Causes of sudden death of Female Dolphin *Turisops truncates* at Magic Land – Egypt

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Abstract

Although cause-of-death information on bottlenose dolphin *Tursiops truncatus* can be located in the literature, few citations include mortality data over a long period of time covering a broad geographic region. This study was organized to analyze all causes of sudden death for a captive female dolphin *Turisops truncates* which was recently died at Magic Land, Egypt. Post mortem examinations proved severe heamorrhage and congestion in internal organs. Bacterial examinations showed the tested internal organs according to their high incidence of pathogens were as follows: liver, lungs, bronchial lymph nodes, colon, stomach, spleen, kidneys, gall bladder, small intestine, trachea and heart. The main isolates were E – coli, Pseudomonas aeruginosa, Klebsiella pneumonia and Pasturella spp. Antibiotic sensitivity tests for the isolated micro organisms revealed that Garamycin (30 ug) and Ampicillin (10 ug) were the most sensitive antibiotics of choice for isolated microorganisms. Parasitological examinations of all organs revealed no presence of any adult parasites or its larval stages. Histopathological examination revealed congestion in pulmonary blood vessels of lungs, interstitial pneumonia with red hepatization. Liver showed congestion in hepatic blood vessels and sinusoid accompanied with thickness in hepatic capsule and vacuolar degeneration and necrosis in hepatocytes. Kidney showed shrinkage in glomeruli, other glomeruli showed hypercellularity of glomerular tuft with severe interstitial hemorrhage in between renal tubules. Heart revealed edema and degeneration of some cardiac musculature. Intestine showed proliferation of goblet cells and infilteration with inflammatory cells associated with edema in the muscular layer. Spleen showed moderate depletion in lymphoid follicle.

Key words: *bottlenose dolphin, E. coli, Pseudomonas aeruginosa, Klebsiella pneumonia and Pasturella spp. .*

Introduction

Bottlenose dolphins can be regarded as sentinel species of the oceans, providing an indication of disease processes and environmental problems **(Bossart, 2006)** that could affect marine animal and human health.

Information on the causes of mortality of bottlenose dolphins *Tursiops truncatus* over many years for a broad geographic region has been sparse. Most of the information in the literature on this topic is limited to mortality of bottlenose dolphins that involved periods of increased mortality or descriptions of pathologic findings in small numbers of individuals **(Lipscomb *et al*., 1996; Bossart *et al*., 2003; McFee and Osborne, 2004)**.

Several studies have described the presence of high levels of contaminants in bottlenose dolphins as a potential cause of reproductive stress and debilitative disease **(Hansen *et al*., 2004; Houde *et al*., 2005; Wells *et al.,* 2005)**.

Bacterial diseases are major cause of death in marine mammals. Bacterial organisms might be considered as secondary invaders in conjunction with parasitic, viral or traumatic injuries. The inhibition of bacteria in the marine mammal's environment is one of the most important factors in maintaining marine mammal health in captivity **(Moller, 2002).**

Bacterial infection of lungs in small Cetaceans is the most frequent systemic disease and is the most common cause of death, infection fre-

quently occurs as a sequel to diseases that result in immunosupression, all form of diseases are seen from per acute hemorrhagic bronchopneumonia to chronic abscesstion **(Sweenwey and ridgway,1975).** Staphlococcus spp., streptococcus spp. were isolated from cases suffered from pneumonia (**Medway et al., 1973).** *Pseudomonas fluorescens* was isolated as the causative agent of sudden death of captivate bottlenose dolphin (*Tursiops truncatus*) at Magic land, Egypt **(Mohamed, 1999)**.

Pathogenic strain of *E. coli* has been reported as causative agent of death in captivates bottlenose dolphin *Tursiops truncatus* at Magic land, Egypt. The histopathological effects on organs showed that interstitial pneumonia, granular degeneration in hepatocyte and sloughing of intestinal villi with necrosis and few lymphocytic infiltration in the epithelial lining (Mohmoud *et al.*, 2001). Moreover, Klebsiella (Gram negative bacilli) was seen associated with pneumonia, encephalitis and ocular infections in marine mammals. Various species of Pseudomonas (gram negative bacilli) have been incriminated in causing bacterial disease in both pinnipeds and cetaceans. It is felt that this organism is an opportunistic pathogen which colonizes wounds and can lead to septicemia **(Moller, 2002).** *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Burkhoderia pseudomallei* have been known to produce septicemia and death, but they are usually associated with other predisposing diseases **(Dierauf and Gulland, 2001; Fowler and Miller, 2003)**. **Bermudez *et al*., (2009)** had defined Lobomycosis (lacaziosis) as a chronic, granulomatous, fungal infection of the skin and subcutaneous tissues that affects humans and members of the family Delphinidae caused by the noncultivable yeast-like organism (Lacazia loboi) of the order Onygenales.

This study was aimed to investigate the cause for sudden death of captive female dolphin *Turisops truncates* which was recently died at Magic Land, Egypt.

Material and Methods

A – Material:

An entertainment captive female dolphin *Turisops truncates* which was recently died at Magic Land, Egypt.

Postmortem Examination:

The whole carcass was examined for the cause of death and recorded any abnormal pathologic macroscopic criteria.

Samples:

I – Selective samples were taken from internal organs: lungs, trachea, heart, bronchial lymph nodes, liver, spleen, kidneys, stomach, gall bladder, small intestine and colon for bacteriological, parasitological and histopathological examinations.

II – Random samples from the frozen horse mackerel (food of dolphin) were also taken for examination.

B – Methods:

Bacteriological Examinations:

Under complete sterilization collected swaps were cultured into nutrient broth at 37º C for 24 hrs. and then sub-cultured into the following (Difico) nutrient agar, 5 % sheep blood agar, Macconkey agar, Eosin Methylene blue plates, SS agar and XLD agar then biochemical tests were carried out as Indole production, Methyl red, Voges- proskawr, citrate, urease & sugar fermentation tests for identification of *E. coli*, Klebsiella pneumonia and *Pseudomonas aeruginosa* identified by API 20 NE according to the instruction of the Bio Merieux Company.

The obtained isolates were identified according to **Quinn *et al*. (1994)**.

Antibiogram Study:

Drug sensitivity test of all isolated bacteria were done using standard disc technique according **Astenholz (2001)** against eight different chemotherapeutic agents. The biomerieux discs were used for in vivo assay namely erythromycin (15 ug), Garamycin (30 ug), Kanamycin (30 ug), Neomycin (30 ug), Oxytetracyclin (10 ug), Spectinomycin (10 ug), Ampicillin (10 ug) and Naldixic acid (30 ug).

Bacteriological examination of fish feeding of the dolphin:
Samples from liver, kidney, heart, spleen and muscles were aseptically streated on Tryptic soya agar and nutrient agar, incubated at 25º C for 18 - 24 hrs. Pure colonies were streated onto soft agar for further studies. Bacterial isolates were identified according to **Balows *et al*., (1992), Cowan and Steel (1993)** and **Baller, (2004)**.

Parasitological Examination:
Several small samples from all organs were examined by dissecting microscope for the presence of adult parasites or their larval stages according to **Kinne (1984)**.
Small intestine and colon were opened and their contents were scraped. After complete thorough washing, several parts throughout the whole intestine and colon were taken and examined for the presence of adult worms.

Histopathological Examination:
Tissue specimens were taken from internal organs: lungs, heart, liver, spleen, kidneys and intestine were collected from female dead captive dolphin at magic land Egypt. Then they were fixed in 10% formaline, dehydrated in ethyl alcohol, clearance in xylol, embedded in paraffin wax and sectioned at 4-5 µ thickness and stained with H&E according to **Bancroft *et al*., (1999)**.

Results
Post mortem Examinations:
The post mortem examination of recently dead dolphin registered severe heamorrhages in internal organs and severe congestion of blood vessels of all organs.
Bacteriological Examinations:
Results registered in Table (1) revealed that the main isolates were *E. coli*, *Pseudomonas aeruginosa*, Klebsiella pneumonia and Pasturella spp.

Table (1). Bacteriological examinations for the different organs of the dead female dolphin.

M.O.	Lung	Trachea	L.N	Liver	Spleen	Kidney	Stomach	Gall Blad.	S. Int.	Colon	T.No. of colonies
Klebsiella pneumonia	1	1	1	1	-	-	-	-	-	-	4
Pasturella spp.	1	-	1	1	1	-	-	-	-	-	4
E. coli	-	-	-	1	-	-	1	1	1	1	5
Pseudomonas aeruginosa	1	-	-	1	-	1	1	1	-	1	6
Total	3	1	2	4	1	1	2	2	1	2	19

Bacteriological examination of fish feeding of the dolphin:
Examinations revealed no pathogenic isolates which might be the cause of dolphin death.
Antibiotic Sensitivity Tests:
As tabulated in Table (2) sensitivity tests for the isolated microorganisms revealed that Garamycin (30 ug) and Ampicillin (10 ug) were the most sensitive antibiotics of choice for isolated microorganisms.

Table (2). Antibiogram of the isolated micro organisms from the dead female dolphin.

Isolates	Naldixic acid	Erythro-mycin	Kanamycin	Neomycin	Gara-mycin	Oxytetra-cyctin	Ampicillin	Spectimo-mycin
E. coli	-	-	+++	+	+++	-	-	-
Klebsiella pneu-monia	-	-	+++	-	+++	-	-	-
Pasturella spp.	-	-	-	-	+++	++	++++	-
Pseudomonas aeruginosa	-	-	+	+	+++	-	+	-

Prasitological Examinations:
Examinations of all organs revealed no presence of any adult parasites or its larval stages.

Pathological Examinations:
Lungs revealed congestion and thickening in wall of pulmonary blood vessel, red hepatization and atelectasis involve most area of the lungs (alveoli are compressed into scarcely recognizable slites) (fig.1). Severe hemorrhage and interstitial pneumonia were observed (fig.2). Focal infiltration with inflammatory cells was seen also (fig.3). Few fibrin threads were observed.

Liver showed congestion in hepatic blood vessels and sinusoidal capilaries, thickness in hepatic capsule, some hepatocytes revealed granular and vacuolar degeneration (fig.4), other hepatocytes showed necrosis, fibrous connective tissue proliferation in portal area were seen (fig. 5).

Kidney showed severe interstitial hemorrhage, degeneration and necrosis in renal tubules (fig. 6). Some tubules showed slouphing of some lining epithelium, renal glomeruli revealed proliferation and vacuolar degeneration of glomerular tuft capillaries, bacterial colonies were observed in glomeruli and tubules (fog. 7 A&B).

Heart showed congestion in myocardial blood vessels, severe hemorrhage in between cardiac muscle. Some myocardial muscles lost its striation with edema in between cardiac musculature (fig.8). Bacterial colonies were seen in between muscle fibers (fig. 9) and other colonies observed in blood vessel.

Spleen revealed depletition in lymphoid follicles with presence of bacterial colonies in between lymphoid follicles (fig.10).

Intestine showed degeneration and necrosis in intestinal gland, infiltration with inflammatory cells in submucosa with goblet cells proliferation (fig.11). Congestion in intestinal blood vessels and hemorrhages and edema in muscularies mucosa were seen (fig. 12).

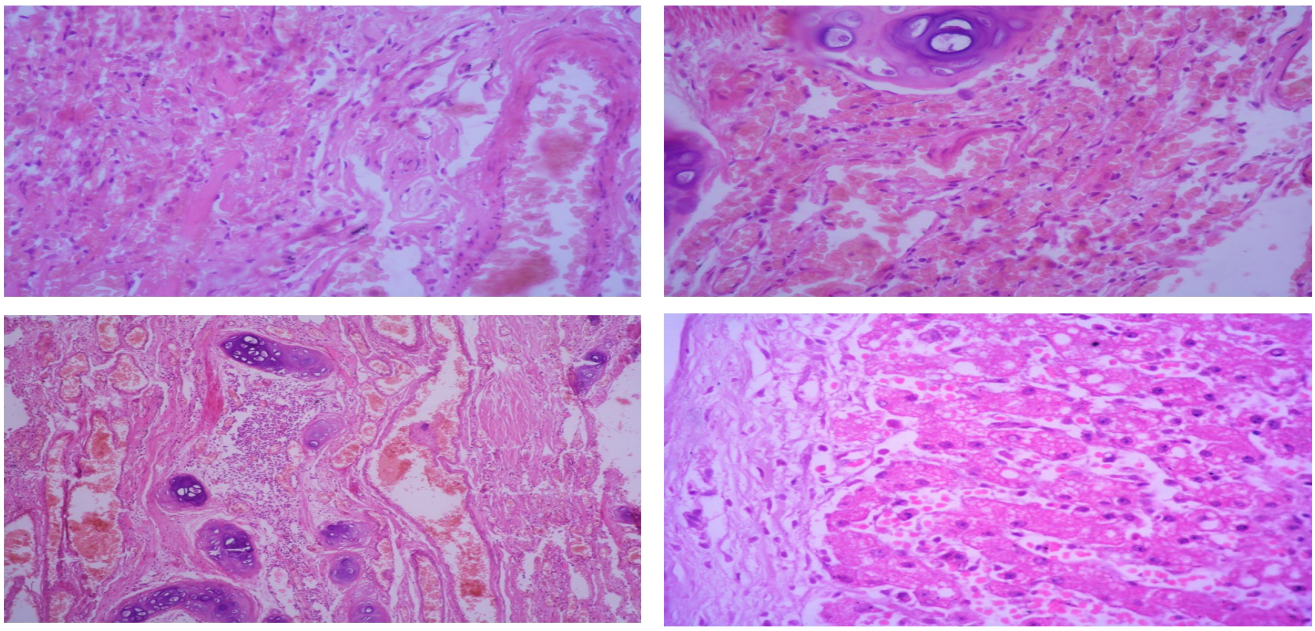

Fig (1). Lung of captive dolphin showed congestion and thickening in wall of pulmonary blood vessel and red hepatization and atelecasis. H & E (X400).
Fig (2). Lung of captive dolphin showed severe hemorrhage and inter4stitial pneumonia.
H & E (X400).
Fig (3). Lung of captive dolphin revealed severe congestion in pulmonary blood vessels and focal infiltration with inflammatory cells. H & E (X100).
Fig (4). Liver of captive dolphin revealed thickness in hepatic capsule, vacuolar degeneration in hepatocyte and congestion of the sinusoidal capillaries. H & E (X400).

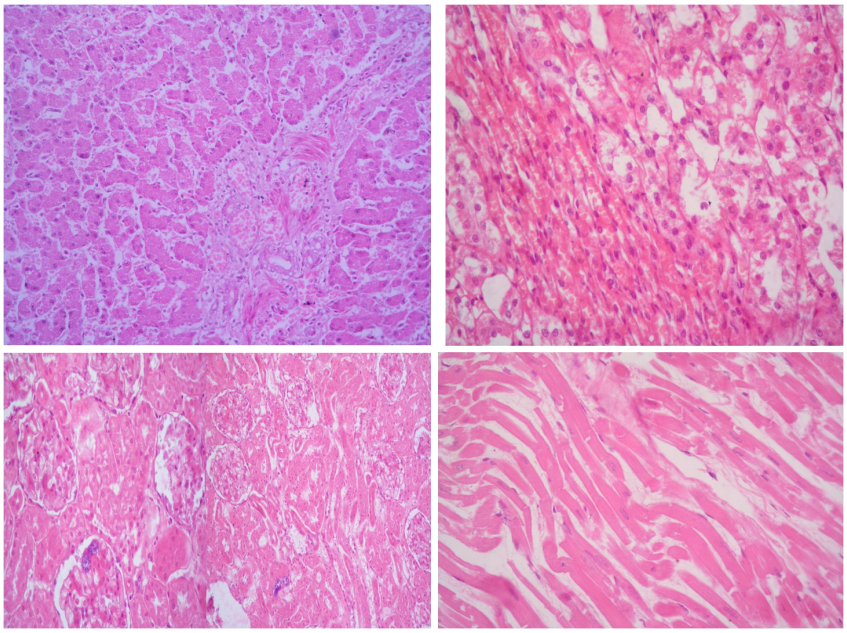

Fig (5). Liver of captive dolphin showed congestion in hepatic blood vessels, fibrous connective tissue proliferation in portal area. H & E (X200).
Fig (6). Kidney of captive dolphin showed interstitial hemorrhage in between renal tubule and necrosis in some renal tubule. H & E (X200).
Fig (7 A &B). Kidney of captive dolphin showed complete sloughing of some lining tubular epithelium (A). Proliferation and vacuolar degeneration of glomeruli tuft capillaries (B) associated with presence of bacterial colonies in some glomeruli and tubule. H & E (AX200, BX 400).
Fig (8). Heart of captive dolphin showed degeneration and edema in between myocardial muscle. H & E (X400).

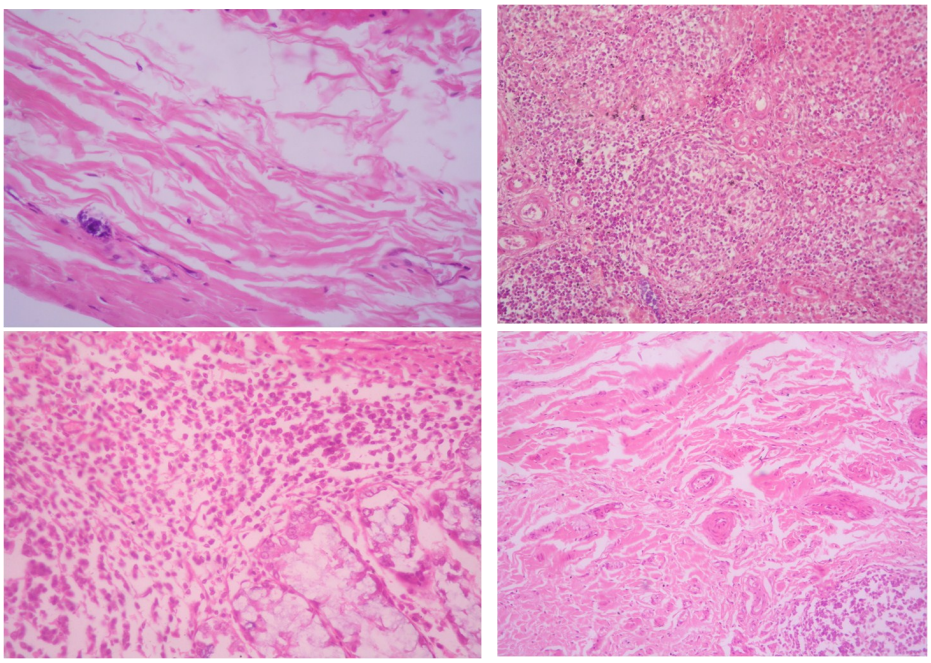

Fig (9). Heart of captive dolphin showed interstitial edema and presence of bacterial colonies in cardiac muscle. H & E (X200).
Fig (10). Spleen of captive dolphin revealed depletion in lymphoid follicle with presence of bacterial colonies in between lymphoid follicle. H & E (X200).
Fig (11). Small intestine of captive dolphin revealed proliferation in mucous producing cells, degeneration and necrosis in intestinal gland, infilteration with inflammatory cells in submucosa.
H & E (X200).
Fig (12). Intestine of captive dolphin showed congestion in intestinal blood vessels and edema in muscularies mucosa and inflammatory cells infilteration. H & E (X200).

Discussion

Marine mammals are slightly susceptible to a wide variety of bacterial pathogens. Majority of the bacterial microorganisms reported by **Carrasco *et al.*, (2011)** already existed in the natural environment and may be of the normal transient flora, some can also act as opportumistic agents causing disease in native populations or animals with compromised immune or nutritional status **(Dunnet *et al.*, 2001)**. Wayne and Thomas 2009 had agreed that bacterial infections accounted for the large majority of fatal infections and emaciation was the leading cause of noninfectious mortality. They added that bacterial infections were the only infectious causes of death for dolphins in the adult age class. Most of these were septicemias with the others being pneumonia, meningitis, myocarditis, peritonitis, enteritis, and placentitis.

Various species of Pseudomonas (gram negative bacilli) have been incriminated in causing bacterial disease in cetaceans and captive dolphin. It is felt that this organism is an opportunistic pathogen which colonizes wounds which lead to septicemia. *Pseudomonas aeruginosa* had caused bronchopneumonia and multiple large cutaneous ulcers in Atlantic bottlenose dolphins. The bacteria are known to progress deep into the cutaneous tissue, causing serious damage to the animal. Many times pseudomonas septicemia causes a characteristic proliferation of gram negative bacilli into the wall of affected blood vessels. If affected animals develop a septicemia **Watson *et al.*, (2002).**

Concerning histopathological examinations which revealed Pneumonia with red hepatization and atelectasis in alveoli of lung associated with severe hemorrhage and congestion of blood vessels, which was in accommodation with the results obtained by **(Mahmoud *et al.*, 2001, Mohamed, 1999** and **Christopher *et al.*, 2007)** who defined Pneumonia as the most common pulmonary disease. Predominant isolates were *Escherichia coli*, Pasteurella, Pseudomonas, Septicemia was encountered, also record pneumonia, fetal atelectasis, congestion, fibrosis and edema. Pneumonia may be attributed to bacterial infection. Cetaceans are unusually prone to bacterial septicemia and may become septic and die within a few hours. In this study, liver showed congestion in hepatic blood vessels and sinusoid, hepatocyte revealed vacuolar degeneration, other necrosis were seen our result coordinate with the result obtained by **(Mohamed *et al.*, 1999** and **Christopher *et al.*, 2007)** which recorded bile duct hyperplasia, hepatocellular necrosis and hepatitis, vacuolar degeneration and centrilobular necrosis.

In the present study kidney revealed degeneration and necrosis in tubule, atrophy in some glomeruli and hypercellularity in other, these accompanied by interstitial hamorrhage. Also recorded edema and degeneration in heart and proliferation in goblet cells of intestinal villi were observed in addition to infiltration with inflammatory cells in submucosa with edma in muscularies mucosa .This agree with **(Christopher *et al.*, 2007)** were recorded tubular necrosis, and glomerular nephrosis, leucocytic infiltration in small intestine, severe hemorrhage in organs .

Finally, we conclude that *Pseudomonas aeruginosa* had caused severe pneumonia, hepatitis together with bacterial colonies in different organs and was the cause of death of the captive dolphin *Tursiops truncates* at Magic Land. Thus we advised those responsible of dolphins at Magic Land to avoid any source of human pollution which is the favorable condition for the flourishing and infection with *Pseudomonas aeruginosa* to dolphin.

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أسباب النفوق المفاجئ لأنثى الدولفين فى مدينة الانتاج الاعلامى (ماجيك لاند)

ا.د/ نشوى سمير الياس د/ سعاد صبرى د/ هبة د/ ثريا ا.د/ ابتسام عبد الغنيم

الملخص العربى

بالرغم من قلة المعلومات عن أسباب نفوق الدولفين البوتلنوز الا انه توجد بيانات قليلة عن بيانات النفوق على فترات طويلة من الزمن فى مناطق كثيرة جغرافيه.

قامت هذة الدراسة على تحليل كل أسباب النفوق المفاجئ لأنثى الدولفين التى نفقت حديثا فى الماجيك لاند. كشفت فحوصات ما بعد الوفاه وجود نزيف حاد واحتقان فى الأعضاء الداخلية.

وأوضحت الاختبارات البكتيرية ترتيب الأعضاء تبعا لارتفاع اصابتها بالميكروبات كالتالى: الكبد – الرئتين – الغدد الليمفاوية بالرئتين – القولون – المعدة – الطحال – الكليتين – المرارة – الامعاء الدقيقة – القصبة الهوائية واخيرا القلب. وقد كان الميكروب الرئيسى هو الاى كولاى يليه السودوموناس ايريجونوسا يليه كليبسيلا نومونيا واخيرا ميكروب الباستريلا. بينما أوضح اختبار الحساسية لتلك الميكروبات ان الجارميسين (٣٠ ميكروجرام) والامبيسلين (١٠ ميكروجرام) هما أكثر المضادات الحيوية فاعلية.

الفحص الطفيلى أثبت خلو جميع الأعضاء من أى طفيليات أو أطوارها.

اظهرت نتائج الفحص الهستوباثولوجى احتقان فى الاوعية الدموية للرئتين والكبد مع وجود انزقة شديدة بالرئتين والكلى كما شوهد التهاب رئوى بينى، و استحالة وتنكرز فى الخلايا الكبد ية مع وجود اديما فى القلب .ايضا اظهرت الدراسة أرتشاح بالخلايا الالتهابية و زيا دة فى الخلايا الكأسية المبطنة للامعاء مصاحب لها اديما .كما شوهد قلة فى الحويصلات الليمفاوية للطحال ايضا شوهدت المستعمرات البكتيرية فى الكلى والقلب والطحا ل.