Field Trial Evaluation of Levofloxacin and Erythropoietin in Treatment of Hemorrhagic Enteritis in Dogs

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Abstract: Enteric fever was a marked problem allover the world in domestic pets and it plays a major role in zoonosis to contact human. The target of our investigation to confirm diagnosis of enteric fever caused by *S. paratyphi A* using rapid Widal test and isolation and identification of the causative organism. Also the present study was aimed to evaluate clinically and clinicopathologically by a field work-up, the therapeutic value of levofloxacin and human erythropoietin. The present study was carried out on fourteen dogs (9 diseased dogs and 5 apparently healthy dogs). Clinical manifestations were severe watery bloody diarrhea (n.=3), acute diarrhea with occult blood in fecal examination (n.=4), high fever (n.=7), prolonged capillary times (n.=7), enophthalmos (n.=7), loss of skin turgor (n.=7), increased respiratory and pulse rates (n.=7). Clinical examination was revealed abdominal distension (n.=3), hepatomegally (n.=2), abdominal tenderness (n.=7), tympanic sound (n.=7) and increased peristaltic movements (n.=7). Clinical remission of signs occurred after treatment with levofloxacin within 14 days. Widal agglutination test was displayed the titer of 7 positive cases ranged from 1:80-1:320 and 2 negative cases (1:40). Isolation and identification was revealed *S. paratyphi A* (100%) and *S. paratyphi B* (22.2%). Significant decreases of PCV %, hemoglobin concentration, erythrocytes and leucocytes count was recorded which was became significant increases after treatment within 8 weeks. Significant increase of ALT activities was denoted hepatic involvement during the infection. Erythropoeitin exerted an excellent therapeutic effect within 4- 8 weeks on basis of erythrogram investigations. It was concluded that combination of levofloxacin and human erythropoietin was the first record for treatment of hemorrhagic enteritis caused by *S. paratyphi A* in companion dogs in Egypt. (n.= number)
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1. Introduction:

The risk of *Salmonella* infections are the zoonotic importance as it can be transferred between human and animals. Many infections are due to ingestion of contaminated food. A distinction is made between enteritis *Salmonella* and typhoid/paratyphoid *Salmonella*, where the latter because of a special virulence factor and a capsule protein (virulence antigen) can cause serious illness, such as *Salmonella enterica subsp. enterica* serovar typhi (*Salmonella* typhi). It is adapted to humans and does not occur in animals (Crump et al., 2004 and Porwollik, 2011). Enteric fever, that is typhoid and paratyphoid fevers, is the common name for infections caused by Salmonella enterica serotypes typhi and paratyphi (Crump et al., 2004).

Salmonella infection, especially a carrier state in dogs is very important to public health, because dogs are usually reared in contact with humans (Bagcigil et al., 2007). In foreign countries, there have been some reports on transmission of Salmonella from dogs to humans. In Japan, there have been many reports on isolation of Salmonella from apparently healthy dogs. Salmonellosis is a zoonotic disease affects both animals and humans; it is a potentially fatal systemic infection and its clinical symptom mainly related to septicemia and enteritis

Salmonella infection in humans usually occurs from pets, other humans and from ingesting contaminated water or animal food products, most often eggs, poultry, and meat (Bhan et al., 2005, Centers for Disease control and prevention, 2005 and Swanson et al., 2007). Any food can become contaminated by feces. After the ingestion of organisms, the likelihood of infection developing, as well as the severity of infection, is related to the dose and virulence of the Salmonella strain and the status of host defense mechanisms.

Salmonellae are motile, gram-negative, non-spore-forming bacilli and can be differentiated into more than 2000 serotypes (serovars) by their somatic (O) antigens. There are six serogroups: A, B, C1, C2, D, and E. There are three species of Salmonellae that cause paratyphoid (Salmonella enterica serovar paratyphi): Salmonella paratyphi A, S. paratyphi B (or S. schotmulleri) and S. paratyphi C (S. hirschfeldii) after Maskalyk (2003), Bhan et al. (2005) and Effa & Bukirwa (2008). As a result of modern sewage and water treatment facilities, these diseases have become rare in developed countries but

remain a problem in countries without adequate sanitation and a safe water supply (Steffen et al., 2003).

The extent of infection of Salmonella species depends on the bacterial type and its strain. Salmonella possesses two antigens or weapons to protect it and cause destruction, these include Heat stable cell wall lipopolysaccharide known as Somatic or 'O' antigens and flagellar or H antigens formed from structural proteins, which make up the hair like filaments from the movement of the bacteria. (Lesser and Miller, 2001)

After ingestion of Salmonella species along with contaminated food, the bacterium overcomes the body's defense mechanism of gastric acidity (Connor and Schwartz, 2005). However, their main place of settlement is in the intestine where they cause inflammation and ulcers (Rene and Pines, 2002).Thus there is often a lesion in the intestinal tract with the consequent danger of haemorrhage and intestinal perforation (Turk and Porter, 1982). Perforation of the intestine which is followed by the leakage of the intestinal contents into the abdominal cavity (peritonitis) is a frequent cause of death from typhoid fever (Rene and Pines, 2002). The disease presents a clinical dilemma because of its varied manifestations and serious complications to various body organs (Qasmi et al., 2010)

Diagnosis of Salmonellosis is carried out by isolation of Salmonella spp. from blood, feces, urine or other body fluids (Porwollik, 2011). Moreover, salmonella infections can be diagnosed using serological tests such as the traditional Widal test ; "a test involving agglutination of typhoid bacilli when they are mixed with serum containing typhoid antibodies from an individual having typhoid fever; which may be used to detect the presence of Salmonella typhi and S. paratyphi." Newer diagnostic serological tests (e.g.IDL Tubex test, Typhidot test, IgM dipstick test) have been reported to give improved sensitivity and specificity in initial evaluations, but are not yet widely utilized. However, these tests have some limitations as a positive test result is dependent on an antibody response by the animal which may take up to 7-10 days to develop, in addition to the antigenic cross- reactions (Chin, 2000).

From clinicopathological concern, severe leucopenia and thrombocytopenia are common in typhoid and paratyphoid fever with moderately raised liver enzymes (Okafor, 2005). The present paper deals with cases of Salmonella Paratyphi A infection of a household dogs manifesting severe bloody diarrhea. Bacteriological examination, Widal agglutination test as well as hematological and biochemical analyses were studied in these cases.

The present study was aimed to diagnose the infection caused by Salmonella (paratyphoid fever) and to choice a satisfactory therapeutic approach. Differential diagnosis of hemorrhagic diarrhea caused by paratyphoid fever is difficult as large number of etiologies and need careful history taking, professional clinical examination and laboratory investigations. Causes of acute diarrhea were tremendous as internal worms, Coccidia, Giardia, Entamoeba histolytica, Salmonellae, Campylobacter, Clostridium perfringens, Corona virus, Parvo virus, Canine distemper, acute hepatic failure (canine hepatitis virus), acute renal failure (leptospirosis) and acute pancreatitis. But life threatening hemorrhagic enteritis was hookworms, whipworms, Canine Parvovirus, acute pancreatitis, acute hepatic failure (canine hepatitis virus), acute renal failure (leptospirosis), Campylobacter, Clostridium perferingens and Salmonellae (Burrows et al., 1995 and Leib & Monroe, 1997).

2. Materials and Methods:

Animals

The present study was carried out on fourteen dogs which were admitted to veterinary clinics in Giza governorate (9 diseased dogs and 5 apparently healthy dogs). All dogs were ranged from 3.8- 13.2 years. The breeds of diseased dogs were 5 Labrador retriever dogs (3 females and 2 males), 2 German shepherd dogs (one female and one male) and one Greatdane dog (one male), while the breeds of apparently healthy dogs were 3 Labrador retriever dogs (2 females and one male) and 2 German shepherd dogs (2 males). Dogs' weight ranged from 37.5- 47 kg. An accurate medical history of previous treatments and routine health care, such as deworming and vaccination programs was recorded. All investigated dogs were vaccinated annually against Canine distemper virus, Corona virus, Parvo virus, Adeno virus 2, Parainfleunza and L. icterohemorhagica and L. canicola (by Pfizer) and received Drontal® plus (50 mg praziquantel, 150 mg Febantel, 144 mg pyrantel- Embonat, made in Germany by Bayer) as internal worm prophylaxis. The diseased dogs were fed on a commercial dog food (German formula). Thorough diagnostic plan must be followed to reach a diagnosis efficiently and evaluated clinically every one week after treatment. Clinical examination was performed by inspection, palpation, percussion and auscultation of abdomen (intestines). Respiratory rate (rate/ min), pulse rate (rate/ min), rectal temperature (ºC), lymph nodes and mucous membranes were thoroughly examined and evaluated (Leib and Monroe, 1997).

Experimental design and sampling:

The blood samples were obtained from anterior median vein. Two blood samples were collected from each dog. The first blood sample was collected on EDTA which used for hematological examination and the second blood sample was collected into plain centrifuge tube for serum separation for biochemical analysis and Widal test. The same blood samples were taken at 4 and 8 weeks post treatment (p.t.) from all dogs except one case died at first day pre-treatment.

Fecal examination:

Fecal samples were examined macroscopically for presence of blood, undigested food or mucous. Thin fecal smears were examined under the microscope for any parasitic ova or coccidia oocysts (Thiopont et al., 1986). The dogs were re-examined at 4 and 8 weeks post treatment (p.t.).

Widal agglutination test:

Serological identification was done by using polyvalent and monovalent O and H antisera (Difco) according to Membrebe, and Chua (1999).

Salmonella isolation and identification:

Fecal swabs were collected from infected and non infected dogs at day 0 and 4 weeks (p.t.) for Salmonella isolation and identification. The inoculated Tetrathionate broth tubes with fecal swabs were incubated for 16 hours at 37ºC. A loopfull of the broth was streaked onto XLD, MacConkey and SS agar plates and incubated at 37ºC for 36-48 hours and the suspected colonies were identified morphologically, Gram's staining and biochemically using the API-20E kit system (Biomeraux, France) after Morifnigo et al. (1986) and Chirino-Trejo (1999).

Hematological examination:

Estimation of erythrocytic count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV) and; total and differential leukocytic counts were performed according to Fieldman et al. (2000).

Biochemical analysis:

Serum samples were used for determination of the activities of Alanine amino transferase (ALT) according to Reitman & Frankel (1957). Activities of both of alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) were studied according to Babson et al. (1966) and Dumas & Biggs (1972), respectively. Bilirubin was measured according to Doumas et al. (1973). Total proteins and serum albumin according to Weicheselbaun (1964) and Dumas & Biggs (1972) respectively. Serum globulin was calculated by subtracting the obtained values of albumin from values of total proteins. Blood urea nitrogen and serum creatinine were assayed according to Patton & Grauch (1977) and Fabiny & Eringhausen (1971) respectively. Serum biochemical parameters were assayed using commercial diagnostic kits supplied by Stanbio-Laboratory, USA.

Intensive therapeutic plan must be managed specially in life threatening diarrhea. Fluid therapy was the first emergency treatment by intravenous injection of dextrose® 5% (dextrose 5% solution, by Otsuka) which was calculated on basis of body weight and skin fold test, in addition to the use of rehydran N® sachets (Kcl, Cacl2 and Nacl, by CID) after Leib & Monoroe (1997). Tavanic® tablets (Levofloxacin 500 mg/ tablet, by Aventis) used as broad spectrum antibacterial, one tablet /24 hours for 5 days (Nichterlein et al., 1998). Erypoietin® vial (Epoetin beta, recombinant humane erythropoietin, by Amoun) in a dose of 1 vial weekly/ 8 treatments beside adjunct therapy by Sytron® syrup (Iron 27.5 mg/ 5ml and sodium iron edelate 190 mg/ 5ml, by PD) in a dose of 5 ml bid for treatment of anemic cases for 8 weeks and Halorange plus® syrup (Vitamins A, E, D, K and C, by Tetra.) in a dose of 5 ml bid for 3 weeks (Oishi et al., 1995).

Statistical analysis was performed by statistical Package for Social Sciences (SPSS). Mean and standard deviation are descriptive values for quantitative data. ANOVA (Analysis Of Variance) was used for testing means of more than two groups by computer program according to the method described by Irwan (1996).

3. Results:

Clinical presentation was severe and sudden. 7 Sick dogs arisen suddenly the acute enteritis, low performance, high fever, loss of appetite, the diarrhea, the draining water mucoid excrement and critically 3 ill dogs (senile dogs) arranged the bloody diarrhea. Signs of dehydration (7 cases) were recorded as dry mucous membranes, loss of skin turgor, prolonged capillary refill times and enophthalmos. Mucous membranes were pale, dirty finally because of dehydration, one case then showed signs of shock then died.

Careful clinical examination of abdomen revealed abdominal distension (3 cases) on inspection, abdominal tenderness on palpation (7 cases), tympanic sound on percussion (7 cases), hypermotility (increased peristaltic movements in 7 cases) on auscultation prior treatment and clinical remission of signs after 8- 14 days. Popliteal lymph nodes were of normal size, movable, normal local temperature, symmetrical, firm in consistency, non lobulated in both groups. Mucous membranes were

pale (2 cases), congested (5 cases), rosy red (2 dogs of diseased group and 5 dogs of apparently healthy group) and improvement of color of mucous membranes after 22- 38 days. There were significant increases in respiratory rate, pulse rate, rectal temperature and these parameters returned to normal within 18- 28 days (Table 1).

Table (1): Respiratory rate, pulse rate and rectal temperature evaluation prior treatment in non infected and infected groups

Parameters	Non infected group	Infected group
Respiratory rate (rate/ min.)	28 ± 2.8	57 ± 2.3**
Pulse rate (rate/ min.)	76 ± 4.4	109 ± 3.4**
Rectal temperature (ºC)	38.1 ± 0.3	40 ± 0.4**

**Significant at P value ≤ 0.01

Contact human (owners and attendants) were apparently healthy and showed no signs of paratyphoid or other diseases during 3 months of field evaluation.

Fecal examination:

The fecal samples were examined macroscopically and Samples showed blood and mucous with offensive odour in the infected group at day zero (pretreatment). Microscopically neither parasitic ova nor coccidia oocysts were detected in the specimens but there was only occult blood in 4 cases.

Widal agglutination test

Seven cases out of nine were positive for salmonella paratyhi A by Widal agglutination test, the titer of positive cases ranged from 1:80 (4 out of 9, 44.4%)-1:160 (2 out of 9, 22.2%), only one case that died after 4th weeks post treatment showed a titer of 1:320 (one out of 9, 11.1%). Two cases were negative for salmonella paratyhi A by Widal agglutination test (1:40, 22.2%)

Salmonella isolation and identification:

Bacteriological examination of the 9 fecal swabs collected from the infected dogs' revealed presence of Salmonellae in all infected dogs. Isolated Salmonellae from the examined fecal swabs collected from infected dogs were identified as *S. paratyphi A* and *S. paratyphi B* (Table 2). The most prevalent Salmonella species was *S. Paratyphi A*, in all cases from the infected dogs (100%) but *S. paratyphi B* (22.2%). All fecal swabs collected from apparently healthy group and infected group at 4th weeks post treatment (p.t.) were bacteriologically negative.

Table (2): Isolated Salmonellae paratyphi A and S. paratyphi B in different dog breeds

Dog breed	*S. paratyphi A*	*S. paratyphi B*
German Shepherd	2 (100%)	0 (0%)
Labrador retreiver	5 (100%)	2 (40%)
Greatdane	One (100%)	0 (0%)

Erythrogram

Mean values of the erythrogram [packed cell volume (PCV %), hemoglobin concentration (Hb), erythrocytes count (RBCs), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC)] of different experimental groups are illustrated in table (3).

In comparison to the mean values of apparently healthy dogs (non infected group) to the infected group at day zero (pretreatment), PCV %, Hb concentration, RBCs, and platelet count values showed significant decreases while, MCV and MCHC values revealed no significant changes. Microscopical examination of the stained blood film revealed hypochromacia in addition to the appearance of target cells. At 4th and 8th weeks (p.t.), PCV %, Hb concentration, RBCs, MCHC showed significant increases. Platelet count values showed insignificant change at 4th and 8th weeks (p.t.) in comparison to non infected group.

Leukogram

Mean values of the leukogram [total leukocyte count (TLC), neutrophil, lymphocyte, eosinophil and monocyte counts] of different groups are illustrated in table (3).

Compared to the apparently healthy group, the infected group at day zero (pretreatment) showed significant leucopenia with significant neutropenia and lymphopenia while, at 4th and 8th weeks (p.t.) showed significant increase in leukogram when compared to the infected group at day zero (pretreatment).

Table (3): Erythrogram and Leukogram in dogs of non-infected and infected groups (at zero day, 4th week and 8th week p.t.) with *S. paratyphi*

Parameters	Non infected dogs	Zero day	4th week (p.t.)	8th week (p.t.)	LSD
PCV (%)	43.33 ± 1.53 a	32.33 ± 2.52 c	38.67 ± 1.53 b	45.33 ± 2.08 a	3.69
Hb (g/dl)	15.27 ± 0.55 a	11.10 ± 0.75 c	14.15 ± 0.25 b	15.65 ± 0.30 a	0.95
RBCs (x10^6/µl)	6.98 ± 0.16 a	5.23 ± 0.35 c	5.95 ± 0.27 b	7.13 ± 0.18 a	0.47
MCV (fl)	62.06 ± 0.87 bc	61.73 ± 0.68 c	64.99 ± 0.51 a	63.55 ± 1.35 ab	1.71
MCHC (g%)	35.23 ± 0.31 ab	34.33 ± 0.65 b	36.47 ± 0.64 a	34.55 ± 0.96 b	1.28
Platelets (x10^3/µl)	229.00 ± 75.72 a	123.33 ± 10.41 b	209.33 ± 61.98 ab	264.33 ± 29.26 a	96.65
TLC (x10^3/µl)	8.80 ± 0.52 a	4.04 ± 0.86 c	6.96 ± 0.33 b	9.03 ± 1.15 a	1.47
Neut. (x10^3/µl)	5.01 ± 0.29 a	1.81 ± 0.39 c	3.96 ± 0.19 b	5.14 ± 0.65 a	0.84
Lymph. (x10^3/µl)	2.99 ± 0.18 a	1.93 ± 0.41 b	2.36 ± 0.11 b	3.06 ± 0.39 a	0.57
Mono. (x10^3/µl)	0.61 ± 0.04 a	0.20 ± 0.04 c	0.48 ± 0.02 b	0.63 ± 0.08 a	0.09
Esino. (x10^3/µl)	0.17 ± 0.01 a	0.08 ± 0.02 c	0.13 ± 0.01 b	0.19 ± 0.02 a	0.03

Means with different superscripts (a, b,c) are significantly different at P value < 0.05

Serum biochemical evaluation

Statistical analysis of different serum biochemical parameters is illustrated in table (4)

The results of the infected group at day zero, 4th and 8th weeks (p.t.) showed insignificant changes in BUN and creatinine. Serum total protein concentrations showed significant decrease in A/G ratio which attributed to the presence of significant hypoalbuminemia. The hepatic enzymes (ALT, ALP and GGT) were significantly increased than the control group in association with the hyperbilirubinemia. On the other hand, the infected group at 4th and 8th weeks (p.t.) showed significant decreases in all serum biochemical parameters when compared to infected group at day zero (pretreatment) except serum total protein and albumin concentrations showed significant increases.

Table (4): Serum biochemical parameters in dogs of non-infected and infected groups (at zero day, 4th week and 8th week p.t.) with *S. paratyphi*

Parameters	Non infected dogs	Zero day	4th week (p.t.)	8th week (p.t.)	LSD
Total proteins (g/dl)	8.40 ± 0.34 a	6.49 ± 0.23 c	7.74 ± 0.24 b	8.69 ± 0.15 a	0.47
Albumin (g/dl)	3.79 ± 0.14 a	2.17 ± 0.15 b	3.61 ± 0.32 a	3.91 ± 0.07 a	0.36
Globulin (g/dl)	4.61 ± 0.21 ab	4.33 ± 0.19 bc	4.12 ± 0.22 c	4.78 ± 0.22 a	0.39
A/G ratio	0.82 ± 0.01 a	0.51 ± 0.04 b	0.87 ± 0.12 a	0.82 ± 0.05 a	0.13
ALT	26.00 ± 5.29 b	40.00 ± 5.00 a	30.33 ± 3.21 b	25.67 ± 4.93 b	8.81
GGT	38.00 ± 3.61 b	57.00 ± 3.00 a	40.00 ± 2.00 b	37.33 ± 5.86 b	7.31
ALP	26.67 ± 12.66 b	58.33 ± 10.07 a	32.33 ± 5.86 b	26.67 ± 10.69 b	19.07
T.bilirubin (mg/dl)	0.23 ± 0.05 c	0.39 ± 0.02 a	0.31 ± 0.01 b	0.29 ± 0.02 b	0.05
Urea (mg/dl)	18.67 ± 1.53 a	18.33 ± 4.16 a	17.00 ± 3.61 a	19.00 ± 2.65 a	5.93
Creatinine (mg/dl)	1.10 ± 0.10 a	1.00 ± 0.10 a	1.00 ± 0.20 a	1.03 ± 0.15 a	0.27

Means with different superscripts (a, b,c) are significantly different at P value < 0.05

4. Discussion

Enteric fever is a major global public health problem and growing international travel leads to an increase in the risk of contracting infectious diseases that are endemic in the country of destination (Crump et al., 2004, Bhan et al., 2005 and Swanson et al., 2007).

Many people believe that they can feed their dogs any type of meat; raw or cooked, and they will be able to devour and enjoy it, while most of the time that may be not true, sometimes eating raw meats can affect the dog health, especially if he already has an underlying problem to begin with. Dogs normally can fight off bacteria, but if they have a weak immune

system because of other health problems or senility, they may end up getting really sick by eating raw meat and get salmonella (Bagcigil et al., 2007).

Contact human (owners and attendants) in the present field study were apparently healthy and showed no signs of paratyphoid or other diseases during 3 months of field evaluation as restrict hygienic measures and quarantine of infected dogs.

In Dogs, they can get salmonella poisoning in a number of ways, including eating raw or uncooked meat or eggs, accessing rotten food in a trash can or coming into contact with a bird feeder or bird feces (song birds and pigeons often carry salmonella bacteria) after Bagcigil et al. (2007).

The rock pigeon (*Columba livia*) may serve as a reservoir for several pathogenic agents that can be transmitted to poultry, wildlife, domesticated pets, and/or humans via excreta, secretions, or dust from feathers. In addition, ingestion of infected pigeons by wild and domestic animals can also transmit these pathogenic agents (Eliane et al., 2010).

Clinical presentations recorded in the present research work were severe watery bloody diarrhea, high fever, prolonged capillary times, enophthalmos, loss of skin turgor, increased respiratory and pulse rates. Clinical examination was revealed abdominal distension, hepatomegally, abdominal tenderness, tympanic sound and increased peristaltic movements. Stimulation of crypt enterocytes can result in secretion of large volumes of fluid that exceed the absorptive ability of the intestine. This occurs most commonly with infectious diseases as salmonellosis and colibacillosis, but byproducts of bacterial overgrowth can also stimulate intestinal secretion. Byproducts of bacterial overgrowth can cause abnormal motility (Burrows et al., 1995).

In our study seven cases out of nine were positive for salmonella paratyhi A by Widal agglutination test (Widal test is positive if the titer is 1:80 or more), the titer of positive cases ranged from 1:80-1:160, while by bacteriological examination *Salmonella paratyphi A* (100%) and *S. Paratyphi B* (22.2 %) were identified and the most prevalent Salmonella species was *S. Paratyphi A*, in all cases from the infected dogs. The titer of the same 7 cases became stable (1: 80) after 4 and 8 weeks of treatment and negative culture results after 4 weeks denoted a state of immunity. Crump et al. (2004) and Su et al. (2004) recorded that of the three types of *S. paratyphi* (A, B, and C), B is the most common. In other study, 39 isolates of *Salmonella Paratyphi B* from animals/environment and one of human origin were characterized to understand epidemiological distribution of this pathogen (Agarwal et al., 2003).

Widal agglutination test alone is not a very specific test for diagnosis of salmonella infection, since the dogs are often exposed to other bacteria (e.g. Salmonella enteritidis, Salmonella typhimurium and some types of E. coli) in this species that induce cross-reactivity; many animals have antibodies against these enteric pathogens, which also react with the antigens in the Widal test, causing a false-positive result. Test results need to be interpreted carefully in the light of past history of enteric fever, typhoid vaccination, and the general level of antibodies in the populations in endemic areas of the world. Widal agglutination test should be used as an adjunct to culture methods for definitive and accurate diagnosis for salmonella infection (Chew et al., 1992 and Olopoenia & King, 2000).

One case died at first day of treatment showed a titer of 1:320 in Widal agglutination test, it may be explained that the bacterium overcomes the body's defense mechanism as recorded by Connor & Schwartz (2005) which mentioned that after ingestion of Salmonella species along with contaminated food causing severe haemorrhage and intestinal perforation after settlement of the bacterium in the intestine. Perforation of the intestine followed by leakage of the intestinal contents into the abdominal cavity was resulted in severe peritonitis and death (Rene & Pines, 2002).

The significant leucopenia commonly observed in paratyphoid fever could be attributed to invasion of haemopoietic organs such as lymph nodes, spleen, tonsils and bone marrow by *S. paratyphi A* which radically slowed down the rate of granulopoiesis. The invasion of the above organs by *S. paratyphi A* which can also depress the rate of haematopoiesis may also explain the observed non regenerative anemia (normocytic normochromic anemia) and thrombocytopenia seen in typhoid and paratyphoid patients. The anemia and thrombocytopenia may be also resulted from prolonged bleeding lesions in the intestinal tract which manifested by the bloody diarrhea (Connor & Schwartz, 2005 and Okafor, 2005).

Analysis of serum liver enzymes revealed significant increase in serum alanine amino transferase (ALT). Increased activities of ALP and GGT in association with the hyperbilirubinemia observed in Salmonella infected groups, these changes denoted hepatic involvement which may be attributed to entering of salmonella to the small intestine wall, and then Salmonella invaded through the lymphatic system and after a period of multiplication invades the blood stream (Khosla 1990, Schwartz, 1994 and Rajagopal et al., 2002). From blood stream the bacteria invaded the liver, gall bladder, spleen, kidney and bone marrow where it multiplied and caused infection of these organs. Decreased serum albumin and total protein due to loss of protein from the

intestinal mucosa, to impaired dietary intake or may be attributed to its reduced production as a result of hepatic involvement (Chin, 2000)

Soon after the diagnosis by rapid Widal test, we prescribed orally 500 mg of Levofloxacin to the dog once daily for 5 days, Commercially available recombinant human erythropoietin (r-HuEPO) at a dosage of 1000 U/dog, once per week until PCV achieved the target range with iron supplement within 8 weeks. Significant increases of respiratory and pulse rates were attributed to anemia so they were improved after treatment by erythropoietin and iron. Control measures such as cleaning and disinfection of the environment were done on daily basis. Also, fluid therapy was very important for rehydration. Vit. A, E and C as adjunct therapy improved healing process of intestinal mucosa. A disinfectant solution containing sodium hypochlorite was sprayed on the concrete floor and the kennel, where diarrheal feces were excreted during the incidence (Oishi, 1995 and Nichtelein et al., 1998). Clinical remission of clinical signs within 14 days of treatment denoted suitable antibacterial with a satisfactory dose and an appropriate duration.

After 4th weeks (p.t), fecal samples were collected for bacteriological examination from all dogs at the same time, and they were negative for Salmonella. Treatment with Levofloxacin (fluoroquinolone) appeared active against bacteria only in 5 days. It functions by inhibiting DNA gyrase, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division. Moreover, fluoroquinolones have emerged as the mainstay of therapy for invasive infection associated with typhoidal and nontyphoidal Salmonella enteric serotypes (Booker et al., 2005). Also in tissue culture cells infected with S. typhimurium and paratyphimurium, levofloxacin was slightly more effective than ciprofloxacin in animal models of infection so, levofloxacin is a candidate for the treatment of infections caused by facultative intracellular Gram-positive and Gram-negative bacteria. (Nichterlein et al., 1998, Huang & Dupont, 2005 and Kedhiravan et al., 2005).

At 4th and 8th weeks (p.t), Mean PCV, RBCs count and Hb concentration increased significantly and gradually during the first 4th weeks of treatment. Erythrocyte indices of dogs in our work-up showed a normocytic and normochromic anemia at the pretreatment period and during 8th weeks of treatment with r-HuEPO they became normal. All dogs received daily oral iron supplementation during treatment with r-HuEPO.

In the present study, treatment with r-HuEPO in anemic dogs appeared to stimulate erythrocyte production. The response to r-HuEPO was found since the first week of treatment at the initial dosage of approximately 1000 U/ dog per week. All dogs showed decrease in liver enzymes as, r-HuEPO has shown a benefit in reduction of the liver injury experimentally induced by ischemia–reperfusion in the rat (Sepodes et al., 2006). Many previous literatures have shown the effective and beneficial use of r-HuEPO for treatment of anemia in animals, including in nephrectomized dogs and anemic dogs. Treatment with r-HuEPO also reduces the clinical signs of depression and weakness, increases appetite and physical activity (Oishi et al., 1995 and Assarasakorn et al., 2008).

In conclusion, the asymptomatic intestinal carrier state (2 cases) may result from inapparent infection or may follow clinical disease. It is usually self-limited to several weeks to months, with the incidence of positive stool cultures rapidly decreasing over time. But in infected cases, after an incubation period of 5 to 21 days (generally 7 to 14 days), fever and malaise developed. A small proportion of dogs may have diarrhea during the incubation period. The fever tends to rise in stepwise fashion over the first few days to a week and then became sustained, usually at 39.4 to 40°C or higher. After 2 weeks of illness, the severe complications of intestinal hemorrhage or perforation may be observed as in dead case. The illness usually resolves by the end of the fourth week in an untreated patient. Relapse may occur in untreated as well as treated patients, but the illness is milder than the original episode as recorded by Huang & Dupont (2005). The most affected cases were 3 senile dogs (immunocompromized) and from them one case died. Also, Nicolas et al. (2009) mentioned that mortality from Salmonella is not uncommon and is most likely to occur in the very young, the very old, and the immunocompromised.

5. Conclusion:

The present investigation emphasized the potent spread of Salmonellae bacilli in developed countries. Paratypoid infection in the present study was incriminated to be the cause of hemorrhagic diarrhea in dogs. Since the dog can be a very important source of human Salmonella infection, strict control measures should be taken when the animal is confirmed to be infected with the pathogen. In the present investigation, control measures such as cleaning and disinfection of the surrounded environment have been done successfully because the dog was kept mainly on the concrete floor. In conclusion, Levofloxacin (fluoroquinolone) in our investigation were the first record as prescription against paratyphoid infection in companion dogs in Egypt. On the other hand, treatment with r-HuEPO

stimulated erythrocyte production in dogs with normocytic normochromic anemia seen in paratyphoid cases. PCV reached the target range within 8 weeks of treatment.

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5. References:

1. Agarwal, Meenu, Singh, B.R., Santoshi, M.C., Singh, V.P., Chandra, Mudit (2003). A study on biotyping, bacteriocin typing and drug resistogram of Salmonella paratyphi B isolates from animals, their products and environment. *Indian journal of Comparative Microbiology, Immunology and Infectious Diseases 24, 2.*
2. Assarasakorn S. & Kaewthamasorn M. & Niwetpathomwat A. (2008). A retrospective study of clinical use of recombinant human erythropoietin for treatment of anemia in dogs with renal failure in Bangkok, Thailand. *Comp Clin Pathol* 17,165– 170.
3. Babson, L.A., Greeley, S.J., Coleman, C.M. & Philips, G.D. (1966). Colorimetric method for estimation of serum alkaline phosphatase. Clinical Chemistry, 12: 482-490.
4. Bagcigil A., Ikiz S., Dokuzeylul B., Basaran B., Or, E. & Ozgur, N. (2007). Fecal shedding of salmonella spp in dogs. *J. Vet. Med. Sci*. 69 (7): 775- 777
5. Bhan, M.K., Bahl, R. & Bhatnagar, S. (2005). Typhoid and paratyphoid fever. *Lancet*. 366, 749-762.
6. Booker, B. M., Smith, P. F., Forrest, A., Bullock, J., Kelchlin, P., Bhavnani, S. M., Ronald, N., Jones & Ambrose' P. G. (2005). Application of an in vitro infection model and simulation for reevaluation of fluoroquinolone break points for *Salmonella enterica* serotype typhi. *Antimicrob. Agents Chemother*. 49 (5): 1775– 1781.
7. Burrows, C.F., Batt, R.M. & Sherding, R.G. (1995). Diseases of the small intestine. In: Ettinger, S.J., Feldman, E.C., eds. *Textbook of Veterinary Internal Medicine. Philadelphia, W.B. Saunders.* 1169- 1232.
8. Centers for Disease Control and Prevention (2005). Outbreak of multidrug-resistant Salmonella typhimurium associated with rodents purchased at retail pet stores– United States, December 2003 – October 2004. *MMWR Morb. Mortal Wkly Rep*. 54, 429- 433.
9. Chew, S.K., Cnizi, M.S.P., Irim, Y.S. & Monteiro, E.H. (1992). Diagnostic value of the widal test for typhoid fever in Singapore. Jour. *Trop. Med. Hygie*. 95, 288– 291
10. Chin, J. (2000). Control of Communicable Diseases Manual, 17th edition. *American Public Health Association.*
11. Chirino-Trejo, J.M. (1999). A more sensitive procedure for the detection of Salmonella carriers in swine. *Proc. Western Can. Assoc. Swine Pract.* 50- 53.
12. Connor B. A. & Schwartz E. (2005). Typhoid and paratyphoid fever in travelers. *Lancet Infect. Dis.* 5, 623–28
13. Crump, J.A., Luby, S.P. & Mintz, E.D. (2004). The global burden of typhoid fever. *Bull World Health Organ*. 82, 346- 353.
14. Doumas, B.T., Perry, B.W., Sasse, E.A. & Straumfjord, J.V. (1973). Colorimetric method for estimation of bilirubin. *Clinical Chemistry*, 19, 984- 993.
15. Dumas, B.T. & Biggs, H.G. (1972). "Standard Methods of Clinical Chemistry". Academic Press, N.Y., U.S.A.
16. Effa, E.E. & Bukirwa, H. (2008). Azithromycin for treating uncomplicated typhoid and paratyphoid fever (enteric fever). *Cohrane Database of Systemc Reviews* Issue 4. Art No. CD006083, pages 2-3.
17. Eliane de Sousa, Angelo Berchieri Júnior, Aramis Augusto Pinto, Rosangela Zacarias Machado, Adriano de Oliveira Torres Carrasco, José Antonio Marciano, Karin Werther (2010). Prevalence of Salmonella spp. Antibodies to Toxoplasma gondii, and Newcastle Disease Virus in Feral Pigeons (Columba livia) in the City of Jaboticabal, Brazil. *Journal of Zoo and Wildlife Medicine* 41 (4): 603- 607.
18. Fabiny, D.L. & Ertingshausen, G. (1971). Colorimetric method for estimation of creatinine. *Clin. Chem.* 17: 696.
19. Fieldman, B.F.; Zinkl, J.G. & Jain, N.C. (2000). "Schalm's Veterinary Hematology" 5th ed. Lea and Fibiger, Philadelphia, USA.
20. Huang, D.B. & DuPont, H.L. (2005). Problem pathogens: Extra-intestinal complications of Salmonella enterica serotype typhi infection. *Lancet. Infect. Dis.* 5, 341- 348.
21. Irwan, T.M. (1996). In Applied Linear Statistical models, Neter, Kutner, Neachtsheim Wasserman. Fourth edition.

22. Kadhiravan, T., Wig, N., Kapil, A., Kabra, S.K., Renuka, K. & Misra A (2005). Clinical outcomes in typhoid fever: adverse impact of infection with nalidixic acid– resistant Salmonella typhi. *B.M.C. Infect. Dis.* 5 (1): 37.
23. Khosla, S.N. (1990). Typhoid hepatitis. *Postgrad. Med. J.* 66: 923– 25.
24. Leib, M.E. & Monroe W.E. (1997). Diseases of the intestines. *Practical Small Animal Internal Medicine* 685- 760.
25. Lesser, C.F. & Miller S.I. (2001). Salmonellosis. In: Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL editor (s). *Harrison's Principles of Internal Medicine. 15th Edition.* Vol. 1, New York:
26. Maskalyk, J. (2003). Typhoid fever. *Canadian Medical Association Journal* 169 (2):132.
27. Membrebe, F.A. & Chua, J.A. (1999). The clinical utility of typhoid in the diagnosis of typhoid fever. *Philipp J Microbiol Infect Dis* 28,1- 4
28. Morifnigo, M.A., Borrego, J.J. & Romero, P. (1986). Comparative study of different methods for detection and enumeration of *Salmonella* spp. in natural waters. *J. Appl. Bacteriol.* 61:169-176.
29. Nichterlein, T., Bornitz, F., Kretschmar, M. & Hof, H. (1998). Successful treatment of murine listeriosis and salmonellosis with levofloxacin. *J. Chemother.* 10 (4): 313- 9.
30. *Nicolas Lapaque, Thierry Walzer, Stéphane Méresse, Eric Vivier & John Trowsdale (2009). Interactions between human NK cells and macrophages in response to Salmonella infection.* The Journal of Immunology, *Volume: 182, Issue: 7.*
31. Oishi, A., Hamada, S., Sakamoto, H. & Shimizu, R. (1995). Preventive effects of recombinant human erythropoietin administration on anemia associated with repeated hemodialysis in nephrectomized dogs. *J. Vet. Med. Sci.* 57, 989– 990.
32. Okafor, A.I. (2005). Haematological alterations due to typhoid fever in Enugu Urban- Nigeria. Malaysian Journal of Microbiology, 3 (2): 19- 22
33. Olopoenia, L.A. & King A.L. (2000). Widal agglutination test – 100 years later: still plagued by controversy. *Postgrad. Med. J.* 76, 80– 84.
34. Patton, C.G. & Crouch, S.R. (1977). Enzymatic colorimetric method of urea determination. *Anal. Chem.* 49, 464- 469.
35. Porwollik, S. (2011). *Salmonella:* from genome to function. *Caister Academic Press* ISBN 978- 1- 904455- 73- 8.
36. Qasmi, S., Ahmed, A., Zaidi, S. & Ahmed, O. (2010). Incidence and diagnosis of typhoid/ paratyphoid fever in Karachi. *Infectious Diseases Journal of Pakistan* 19 (3):196- 198
37. Rajagopal, A., Ramasamy, R., Mahendran, G. & Thomas, M. (2002). Hepatic abscess complicating paratyphoid infection. *Tropical Gastroenterology*; 23 (4):181– 2.
38. Reitman, S. & Frankel, S. (1957). A colorimetric method for the determination of oxaloacetic transaminase and serum glutamic pyruvic transaminase. *Am. J. Clin. Path.* 28: 56– 60.
39. Rene, D. & Pines, M. (2002). Health and Diseases In: *Time Life International* pp 22-23; N. V. Publishers, Netherland 54- 55.
40. Schwartz, E., Jenks, N.P., Shlim, D.R. (1994). 'Typhoid hepatitis' or typhoid fever and acute viral hepatitis. *Trans. R. Soc. Trop. Med. Hyg*. 88, 437– 38.
41. Sepodes, B., Maio, R., Pinto, R., Sharples, E., Oliveira, P., McDonald, M., Yaqoob, M., Thiemermann C. & Mota-Filipe, H. (2006). Recombinant human erythropoietin protects the liver from hepatic ischemia– reperfusion injury in the rat. *Transpl. Int.* 19: 919– 926.
42. Steffen, R., de Bernardis, C. & Banos, A. (2003). Travel epidemiology– a global perspective. *Int. J. Antimicrob. Agents* 21, 89- 95.
43. Su, L.H., Chiu, C.H., Chu, C. & Ou, J.T. (2004). Antimicrobial resistance in nontyphoid Salmonella serotypes: A global challenge. *Clin Infect Dis* , 39:546-551.
44. Swanson, S.J., Snider, C., Braden, C.R., Boxrud, D., Wunschmann, A., Rudroff, J.A., Lockett, J. & Smith, K.E. (2007). Multidrug-resistant Salmonella enterica serotype typhimurium associated with pet rodents. *N. Engl. J. Med*. 356, 21- 28.
45. Thiopont, D., Rochette, F., Vanparijs, O.F.J. (1986). Fecal floatation concentration, direct method. *Diagnosing Helminthiasis by coprological examination second edition textbook* 32- 34.
46. Turk, D.C. & Porter, I.A. (1982). Medical Microbiology 3rd ed. *English Universities Press Ltd* pp. 30-35.
47. Weichselbraun, D.E. (1964): Colorimetric method for determination of total proteins. *Am. J. Clin. Path.* 40, 16-40.

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