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# Mastitis in dairy buffalo and cattle in Egypt due to *Clostridium perfringens*: prevalence, incidence, risk factors and costs

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**Summary**

Although *Clostridium perfringens* is recognised as an important cause of clostridial enteric diseases, there is only limited knowledge about the association of particular *C. perfringens* toxinotypes (types A to E) with mastitis in domestic animals. In this study, mastitis was detected in 213/623 (34.12%) and 8/83 (9.64%) of the quarter milk samples collected from cases of clinical mastitis in cows and buffalo, respectively. The micro-organism was isolated in an incidence of 16/357 (4.48%) of milk samples from cows and 1/25 (4.0%) of samples from buffalo. Infection in one quarter was the most typical situation found (83% in cows and 87% in buffalo). *Clostridium perfringens* infection was also correlated to the season, with the highest proportion of isolates being found during spring (10.71%) and winter (7.07%). Using the classical toxin neutralisation typing method, 17 strains, isolated from cow and buffalo milk, were identified as *C. perfringens* type A, and selected for molecular analysis. Polymerase chain reaction detected the α/*cpa* gene while the β/*cpb* and ε/*etx* genes went undetected. The authors believe that *C. perfringens* has the potential to produce disease on its own or to predispose the udder to disease caused by major mastitis and environmental pathogens.

**Keywords**

α/*cpa* gene – Alpha-toxin – Bovines – Buffalo – *Clostridium perfringens* – Dairy cattle – Egypt – Mastitis.

## Introduction

Mastitis is considered to be one of the most important health problems in dairy cattle (18). It can be defined as an inflammation of the mammary gland, resulting from the introduction and multiplication of pathogenic micro-organisms in this gland. The main causative bacteria include: *Staphylococcus aureus*, *Streptococcus agalactiae* (both of which are contagious), coliforms, *Streptococci* and *Enterococci*. All of these pathogens are found in the environment of the animals (water, feed, bedding, manure and soil). These major pathogens can cause clinical mastitis, which can lead to swelling or pain in the udder, changes in milk composition and appearance, increased rectal temperature, lethargy, anorexia and even death (11).

Sub-clinical mastitis does not lead to visible changes in milk or the udder, although it is characterised by reduced milk yield, altered milk composition and the presence of inflammatory components and bacteria in milk. Both forms of mastitis can have serious economic consequences, due to loss of production and premature culling of affected animals (11). Several other pathogens have been isolated in mastitic cattle mammary glands. These include *Actinomyces pyrogenes*, *Clostridium perfringens* and other coliforms, such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Pasteurella haemolytica*, among others.

The *Clostridium* spp. are relatively large, Gram-positive, rod-shaped bacteria. *Clostridia* are ubiquitous, obligate anaerobic organisms. *Clostridium perfringens* is a pathogen for humans and animals (4, 37). Diseases caused by this organism are, in many cases, life-threatening or fatal. At the same time, it is part of the ecological community of the intestinal tract of humans and animals.

Mastitis is responsible for about 70% of avoidable economic losses incurred by commercial dairy farmers (34). Mastitis is a disease that affects a large number of dairy cattle throughout the world. A survey conducted in the major milk-producing countries indicates that each year, clinical mastitis afflicts 15% to 20% of cows (29). In Canada and the United States of America (USA), it is thought that 50% of cows have one or more infected quarters. In Denmark, it is estimated that mastitis is the cause of 30% to 40% of veterinary interventions (13, 15). There is a relative scarcity of published data on the prevalence of *C. perfringens* as a cause of mastitis, given that a survey of the literature revealed that the *C. perfringens* type A micro-organism has been recorded as being associated with many bovine mastitis-related incidents (21, 27, 28, 40).

Five toxin types (A to E) are based on the existence of up to three of the four so-called major toxin (α, β, ε, i) genes (*plc*, *cpb, etx, iap/ibp*) (37). *Clostridium perfringens* enterotoxin (CPE) is an important virulence factor for both *C. perfringens* type A food poisoning and several non-food-borne human gastro-intestinal diseases (9). Recent studies have indicated that *C. perfringens* isolates associated with food poisoning carry a chromosomal *cpe* gene, while non-food-borne human gastro-intestinal disease isolates carry a plasmid *cpe* gene (10, 12, 23).

On the national level, there is considerable need to identify the prevalence of the different types of *C. perfringens* in the milk of mastitic animals in the Egyptian environment, with special reference to the α/*cpa* gene in the buffalo and cattle of Egypt. Thus, the objective of this study was to identify the causal *C. perfringens* strain and study, for the first time, certain epidemiological and clinical features of clinical mastitis in buffalo (not recorded internationally) and cattle kept for meat and milk production in Egypt.

# Materials and methods

## Sampling and bacteriological examinations

A veterinarian performed clinical examinations of all selected buffalo and cows. The examination included an assessment of the general condition of the animal, including appetite and body temperature. The weight was estimated by measuring the heart girth (19). The udder was examined by palpation, and teat injuries were recorded. Clinical data included the rectal temperature, an assessment of the severity of systemic signs (graded as early or late) and local clinical signs of the affected gland, including whether or not gangrene was present (i.e. a cold and blue udder and teat skin).

## Milk samples

Udder secretions were collected and clinical data recorded from apparently healthy animals, as well as those with sub-clinical and clinical mastitis. Udder quarter milk samples were collected aseptically from the clinically affected glands in 10 ml sterile plastic vials from each animal on two occasions, in accordance with International Dairy Federation standards. In addition, samples were taken from the clinically unaffected gland with unilateral clinical mastitis. The California mastitis test (CMT) was recommended by the American Public Health Association (23, 30) as a method to detect sub-clinical mastitis from samples collected from apparently normal quarters. This test was performed beside the cow, according to the recommendations of the manufacturer, on quarter foremilk samples. The CMT results were recorded as 0 (negative), 1 (trace or +), 2 (++), or 3 (+++). None of the samples came from animals with arthritis, conjunctivitis or pneumonia; therefore, the mammary secretions were not checked for the presence of mycoplasms.

## Bacteriological examination

The randomly collected milk samples were centrifuged at 3,000 revolutions per minute for 20 min (35). The cream and supernatant were discarded, then the sediment of each milk sample was inoculated into cooked meat broth (CMB). The samples were cultured aerobically and anaerobically, as described by Hirvonen *et al.* (19). After an overnight anaerobic incubation at 37ºC, using anaerobic jars containing 95% $H_2$ and 5% $CO_2$, a loopful was streaked onto sheep blood agar plates, containing 150 µg/ml neomycin sulphate, and incubated anaerobically at 37ºC for a further 24 h (30). *Clostridium perfringens* was differentiated from other *Clostridium* spp. by:

– colony morphology

– immobility

– the presence of a double zone of haemolysis

– further confirmatory biochemical tests, as necessary (26).

Milk samples from which bacteria other than *C. perfringens* were cultured were discarded (n = 191). Directly after culturing, all milk samples were stored at – 20ºC. The 33/224 milk samples in which *C. perfringens* was found were thawed at room temperature and then cultured again.

### Animal model

Immature BALB/c mice, with an average weight of 15 g to 20 g (used for the toxin neutralisation test), and mature guinea pigs, with an average weight of 350 g to 450 g (used for the dermo-necrotic test), were maintained in air-conditioned (22 ± 1ºC) quarters under uniform husbandry conditions with a light cycle from 06:00 to 20:00. The animals were quarantined and acclimatised for seven days, prior to the experimental treatment. During this acclimatisation period, the animals were given a pellet diet and tap water *ad libitum*. General procedures for animal care and housing were in accordance with the United States Department of Agriculture, through the Animal Welfare Act (7USC 2131) 1985 and Animal Welfare Standards incorporated into Title 9 of the Code of Federal Regulations, Part 3, 1991.

The isolated *C. perfringens* strains were then typed for their toxigenicity by the mice neutralisation test (6) and dermo-necrotic test in guinea pigs (39).

## Molecular typing

### Deoxyribonucleic acid isolation

A starter culture (6 ml) of each *C. perfringens* isolate was prepared by overnight growth at 37ºC in CMB, as described previously (38). For deoxyribonucleic acid (DNA) isolation, the bacterial pellet was re-suspended in 400 µl Tris-ethylenediamine tetra-acetic acid (EDTA) (pH 8.0) buffer (Tris-HCl [Sigma] 10.0 mM; EDTA [Sigma] 1.0 mM). The reference strains used in this study, *C. perfringens* types A (ATCC 13124), B (ATCC 3626) and D (NCTC 8346), acted as controls for the different toxins. *Rhodococcus equi* (a field isolate) and *S. aureus* (ATCC 29737) were used for quality assurance and polymerase chain reaction (PCR) specificity.

### Preparation of bacterial specimens and polymerase chain reaction amplifications

Total *C. perfringens* DNA was isolated from the overnight MCB cultures by a previously described protocol (38). Template DNA from bacterial cultures was thus obtained by direct boiling. The purity and concentrations of the DNA were determined using a spectrophotometer (S110). The specific oligonucleotide primers for PCR amplification of the toxin genes are listed in Table I (7). Polymerase chain reaction assays were performed with the DNA Thermal Cycler.

The reactions were performed in 50-µl volumes, containing: 1.0 µl of extracted DNA template from bacterial cultures or 5 µl of the extracted DNA from the milk sample, 45.0 µl of *Taq* PCR mixture (10 mM Tris-HCl [pH 9.0], 4.0 mM $MgCl_2$, 50 mM KCl, 0.005% Tween 20, 0.005% Nonidet P-40 detergent, each deoxynucleoside triphosphate at a concentration of 170 µM, 0.25 µM each oligonucleotide primer, and 1.25 U of *Taq* DNA polymerase [Fermentas]). The reactions were subjected to 39 cycles of amplification, consisting of 60 s of denaturation at 94ºC, 60 s for primer annealing at the respective temperature (Table I), and 30 s of chain extension at 72ºC. The ramping time used to raise the temperature from the annealing temperature to 72ºC is given in Table I. Evaporation within the tube was prevented by the addition of mineral oil.

### Sodium dodecyl sulphate polyacrylamide gel electrophoresis

The PCR products were analysed by applying 20 µl on a 2.0% agarose gel for electrophoresis and visualised with ethidium bromide and an ultraviolet transilluminator. Negative control experiments were performed with all of the reagents except the template DNA.

**Table I**
**Specific oligonucleotide primers for polymerase chain reaction amplification of the genes for *Clostridium perfringens* toxins alpha, beta and epsilon**

| Toxin/gene | Primer | Oligonucleotide sequence | Fragment length (bp) | Annealing temperature (ºC) | Ramping time (s) [a] |
|---|---|---|---|---|---|
| α/*cpa* | CPALPHATOX1-L | 5'-AAG ATT TGT AAG GCG CTT-3' | 1,167 | 56 | 60 |
| | CPALPHATOX1-R | 5'-ATT TCC TGA AAT CCA CTC-3' | | | |
| β/*cpb* | CPBETATOX1-L | 5'-AGG AGG TTT TTT TAT GAA G-3' | 1,025 | 39 | 90 |
| | CPBETATOX1-R | 5'-TCT AAA TAG CTG TTA CTT TGT G-3' | | | |
| ε/*etx* | CPETOXIN1-L | 5'-AAG TTT AGC AAT CGC ATC-3' | 961 | 46 | 60 |
| | CPETOXIN1-R | 5'-TAT TCC TGG TGC CTT AAT-3' | | | |

a) Ramping time from the annealing temperature to an extension temperature of 72ºC
bp: base pair
s: seconds

### Statistical analysis

The results are shown as the means ± standard deviations for these experiments. The statistical procedures were according to Snedecor (36). The student t-test was used, in addition to analysis of variance (F test).

## Results

### Clinical manifestations

*Clostridium perfringens* causing acute bovine mastitis was characterised by a rapid onset of acute oedematous swelling of the affected quarter. The affected quarter was bluish-black in colour, with a clear line of demarcation between the necrotic gangrenous tissue and healthy tissue. The affected skin area was cold to the touch and tended to peel off, with oozing of a serous fluid, while the milk was usually bloody and watery. The affected animals observed during this study were treated with different medications and protocols to no effect, resulting in the sloughing of the affected udder tissues. The affected farms did not lose any of these animals (Figs 1 & 2). Aerobic culturing was negative in the mastitic samples.

### Incidence of *Clostridium perfringens* isolated from the milk samples of sub-clinical and clinical mastitic animals

Table II indicates that, in the sub-clinical stage, four isolates of *C. perfringens* were recovered, in total, from the cows (3.92%), while no *C. perfringens* isolates were recovered from the buffalo. On the other hand, the incidence of *C. perfringens* isolated from the clinically affected quarters and milk samples was 12 (4.7%) from the cows and one (12.5%) from the buffalo.

### Seasonal incidence of *Clostridium perfringens* mastitis

*Clostridium perfringens* infection was correlated to the seasons of the year (Table III), with the highest proportion of isolates being recovered in spring (10.71%) and winter (7.07%).

### Typing *Clostridium perfringens* and differentiation between toxigenic and non-toxigenic strains

Seventeen *C. perfringens* strains were recovered from sub-clinical and clinical mastitic animals (16 isolates from cows and one isolate from buffalo). All suspected *C. perfringens* strains were identified for their biochemical reactions. The results in Table IV, employing Nagler's reaction, pathogenicity testing in guinea pigs and passive haemagglutination, indicate that, out of the four tested *C. perfringens* isolates from the sub-clinically mastitic cows, two were toxigenic and two were non-toxigenic. On the other hand, from the clinically mastitic cows, eight of the 12 *C. perfringens* isolates were recorded as toxigenic and four as non-toxigenic. The single *C. perfringens* isolate recovered from the clinically mastitic buffalo was found to be toxigenic.

Using the dermo-necrotic test in guinea pigs to type the *C. perfringens* strains recovered from mastitic animals, two *C. perfringens* isolates recovered from sub-clinically mastitic cows were identified as type A (100%). Eight isolates recovered from clinically mastitic cows were identified as:

- five type A (62.5%)
- one type B (12.5%)
- two type D (25%).

**Fig. 1**
**The teat of a lactating buffalo affected with gangrenous theilitis**
The photograph clearly shows the dark, blackish discoloration, with a clear line of demarcation (early stages)

**Fig. 2**
**The teat of a lactating buffalo affected with gangrenous theilitis, showing sloughing of the affected tissue (late stages)**

**Table II**
**Incidence of *Clostridium perfringens* recovered from sub-clinical and clinical mastitic milk samples (based on quarter milk samples)**

| Animals | Sub-clinically mastitic milk samples | | | Clinically mastitic milk samples | | |
|---|---|---|---|---|---|---|
| | No. of examined QMS | No. of positive QMS | Percentage of positive QMS | No. of examined QMS | No. of positive QMS | Percentage of positive QMS |
| Cows | 102 | 4 | 3.92% | 255 | 12 | 4.7% |
| Buffalo | 17 | 0 | 0% | 8 | 1 | 12.5% |

QMS: quarter milk samples

**Table III**
***Clostridium perfringens* isolates recovered from the examined milk samples during the four seasons**

| Season | Sub-clinical mastitis | | | Clinical mastitis | | | Total number of isolates | Percentage of isolates |
|---|---|---|---|---|---|---|---|---|
| | Number of examined animals | Number of isolates | Percentage of isolates | Number of examined animals | Number of isolates | Percentage of isolates | | |
| Summer | 34 | 1 | 2.94% | 57 | 3 | 5.26% | 4 | 4.39% |
| Autumn | 20 | 1 | 5% | 57 | 1 | 1.75% | 2 | 2.59% |
| Winter | 25 | 2 | 8% | 74 | 5 | 6.76% | 7 | 7.07% |
| Spring | 3 | 0 | 0% | 25 | 3 | 12% | 3 | 10.71% |
| **Total** | **82** | **4** | **4.88%** | **213** | **12** | **5.63%** | **16** | **5.4%** |

**Table IV**
**Differentiation between toxigenic and non-toxigenic isolates of *Clostridium perfringens* by using Nagler's reaction, pathogenicity testing in guinea pigs and passive haemagglutination**

| Mastitic animals | Total number of tested isolates | Toxigenic | | Non-toxigenic | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| Sub-clinically mastitic cows | 4 | 2 | 50% | 2 | 50% |
| Clinically mastitic cows | 12 | 8 | 66.66% | 4 | 33.33% |
| Clinically mastitic buffalo | 1 | 1 | 100% | 0 | 0% |

The *C. perfringens* strain isolated from the buffalo was identified as type A (100%) (Table V).

## Amplification of a 1167 base pair fragment of alpha-toxin gene from the extracted deoxyribonucleic acid of *Clostridium perfringens*, using CPALPHATOX1-L and CPALPHATOX1-R primers

The specificity of the oligonucleotide primers was confirmed by the positive amplification of a 1167 base pair (bp) fragment from the extracted DNA of *C. perfringens*. On the other hand, no amplification was observed with the extracted DNA of the other standard bacterial strains previously mentioned in Table I. This is shown in Figure 3. Lanes 1, 2, 3 and 4 reveal positive amplification of the alpha-toxin (α-toxin) gene from the extracted DNA of

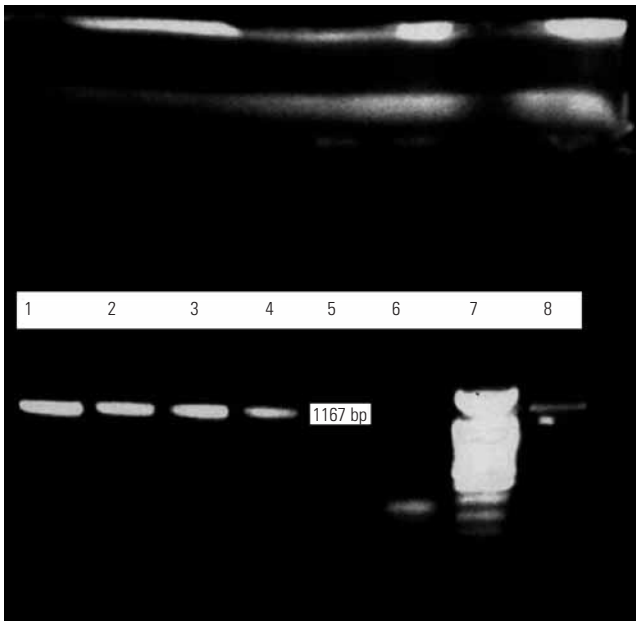


**Fig. 3**
**Agarose gel electrophoresis showing amplification of a 1167 base pair fragment of the alpha-toxin gene from the extracted deoxyribonucleic acid of *Clostridium perfringens* isolates (lanes 1, 2, 3 and 4)**
Lane 7 shows the 100 base pair (bp) 1.5 kilobase deoxyribonucleic acid (DNA) ladder. Lane 8 shows amplification of the 1167 bp fragment of alpha-toxin (α-toxin) gene from the extracted DNA of the *C. perfringens* standard strain and lanes 5 and 6 show no amplification of the 1167 bp of the α-toxin gene from the extracted DNA of *Rhodococcus equi* and *Staphylococcus aureus*

**Table V**
**Typing of toxigenic *Clostridium perfringens* isolates recovered from mastitic cows and buffalo, by the dermo-necrotic test**

| Mastitic animals | Number of tested isolates | Types of toxigenic isolates | | | | | |
|---|---|---|---|---|---|---|---|
| | | Type A | | Type B | | Type D | |
| | | No. | % | No. | % | No. | % |
| Sub-clinically mastitic cows | 2 | 2 | 100% | 0 | 0% | 0 | 0% |
| Clinically mastitic cows | 8 | 5 | 62.5% | 1 | 12.5% | 2 | 25% |
| Clinically mastitic buffalo | 1 | 1 | 100% | 0 | 0% | 0 | 0% |

*C. perfringens* isolates, while lane 7 shows the 100 bp ladder and lane 8 shows positive amplification of the 1167 bp fragment of α-toxin gene from the extracted DNA of the standard strain of *C. perfringens*. Lanes 5 and 6 reveal negative amplification of two standard strains other than *C. perfringens*.

The results, also seen in Figure 4, reveal positive amplification of the 1167 bp fragment of α-toxin gene from the extracted DNA of *C. perfringens* isolates (lanes 1 to 6), while lane 7 shows positive amplification of the 1167 bp fragment of α-toxin gene from the extracted DNA of the standard strain of *C. perfringens*, type A.

Fifteen milk samples were selected for testing with the same primers. Of these, six milk samples were obtained from animals with clinical mastitis, which were infected with *C. perfringens*, as confirmed after bacteriological examination. Nine milk samples came from animals with clinical mastitis which were suspected to be infected with *C. perfringens*. However, bacteriological examination did not reveal any *C. perfringens* in these samples.

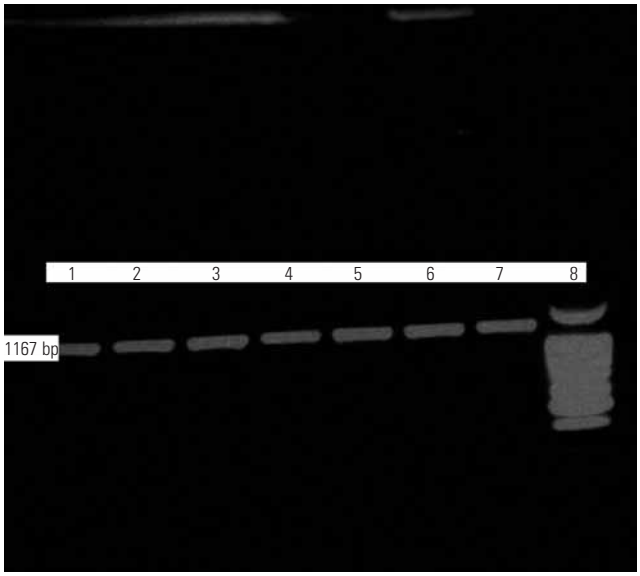


**Fig. 4**
**Agarose gel electrophoresis showing amplification of a 1167 base pair fragment of the alpha-toxin gene from the extracted deoxyribonucleic acid of *Clostridium perfringens* isolates (lanes 1, 2, 3, 4, 5 and 6)**
Lane 7 shows amplification of the 1167 base pair (bp) fragment of the alpha-toxin gene from the extracted deoxyribonucleic acid of a *C. perfringens* standard strain and lane 8 shows the 100 bp-1.5 kilobase DNA ladder

Figure 5 shows the positive amplification of the 1167 bp fragment of α-toxin gene from the extracted DNA of the six milk samples which proved to be infected with *C. perfringens*, as confirmed bacteriologically (100%). Yet, of the nine milk samples which did not reveal any *C. perfringens* after bacteriological examination, two revealed positive amplification with DNA extraction.

## Amplification of the 1025 base pair fragment of the beta-toxin gene from the extracted deoxyribonucleic acid of *Clostridium perfringens* isolates using CPBETATOX1-L and CPBETATOX1-R primers

The extracted DNA of *C. perfringens* isolates was tested with CPBETATOX1-L and CPBETATOX1-R. The results (see Fig. 6) reveal a positive amplification of the 1025 bp fragment of the beta-toxin gene (β-toxin) from the extracted DNA of a *C. perfringens* type B standard strain (lane 1). Lanes 3, 4 and 5 show no amplification of the 1025 bp fragment of the β-toxin gene.

## Amplification of the 961 base pair fragment of epsilon-toxin gene from the extracted deoxyribonucleic acid of *Clostridium perfringens* isolates, using CPETOXIN1-L and CPETOXIN1-R primers

Extracted DNA of the *C. perfringens* isolates was examined with CPETOXIN1-L and CPETOXIN1-R primers. The results, in Figure 6, reveal positive amplification of the 961 bp fragment of epsilon toxin (ε-toxin) gene from the extracted DNA of the *C. perfringens* type D standard strain (lane 2). However, no amplification of this 961 bp fragment of ε-toxin is seen in lanes 7 and 8.

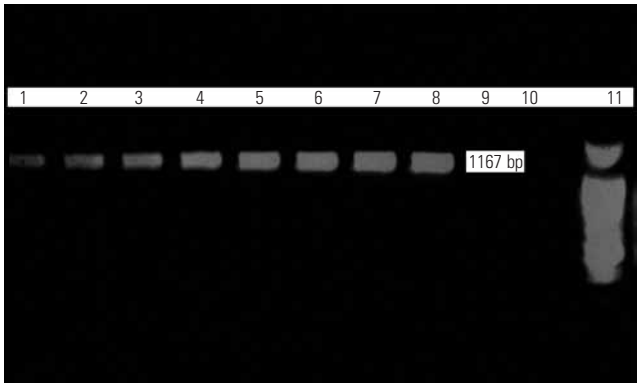


**Fig. 5**
**Agarose gel electrophoresis showing amplification of the 1167 base pair fragment of alpha-toxin gene from the extracted deoxyribonucleic acid of *Clostridium perfringens* positive milk samples (lanes 1, 2, 3, 4, 5 and 6)**
Lanes 7 and 8 show amplification of the 1167 base pair (bp) fragment of alpha-toxin gene from the extracted deoxyribonucleic acid (DNA) of *Clostridium perfringens* culture-negative milk samples, while lanes 9 and 10 show no amplification. Lane 11 shows the M15 set of 100 bp DNA ladder

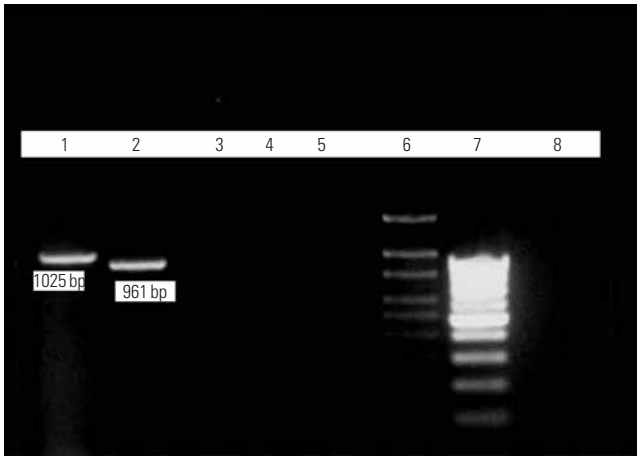


**Fig. 6**
**Agarose gel electrophoresis showing amplification of the 1025 base pair fragment of beta-toxin gene from the extracted deoxyribonucleic acid of *Clostridium perfringens* type B (lane 1)**
Lane 2 shows amplification of the 961 base pair (bp) fragment of epsilon-toxin (ε-toxin) gene from the extracted deoxyribonucleic acid (DNA) of the *Clostridium perfringens* type D standard strain.
Lane 6 shows the 100 bp-1.5 kilobase DNA ladder, while lane 7 shows the M15 set of 100 bp DNA ladder. Lanes 3, 4 and 5 show no amplification of the 1025 bp fragment of beta-toxin gene from the extracted DNA of *C. perfringens* isolates, while lane 8 shows no amplification of the 961 bp fragment of ε-toxin gene from the extracted DNA of the *C. perfringens* isolate

# Discussion

Osman and colleagues conducted a series of studies on the contagious and environmental species of bacteria associated with mastitis in cows, buffalo and camels (1, 2, 3, 20, 24, 25, 41).

Using the culture methods of the present study, Osman *et al.* found that *C. perfringens* type A was the most prevalent cause of mastitis. More than 4.48% (62.5% toxigenic) and 4.0% (100% toxigenic) of the raw cow and buffalo milk samples, respectively, contained the *C. perfringens* type A strain, raising the hypothetical possibility of milk consumption inducing enterotoxaemia in humans. The prevalence of *C. perfringens* in raw milk was found to reach high levels in Gambia (22.3%), Guinea (39.1%) and Senegal (11.2%), by Hempen *et al.* (17). *Clostridium perfringens* type A is responsible for various diseases, including gas gangrene, food poisoning and diarrhoea in humans, as well as enterotoxaemia and haemorrhagic gastro-enteritis in many domestic and wild animals (37). The presence of *C. perfringens* is a public health problem, since people can be infected by ingesting contaminated food, and milk is a major part of the human diet, especially for children. It is assumed that faecal contamination of raw milk is the principal source of contamination, since *C. perfringens* is present in the intestinal tract of cattle.

According to Lorentzon (22), the most common cause of mastitis is a bacterial infection through the teat canal. Environmental conditions affect the rate and magnitude of bacterial growth in the surroundings of the animal. Exposure to manure in cow housing areas can influence the rate of clinical mastitis (33). The most obvious source of environmental infections is the bedding, because the udders come into direct contact with bacterial populations in the bedding (42).

The results of the present study indicate that *C. perfringens* type A is the predominant cause of mastitis in cows and buffalo in Egypt. Conversely, the finding that no *C. perfringens* type E strain was identified in this survey agrees with previous data (37), confirming that *C. perfringens* type E is rare as a causative agent of mastitis. It should be noted that Baldassi *et al.* (5) isolated two strains of *C. perfringens* from the bovine udder but unfortunately did not indicate their types.

In this study, all isolates of *C. perfringens* found in the milk samples of apparently healthy and mastitic cows carried only the α/*cpa* gene belonging to the α-toxin (8). The regulation of α-toxin production appears to be a crucial pathogenicity factor of type A strains of *C. perfringens* (16). Furthermore, the study showed that only one toxin type of *C. perfringens* was found in the given herds (type A, with its chromosomal α/*cpa* gene). Toxin gene typing by PCR has the advantage that it can be performed directly from primary culture colonies, and thus can detect toxin genes which are unstable, such as *cpb* and *cpb*2. These unstable toxin genes might be lost during the cultivation process

needed for the biological method (the mouse model). Moreover, toxin gene detection can also measure the presence of virulence factors that are tightly regulated and specifically expressed during infection, and so remain undetected by phenotypic methods in culture.

Sarker *et al.* (31) demonstrated that all survivors of heating retained the *cpe* gene in its original plasmid or chromosomal location and could still express CPE. These results suggest that chromosomal *cpe* isolates are strongly associated with food poisoning; at least in part because their cells and spores possess a high degree of heat resistance. This will enhance their survival in incompletely cooked or inadequately warmed foods, such as milk.

# Conclusion

There were no previous national data on the prevalence of different types of *C. perfringens* in the milk of mastitic animals in the Egyptian environment. The objective of this work was to identify the causal *C. perfringens* strain or strains, and study certain epidemiological and clinical features of clinical mastitis in buffalo, for the first time (not recorded internationally), and cattle kept for meat and milk production in Egypt.

In developing countries, backyard farms are those with fewer than ten animals, which do not fall into any of the categories of commercial farming. The expansion of domestic herds is limited by:

– land availability

– the limited supply of labour skilled in animal husbandry techniques

– the availability of low-cost agricultural byproducts used for cattle feed

– the ability to afford only unskilled, low-cost labour

– a lack of capital and infrastructure

– the economic circumstances of small landholders, who often raise cattle in 'backyard' farming operations.

All these factors are very important in milk production.

Environmental mastitis bacteria are present in great numbers in the surroundings of the cow, such as bedding, manure and mud, increasing its susceptibility to opportunistic organisms and the likelihood of these environmental bacteria invading the udder and causing mastitis (14, 32).

Acute gangrenous mastitis due to *C. perfringens* type A is rarely reported and identified in the diagnostic laboratory (32). Therefore, the precise defining of the toxin type of *C. perfringens* should simplify and hasten identification and the consequent development of adequate vaccines which fit the epidemiological situation. Toxin gene typing by PCR is a rapid and efficient method for epidemiological investigation of clostridial animal diseases, which are also strongly associated with food poisoning outbreaks in humans.

For the epidemiologist and veterinarian, every *C. perfringens* mastitis outbreak is a rare and unforgiving challenge: a public health emergency that requires rapid recognition and smooth cooperation between authorities to prevent additional cases. Just as veterinarians have to be prepared to quickly make the correct diagnosis in outbreaks of *C. perfringens* mastitis, the epidemiologist must prepare for such outbreaks by surveying potential *C. perfringens* hazards in the community.

# Mammite due à *Clostridium perfringens* dans des troupeaux de bufflonnes et de vaches laitières en Égypte : prévalence, incidence, facteurs de risque et coûts associés

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**Résumé**

Si *Clostridium perfringens* est un agent bien connu pour être à l'origine de maladies entériques clostridiennes, la manière exacte dont chaque toxinotype (types A à E) s'associe à l'apparition de mammite chez les animaux d'élevage reste mal connue. Dans l'étude présentée ici, l'agent de la mammite a été détecté dans 34,12 % (213/623) et 9,64 % (8/83) des prélèvements de lait de quartier provenant de vaches et de bufflonnes cliniquement atteintes de mammite. L'isolement du micro-organisme a révélé une incidence de 4,48 % (16/357) dans les échantillons de lait de vache et de 4,0 % (1/25) dans les échantillons de lait de bufflonne. La situation prépondérante était l'infection d'un seul quartier, ce qui était le cas chez 83 % des vaches et 87 % des bufflonnes. Une variation saisonnière a été mise en évidence avec une prévalence de l'infection à *C. perfringens* plus importante au printemps (10,71 %) et en hiver (7,07 %). Au total, 17 souches de *C. perfringens* isolées du lait de vache et de bufflonne et caractérisées au moyen de la méthode classique de neutralisation de la toxine se sont révélées être de type A. Ces souches ont été soumises à une analyse moléculaire. L'épreuve d'amplification en chaîne par polymérase a permis de détecter le gène $\alpha$/*cpa* ; en revanche, les gènes $\beta$/*cpb* et $\varepsilon$/*etx* n'ont pas été détectés. Les auteurs estiment que la présence de *C. perfringens* est à elle seule potentiellement pathogène ; en outre, elle est un facteur prédisposant aux infections de la mamelle dues à des mammites graves ou à des agents pathogènes présents dans l'environnement.

**Mots-clés**

Bétail laitier – Bovin – Buffle – *Clostridium perfringens* – Égypte – Gène $\alpha$/*cpa* – Mammite – Toxine $\alpha$.

# Prevalencia, incidencia, factores de riesgo y costos de la mastitis por *Clostridium perfringens* en búfalas y vacas lecheras en Egipto

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**Resumen**

Si bien se ha demostrado que *Clostridium perfringens* es un importante agente de enfermedades entéricas clostridianas, aún no se conoce con certeza la influencia exacta de cada uno de sus toxinotipos (tipos A a E) en el desarrollo de mastitis en animales de criadero. En este estudio, los autores detectaron el agente de la mastitis en el 34,12% (213/623) y el 9,64% (8/83) de las muestras de leche provenientes de cuartos de vacas y búfalas con síntomas clínicos de la enfermedad. El aislamiento del microorganismo mostró una incidencia del 4,48% (16/357) en las muestras de leche de vaca y del 4,0% (1/25) de las muestras de búfala. La mayoría de los animales (83% de las vacas y 87% de las búfalas) presentaba un solo cuarto infectado. También se establecieron variaciones

estacionales, con una mayor prevalencia de la infección por *C. perfringens* en primavera (10,71%) e invierno (7,07%). La caracterización con el método clásico de neutralización de la toxina mostró que 17 cepas de *C. perfringens*, aisladas en leche de vaca y de búfala, pertenecían al tipo A. Posteriormente, los autores procedieron al análisis molecular de esas cepas. Si bien con la prueba de reacción en cadena de la polimerasa se detectó el gen α/*cpa*, no se encontraron genes β/*cpb* ni ε/*etx*. En opinión de los autores, la presencia de *C. perfringens* es potencialmente patógena y podría predisponer a infecciones de la ubre, ya sea mastitis graves, o patologías ocasionadas por agentes patógenos presentes en el entorno.

**Palabras clave**
Bovino – Búfala – *Clostridium perfringens* – Egipto – Ganado lechero – Gen α/cpa – Mastitis – Toxina α.

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