous inoculation with fungal spores, intratracheal inoculation with Fungal spores failed to produce abortion, thus seriously questioning Bendixen and Plum's thoughts on a primary pulmonary infection. In this investigation bovine mycotic abortion usually occurs between the sixth to seventh month but ovine mycotic abortion occurs between 2-3 month and the abortions were usually more frequent in winter months. This could be the result of feeding moldy hay to the cattle and sheep. Cases of mycotic abortion appear to be the increase but whether this increase is due to better diagnostic methods, while no other infectious agents were found on microbiologic examination of fetal and stomach contents is to be studied.

Table 1

**Causes of abortion in a survey of 119 cases.**

specimens	Number of cases	Fungi	Contaminated Specimens	Number diagnosis	%
Total	119	60	20	39	50/49
Bovine fetus	60	24	13	23	40
Ovine fetus	59	36	1	16	55/93

Table 11

**Causes of abortion in cattle and sheep in Iran.**

Specimens	Positive case number	Asp. funigatus	Asp. niger	Mucor pusillus	Rhizopus spp	Candida spp	Allerscheria boydii
ovine fetus	36	13	4	6	6	5	2
bovine fetus	24	9	3	4	5	2	1
total	60	22	7	10	11	7	3
%		36.66	11.66	16	18.3	11.66	5

Sheep's number	Age in year	Pregnancy term	Asp. fumigatus spores / ml	Intravenously	Abortion date
1A	2	2month	$3 \times 10^6$		30 days
2A	2	2month	$3 \times 10^6$		30 days
3A	2	2month	$3 \times 10^6$		25 days
4A	2	2month	$3 \times 10^6$		22 days
5A	2	2month	$3 \times 10^6$		28 days
6C	2	2month	—		Control
7C	2	2month	—		Control

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