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# **Mycological and molecular studies on some fungi associated respiratory manifestation in ruminant**

A thesis presented by

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## 7. SUMMARY

A total of 200 samples from large and small ruminant animals (cattle, cow, sheep and goats 50 samples from each) in Garbia governorate. Samples collected from diseased animal suffering from respiratory manifestation. Tissue specimens from lungs were taken from the affected freshly slaughtered animals (100 in numbers). Nasal swabs were aseptically taken from the nasal clift of the respiratory affected animals (100 in number).

The prevalence of fungi associated with pneumonia were 9, 10,15 and 6 positive samples from 100 lung samples of buffalo, Cow, Sheep and goat, respectively. The prevalence of fungal isolation from nasal swabs of examined samples from animal suffering of respiratory manifestation, 100 nasal swabs examined samples (25 from buffalo, Cow, Sheep and goat) 12,15,9 and 7 positive samples from buffalo, Cow, Sheep and goat, respectively.

From lung and nasal swabs examined samples 3 mould and 2 yeast genera isolated, *Aspergillus* spp., *Penicillium* spp. and *Mucor* spp. While yeast genera *Candida* spp. and *Cryptococcus* spp.

The mould species were isolated from buffaloes and cow examined samples collected from Garbia governorate and the most isolated mould species from the cow and buffalo samples (lung lesion and nasal swabs ) were *A. fumigatus* from lung lesions and nasal swabs, respectively. While the following isolated strain was *A. niger*, followed by *A. flavus* from lung lesions and nasal swabs, respectively. There were another different species isolated, including *Penicillium* species from lung lesions and nasal swabs, respectively. While the least isolated strain was *Mucor* spp from the buffalo and cow from lung lesions and nasal swabs, respectively.

*Aspergillus fumigatus* takes the majority of the isolated mould strains from the pneumonia samples in lung tissue and nasal swab sheep and goats examined samples, respectively. Followed by *A. flavus*, *A. niger*, *Penicillium* spp. and the least isolated strains was *Mucor* spp.

*Candida albicans* isolated from buffalo lung tissue, buffalo nasal swab, cow lung tissue and cow nasal swab, respectively. Followed by *C. gullermondii* and *C. tropicalis*. The least isolated strains were *Cryptococcus albidus* where isolated only from buffalo and cow lung tissues.

Yeast genera isolated from the lung and swabs samples collected from sheep and goats in different percentages that were *C. albicans*, *C. gullermondii*, *C. tropicalis* and *Cryptococcus albidus*. *C. gullermondii* and *C. tropicalis* were the most predominant isolated yeast genera from the collected samples, followed by *Cryptococcus albidus* was isolated from lung samples collected from sheep and goats, respectively.

The autofluorescence fungal species identified were *A. fumigatus* and *Cryptococcus*, *Candida*, *A. flavus* and *A. niger* within lung tissues and they exhibited strong enough fluorescence that the technique could be helpful. The architectural detail of bright green to yellow green autofluorescence. In control cases (negative mycotic isolation) did not show any autofluorescence.

The proteolytic and hemolytic activities were determined to twenty one isolated mould and yeast strains isolated from mycotic pneumonia examined samples. From our results observed that *A. fumigatus*, *A. flavus*, *A. niger*, *Penicillium* spp., *Mucor* spp., *C. albicans* and *C. tropicalis* produce the large zone for proteolytic, While, *C. gullermondii* and *Cryptococcus albidus* not produce proteolytic activity. Hemolytic activity among the

different isolated moulds and yeast *A. fumigatus*, *A. flavus*, *A. niger* and *C. albicans* produce larger zone for hemolytic activity (25-34mm,20-31mm, 30- 31mm and 23 -26mm ) than *C. tropicalis* (12mm).

Itraconazole and Voriconazole were the effective antifungal against *A. fumigatus* while, Nystatin more effective against *A. flavus*, *A. niger*, *Penicillium* spp., *C. albicans*, *C. guillermondii* and *C. tropicalis* after *Amphotericin B* , while Voriconazole was the least used antifungal in its effect against the tested strains and *Amphotericin B* was only effect on *Cryptococcus albidus*.

Molecular characterization of three *Cryptococcus albidus* isolates which had previously been identified biochemically with RapID yeast plus system was molecularly identified using The PCR primers used Forward CNa-70-S 5 'ATTGCGTCCACCAAGGAGCT-3 and Reverse CNa-70-A 5 'ATTGCGTCCATGTTACGTGGA -3 oligonucleotide primers used in PCR reactions. The size of the resulting amplicons at bp 500.

Five *A. fumigatus* isolates examined by Polymerase chain reaction for detection *A. fumigatus* virulent gene (*aspHS*). PCR products of strains were positive on agarose gel electrophoresis, PCR reactions at bp 180. One of these 5 strains was successfully amplified and sequenced. The obtained sequences were deposited at NCBI under accession no. (Gene bank accession number: **MW546778**).The analysis for the sequence data obtained from the sequence application analyzed and aligned by clustal method using the program DNA star (Lasergene, Wisconsin, USA) for *A. fumigatus* isolated from cow (isolate number *A. fumigatus*. **Fawaz. A. EG. pepi E4** in this study) determined the differences and similarity percentage for the *AspHS* gene.