ISSN 110-2047 ALEX. J. VET. Med. SCIENCE 2010 VOL. 29 NO. 1: (107-114)

MYCOLOGICAL EVALUATION OF IMPORTED FROZEN MEAT

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ABSTRACT

A total of 100 random samples of imported frozen boneless beef meat of different cuts were collected from different Alexandria markets.

The samples were transferred in an insulated ice box to the laboratory with minimum time of delay under aseptic condition, where they were examined mycologically.

The results revealed the isolation of *Aspergillus species, Penicillium species, Cladosporium species, Rhizopus species, Mucor species, Alternaria species, Trichoderma species, Helminthosporium species, Phialophora species, Geotrichum species, Fusarium species* and other fungi species from the examined samples. Also, the results revealed the isolation of some yeast species as *Candida species, Torulopsis species* and *Rhodotorulla species.*

The obtained results showed that the imported frozen meat can be contaminated with yeast and mould due to inadequate hygienic measurements during loading, disloading, handling, preparation and processing from the time of slaughtering till reaching to the consumer.

INTRODUCTION

The increase of human population and the great shortage of animal protein plus the crises of avian influenza and swine influenza which widely spread in Egypt have led the authorities to allow the businessmen to import large quantities of meat from different countries.

But imported frozen meat were subjected to various kind of contamination from different sources during the period that elapses from the time of slaughtering till consumed. Yeast and mould are the most important contaminants of imported frozen meat as they are widely distributed in the environment, and may be found as a part of the normal flora of the meat and meat products or as airborne contaminants and inadequate sanitized equipment.

The presence of mold in high incidence in imported frozen meat indicate bad hygienic measures adopted during handling, preparation and processing. Therefore, advanced countries carry out mold and yeast counts as standard tests of check the general hygienic conditions during meat processing as recommended by **(Foster et al., 1958)**. Such contaminants may affect the food quality or even make it unfit for consumption, thus resulting in economic losses and may constitutes public health hazards.

Yeast and mould can contaminate imported frozen meat through several ways, through the environment inside the slaughter hall including; air movement, walls, floors, utensils, hides and the intestinal contents of slaughtered animals are considered the main sources of such contamination **(Empey and Scott, 1939; Rolle and Klob, 1954; Youssef and El-Tarabishy, 1966; Mansour, 1986; Mansour et al., 1990; Zohri, 1990 and Refai et al., 1993)**. Imported frozen meat may be also affected with mould during long trips in ships due to mishandling and storage defects. Colonization of molds on frozen meat in the form of black spots, white spots and bluish green spots **(Gracey, 1986)**, whereas the most important factors for germination of spores on meat are temperature and relative humidity **(Mislivec et al., 1975)**, these organisms are capable of growth on a variety of substrates and under a diversity of condition of moisture, pH and temperature, therefore meat are susceptible to fungal invasion during some stages of production, processing, transport and storage **(Bullerman, 1979; El-Gendy and Marth, 1980 and Stoloff, 1984)**.

MATERIALS AND METHODS

A total of 100 random samples of imported frozen boneless beef meat of different cuts were collected from different Alexandria markets.

The samples were transferred in an insulated ice box to the laboratory with a minimum time of delay under aseptic condition, where they were examined mycologically to determine the sanitary quality of imported frozen meat.

Duplicate plates of sabouraud's dextrose agar medium **(Cruickshank et al., 1975)** supplemented with chloramphenicol and chlorotetracycline (100 mg of each) as described by **(Koburgher and Norden, 1975)** were inoculated each with one ml from the previously prepared serial dilutions. The inoculated plates were incubated at 25°C for two days before being examined. After 3 days of incubation, the first examination of the plates was done to the degree of mould and yeast growth and then reported on the 5th day.

Identification of moulds genera and species was based on macroscopic and microscopic examination of the isolates and it was carried out according to the methods of **Larone (1976), Frey et al., (1979) and Samson et al. (1995)**. All of the positive mould cultures were purified by subculturing on sabouraud's dextrose agar (SDA) plates, incubated at 25-28°C for 3-5 days and examined.

Identification of the isolated yeasts according to **Lodder and Kreger Van-Rij (1970), Kreger Van-Rij (1984) and Koneman et al. (1992)**.

RESULTS AND DISCUSION

The mycological evaluation of imported frozen meat gives an index about the hygienic quality of the examined samples. High count of mould and yeast in this study may be due to poor sanitary measures during production, transportation, handling and storage of imported frozen meat.

When meat is frozen correctly and maintained at the optimal freezing temperature, it should stay good

forever. However, it is hard to guarantee particularly with home freezing that the meat will remain safe and of good quality for eating but once frozen meat is thawed the microbes come back to life and continue their life's work, which is to multiply and consume **(Wallace , 2003)**.

It's evident from (table, 1) that the mould detected in 92 % of the examined imported frozen meat collected from Alexandria markets and found that the minimum total mould count was 2.0×10^4 and the maximum total mould count was 9.0×10^4 with a mean value of $4.84 \times 10^4 \pm 2.01 \times 10^3$ cfu/g., also the yeast detected in 94 % of the examined imported frozen meat collected from Alexandria markets and found that the minimum total yeast count was 2.0×10^4 and the maximum total yeast count was 9.0×10^4 with a mean value of $5.15 \times 10^4 \pm 1.99 \times 10^3$ cfu/g.

Table (2) revealed that the predominant genera of the isolated mould from the imported frozen meat was *Aspergillus spp.* 68 (73.9 %) followed by *Penicillium spp.* 52 (56.5 %), *Cladosporium spp.* 47 (51.1 %), *Rhizopus spp.* 41 (44.6 %), *Mucor spp.* 36 (39.1 %), *Alternaria spp.* 32 (34.8 %), *Trichoderma spp.* 24 (26.1 %), *Helminthosporium spp.* 21 (22.9 %), *Phialophora spp.* 17 (18.5 %), *Geotrichum spp.* 14 (15.2 %), *Fusarium spp.* 12 (13.0 %), Epidermophyton spp. 9 (9.8 %), *Paecilomyces spp.* 8 (8.7 %), *Trichophyton spp.* 4 (4.3 %).

In this investigation, imported frozen meat was contaminated with many fungal genera and species. The most predominant species were *Aspergillus* and *Penicillium* where they were isolated in high frequencies of occurrence from all the examined imported frozen meat.

Some strains of *Aspergillus* such as *A. fumigatus, A. niger* and *A. nidulans* could produce mycotoxins in meat and constitute public health hazards **(Hanssen and Hagedorn 1969)**. The isolated strains of *Aspergillus flavus* were capable of producing aflatoxins B1 and B2, but not aflatoxin G, when cultured in Searles Lake (SL) media, strains of *Aspergillus versicolor* isolated were found to sterigmatocystin-producing ability in Yeast Extract-Starch (YES) agar **(Hitokoto et al., 1973)**. The discovering in 1960 of the hepatotoxic and hepatocarcinogenic properties of *Aspergillus flavus*, quickly followed by elucidation of the structure of the aflatoxin changed the control strategy in the whole field of mycotoxins. A more quantitative approach is now possible, based primarily upon the chemical determination of the toxins and on studies of their effects in relation to dose **(Goldblatt and Stoloff, 1983)**.

Some species of genus *Penicillium* may induce endocardities, external otomycosis, mycotic keratitis and pulmonary infection **(Washington, 1981)**. Penicillic acid and sterigmatocystin are mycotoxins produced by *Penicillium species* which had a carcinogenic effect **(Mossel, 1982)**. Some species of *Penicillium* have been associated with pulmonary infection, urinary tract infections and yellow rice disease syndrome which are responsible for several cases of death in man **(Banwart, 1980)**.

Mycotoxins suppress the immune system and increase susceptibility to certain diseases. The immunosuppressive effect can be

much stronger in combined exposure to mycotoxins even at low concentrations. Aflatoxins are toxic metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus fungi*, they are probably the best known and most intensively researched mycotoxins in the world. Acute exposure to aflatoxins can result in aflatoxicosis, which manifests as sever, acute hepatotoxicity with a case fatality rate of approximately 25 % **(Cullen and Newberne 1994)**. Aflatoxin B_1 a known human carcinogen, is the most potent and potentially lethal metabolite. The carcinogenic aflatoxins are produced by *Aspergillus flavus*. In regions of the world where aflatoxins are not highly regulated, consumption of aflatoxin-contaminated food is associated with acute liver damage, liver cancer, immune suppression, and nutritional interference **(Williams et al. 2004)**.

Cladosporium species may be encountered in case of chromatomycosis and brain abscess **(Jawetz et al. 1974 and Washington, 1981)**. it is found that *Rhizopus, Mucor, Alternaria, Trichoderma* and *Helminthosporium species* are prevalent contaminates of imported frozen meat they may induce lesions involve the rhinofacialcarnial area, lungs, gastrointestinal tract, skin, as well as, intraocular infection, external otomycosis, orbital cellulitis and deep wound infection **(Mackie and McCartney, 1956; Jawetz et al. 1974; Al-Doory, 1980 and Washington, 1981)**. Also, *Alternaria species* may be incriminated in case of skin and nail infection, conjunctivitis, endocarditis, hyper sensitive pneumonia and palatitis **(Al-Doory, 1980 and Washington, 1981)**.

Table (3) revealed that the predominant genera of the isolated yeast from the imported frozen meat was *Candida species* 61 (64.9 %) followed by *Torulopsis species* 23 (24.5 %) and then *Rhodotorulla species* 16 (17.0 %), respectively.

The spoilage yeast are those which find their way into food because of their wide distribution in nature resulting in undesirable changes in physical appearance of food **(Walker, 1976)**. Some species of yeast constitutes a public health hazard as some species of *Candida species* may cause gastrointestinal disturbances, vulvovaginitis, endocarditis, pulmonary infection and occasionally fetal systemic disease **(Jesenska and Hrdinova, 1981)**. *Candida albicans* is one of the etiological agents of thrush and pathological condition of white patches in the mouth, throat, esophagus and dermatitis of palms and soles **(Wilson et al., 1981)**.

Regarding the public health point of view, some members of *Candida species* are incriminated in cases of pulmonary infection, urinary tract infection, vaginitis, thrush, arthritis, osteomyelitis, dermatitis, endocarditis, meningitis and eye infection **(Mackie and McCartney, 1956; Jawetz et al. 1974; Al-Doory, 1980 and Washington, 1981)**. *Candida albicans* is responsible for meningitis, ophthalmitis, osteomyelitis and focal infection reported in children and infants **(Rippon, 1982)**.

Table (1) Incidence of mould and yeast in imported frozen meat (n = 100):

positive samples					
	No.	**%**	**Min.**	**Max.**	**Mean ± S.E.M**
mould	92	92%	2.0×10^4	9.0×10^4	$4.84 \times 10^4 \pm 2.01 \times 10^3$
yeast	94	94%	2.0×10^4	9.0×10^4	$5.15 \times 10^4 \pm 1.99 \times 10^3$

Table (2):Incidence of mould species isolated from imported frozen meat

Mould isolates	No. of positive samples	%
Aspergillus spp.	68	73.9%
Penicillium spp.	52	56.5%
Cladosporium spp.	47	51.1%
Rhizopus spp.	41	44.6%
Mucor spp.	36	39.1%
Alternaria spp.	32	34.8%
Trichoderma spp.	24	26.1%
Helminthosporium spp.	21	22.9%
Phialophora spp.	17	18.5%
Geotrichum spp.	14	15.2%
Fusarium spp.	12	13.0%
Epidermophyton spp.	9	9.8%
Paecilomyces spp.	8	8.7%
Trichophyton spp.	4	4.3%

Table (3) Incidence of yeast species isolated from imported frozen meat :

Yeast isolates	No. of positive samples	%
Candida species	61	64.9%
Torulopsis species	23	24.5%
Rhodotorulla species	16	17.0%

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Received: February 22, 2010.
Accepted for Publ. : March 1, 2010.