

The Comparative Study of the Treatment by Oxytetracycline and Homeopathy on Induced *Mycoplasma haemofelis* in less than One-year-old Cats

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Abstract: *Mycoplasma haemofelis* which is also called *Haemobartonella felis* is an infectious anemia factor (F.I.A) in cats. The clinical signs of the disease are different and it can be seen as acute, over acute and chronic. In some situations, the *Mycoplasma haemofelis* isn't treated completely by tetracycline and the similar medications and the cat remains the bearer. Due to the fact that *Mycoplasma haemofelis* creates symptoms, the Homeopathic medication (China) will be selected according to the symptoms. The only difference was that the China not only cleaned the clinical symptoms, but also cleared the blood contamination and left no symptoms from neither contamination nor from the disease.

Keywords: Cat, homeopathy, *Mycoplasma haemofelis*, oxytetracycline, treatment

INTRODUCTION

Mycoplasma haemofelis is a small, cell-wall-free gram-negative bacteria, unculturable organism related to mollicute and member of a newly defined group of mycoplasmas that parasitizes the red blood cells of animals and humans, previously ascribed to the genus *Haemobartonella* (Neimark *et al.*, 2001; Foley and Pedersen, 2001; Neimark *et al.*, 2002).

Haemobartonella felis (*H. felis*) 'Ohio strain' or 'large form' is now called *Mycoplasma haemofelis* (Neimark *et al.*, 2001). Haemobartonellosis was first described in 1953 in the United States (Grindem *et al.*, 1990) but the number of studies about incidence and prevalence of the disease and the risk factors in transmission remains limited after 50 years (Sauerwein and Grabner, 1982; Nash and Bobade, 1986). They are located on the surface of red blood cells and can induce hemolytic anemia (Foley and Pedersen, 2001; Neimark *et al.*, 2001).

Feline hemotropic mycoplasmas are described worldwide with varying incidence rates in the wild and domestic cat populations (Tasker *et al.*, 2003; Willi *et al.*, 2006, 2007; Sykes *et al.*, 2008; Gentilini *et al.*, 2009; Van Geffen, 2012). *Mycoplasma haemofelis* (*Mhf*), is the most pathogenic species in cats and can induce severe hemolytic anemia (Berent *et al.*, 1998; Tasker, 2010).

The infection (Acute and Chronic disease) characterised in cats may present pallor, apathy, jaundice, weight loss, fever, extreme fatigue, adenopathy, motor incoordination, depression,

anorexia, splenomegaly, paraplegia, dehydration, hyperesthesia anemia and may cause death (Cooper *et al.*, 1999; Tasker, 2010). The pathogen can be identified as small coccoids, rings or strings on erythrocyte membrane or free in plasma in Giemsa staining of blood smears (Cooper *et al.*, 1999).

The incubation period after infection with *Mycoplasma haemofelis* varies from weeks to months and is followed by cycles of bacteremia which may last for months. Infected erythrocytes are less deformable in circulation and elicit an immune response with later phagocytosis in lymphoid organs. Massive infection or severe anemia may result in death. Other animals will recover but stay carriers despite their immune response to the organism (Giger, 2005).

Diagnosis of haemobartonellosis depends on clinical and hematological findings together with microscopic examination of blood smears and specific serological and PCR testing for the pathogen (Bobade and Nash, 1987; Tasker and Lappin, 2001).

Various antibiotics were reported to be effective in the treatment of haemobartonellosis. Different studies demonstrated that *H. felis* is sensitive to lincomycine (Ojeda and Skewes, 1978), enrofloxacin, oxytetracyclin, doxycyclin and tiarsemid natrium (Sauerwein and Grabner, 1982; Winter, 1993; Baneth *et al.*, 1998) and resistant to azitromycin (Berent *et al.*, 1998; Tasker and Lappin, 2001). Treatment includes antibiotics, such as doxycycline, supportive treatment with blood products in severely anemic animals and possibly corticosteroids to halt immune-mediated destruction of erythrocytes (Harvey, 2006).

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These bacterial pathogens are sometimes present in blood from mammals such as cats, mice and dogs. They grow attached to red blood cells and the only possible diagnosis procedure until the arrival of molecular diagnosis was microscopic examination of blood smears (Foreyt, 1989). This procedure has many drawbacks, since bacterial pathogens may be confused with artifacts or lost after EDTA treatment of collected blood (Berent *et al.*, 1998).

Homeopathy, brilliant therapeutics discovered and developed by the German physician Samuel Hahnemann at the end of the 18th century, at first used for the treatment of human beings, has proven its efficiency in the treatment of several animal species (Khuda-Bukhsh, 2003; Madrewar, 2004).

Homeopathy has demonstrated in many medical areas its effectiveness in practice, but scientific evidence is lacking (Mathie, 2003; Spence *et al.*, 2005). The veterinary homeopathy research literature comprises less than 20 published, peer-reviewed Randomised Controlled Trials (RCTs) (Mathie *et al.*, 2007).

Homeopathic remedies have significant benefits since there are no residues in animal products, nor does homeopathy generate resistant microorganisms. According to the European Committee for Homeopathy (Spence *et al.*, 2005): "If homeopathy is introduced into the livestock farming sector, the European citizen could be better protected from pharmacological residues in animal products." Homeopathy aims to activate self-healing mechanisms of the body. Therefore the healing process might have a longer duration and more attention needs to be paid to find the correct remedy. Lack of knowledge and understanding might be reasons for the limited use of homeopathy in the present livestock sector (Henriksen and Grøva, 2001).

The present work is aimed to study comparative treatment by oxytetracycline and homeopathy rules (less than a one-year-old cat) inoculation of *Mycoplasma haemofelis*.

MATERIALS AND METHODS

Animals and experiment: The experiment was conducted on 30 cats (both males and females) aging less than one-year which were collected from Isfahan and Shahrekord, Iran. The samples were divided into seven groups (n = 5). Including Five treatment and 2 control groups. After being infected by the disease, the five treatment groups were treated by two methods including: Chemical treatment (for one group) and homeopathic (for the remaining 4 groups). Each of the five treatment groups included 5 cats, where as the control groups each had three cats. The control groups were divided into one positive control (healthy) and one negative control (patients) that were not treated.

Sample collection and processing: Blood samples (10 mL) collected from mature cats infected with *Mycoplasma haemofelis* were approved by direct smear and viewed under a light microscope (Olympus Model AU 5400 System, Center Valley, PA). The blood samples were collected by jugular venipuncture into an Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulant tube. The samples were stored at -20°C and transported periodically to the laboratory with a cold pack for analysis.

Homeopathic treatment was done with three different drugs including: Group A or China, Group B or Secale, Group C or Sepia and Group D with Sepia+China. The dose of homeopathic remedies used was 6c (means 10⁻⁶ load dilution has increased of pharmaceutical basic raw material).

In order to prepare and administer the drug, first the beaver containing the homeopathic drug was shaken 50 times. Then, five drops of this drug were added to 100cc of sterile water and shaken for another fifty times. After, 1 table spoon this mixture was poured into the cats drinking water and food. The rest of was disposed and mixture was prepared for the next day. According to above mentioned method.

Chemical treatment was done using oxytetracycline (20 mg/kg- every eight hours) as a antibiotics effective and Prednisolone (2 mg/kg- every twelve hours) as a good corticosteroid.

After injection of infected blood to samples, transferred the disease to they and then of ensuring, began treatment of diseases with both methods. During treatment, controlled vital and clinical signs specimens. Then, to weaken the samples immunity system and let their bodies be infected quickly, one mL of Dexamethazon (8 mg/2 m was injected through their muscles in three stages (Once in 48 h). After a one week of treatment, were taken blood samples were taken and blood smears were prepared. Next, Giemsa staining and direct observed under an light microscope, we determined contamination levels, the continuity and restrictions.

The treatment took tree weeks and this period was selected because of the chemical treatment requirements. The blood samples were taken from all samples at the end of each week. After smear and Giemsa staining, each sample was viewed and reviewed by a light microscope and the level of contamination and the process of treatment was analyzed and recorded.

RESULTS

After four days of contaminating the samples, the chemical symptoms of the disease such as exhaustion, loss of appetite, dehydration, weakness and paleness of their phlegm appeared. After one week, we got blood samples and smear were prepared and after Giemsa staining, *Mycoplasma* infection was observed in all the

Table 1: Treatment by OTC group

By the end of the first week	By the end of the second week	By the end of the third week
2%	1%	0.02%

Table 2: Homeopathic treatment by China drug group

By the end of the first week	By the end of the second week	By the end of the third week
1%	0.2%	0%

Table 3: Homeopathic treatment by Secale drug group

By the end of the first week	By the end of the second week	By the end of the third week
20%	15%	10%

Table 4: Homeopathic treatment by sepia drug group

By the end of the first week	By the end of the second week	By the end of the third week
3%	1%	0.3%

Table 5: Homeopathic treatment by Sepia+China drugs group

By the end of the first week	By the end of the second week	By the end of the third week
1%	0%	0%

samples. After counting the Red Blood Cell (RBC) infected with *Mycoplasma haemofelis*, the average infection ratio covered 20% of the RBC in all the samples. It means on average out of every fifth RBC, one was infected with mycoplasma.

The positive control group (healthy): This group could maintain its normal condition during the experiment and it remained completely healthy both from the clinical point of view and the blood test.

The negative control group (patient): Since no treatment was followed in this group, the symptoms which appeared from the were increasingly severe so that one of the samples in this group died after 10 days of infection, due to the over acute form of the disease. The observed symptoms of this sample were as follows: severe dehydration, anemia and complete paleness of phlegm, weakness, appetite block, hypothermia and finally death.

Treatment by OTC group: After the first week of treatment for this group, we injected the blood and smeared it. Through staining and reviewing these samples, we realized that the level of infection decreased (Table 1). The clinical symptoms at the end of the second week had minimized remarkably. The appetite became normal, dehydration and exhaustion disappeared but the phlegm paleness still remained. At the last week of treatment (End of the third week), the level of blood contamination reached to 0.2% and the clinical symptoms were gone.

Homeopathic treatment by China drug group: After the first week of treatment in this group, we injected the

blood and smear it. Through staining and reviewing these samples, we realized that the level of infection decreased (Table 2) at the end of the second week reduce contamination rate of RBC reduced to 0.2% and no clinical symptoms remained. At the last week of treatment (End of the third week), total of RBC were healthy and couldn't find any sign of the disease, also all sample in this groups became normal and healthy (without any contamination).

Homeopathic treatment by Secale drug group: After the first week of treatment in this group, we injected the blood and smear it. Through staining and reviewing these samples, contamination rate remains and have not changed, too infection rate in all samples of this group is 20%. At the end of the second week contamination rate of RBC were reduced to 15%, however clinical symptoms did not change (clinical symptoms remain). In the last week of treatment (end of the third week), the contamination rate was low (but not completely removed) and only some of the clinical symptoms decreased (Table 3).

Homeopathic treatment by sepia drug group: After the first week of treatment in this group, we injected the blood and smear it. Through staining and reviewing these samples, contamination rate and clinical symptoms were reduced. At the end of the second week contamination rate of RBC decreased to 1%, also clinical signs significantly decreased. At the last week of treatment (end of the third week) (Table 4).

Homeopathic treatment by Sepia+China drugs group: This group was treated with two drugs sepia and china simultaneous (the China drug prescribed in the morning and Sepia drug prescribed in the evening).

After the first week of treatment in this group, we injected the blood and smear it. Through staining and reviewing these samples, contamination rate and clinical symptoms greatly decreased. At the end of the second week and at the last week of treatment (by the end of the third week), no effect of the contamination remained and all RBC were healthy. In addition, clinical signs disappeared (Table 5).

DISCUSSION

The present study is the first experimental study on treatment by homeopathy rules in cat inoculation of *Mycoplasma haemofelis*. Anemia is a commonly encountered laboratory abnormality in cats. Hemolytic anemia in this species is mostly caused by acquired disorders, such as hemobartonellosis and other infections (Adams *et al.*, 1993; Kohn *et al.*, 2006).

Arthropods, such as fleas and ticks, are suspected to play an important role in *Mycoplasma haemofelis*

transmission (Shaw *et al.*, 2004; Woods *et al.*, 2005; Willi *et al.*, 2007; Barrs *et al.*, 2010).

The diagnosis is based on the detection of the organism in an epicytological place on feline erythrocytes on a fresh blood smear. However, this technique has low sensitivity, especially in chronically infected animals, in cats with low parasite burden, or because of the cyclical parasitemia (Harvey, 2006; Tasker, 2006). Organisms rapidly detach from red blood cells *in vitro*, probably reflecting organism death a few hours after blood collection (Tasker, 2006). The more pathogenic form (*Mycoplasma haemofelis*) is rarely detected in cats without anemia (Jensen *et al.*, 2001).

Untreated haemobartonellosis is a potentially lethal infection of cats. Because the symptoms are non-specific and the diagnosis is rather difficult, the infection is commonly overlooked. Therefore, few studies about the subject were present to date (Van-Steenhouse *et al.*, 1993; Huml *et al.*, 1995).

In a study by Dowers *et al.* (2009), treatment with pradofloxacin resulted in a more effective clearance of organisms than with doxycycline (Dowers *et al.*, 2009). Stevenson (1997) reported that incidence of the disease was increased and the success rate of medical treatment was decreased between 1995-97 (Stevenson, 1997). In contrast some other researchers claimed that treatment with oral tetracycline preparations for 14-21 days is still enough to eradicate the pathogen (Fraser *et al.*, 1991; Berent *et al.*, 1998; Tasker and Lappin, 2001).

In a study by Van Geffen (2012), the cat responded well to antibiotic treatment with doxycycline, together with immunosuppressive doses of corticosteroids (Van Geffen, 2012). In a study by Akkan *et al.* (2005), the prevalence of *H. felis* infection was investigated in Van cats. *H. felis* was detected in blood smears preparations of 18 (14.88%) by Papenheim staining. Among biochemical parameters aspartate amino transferase (AST), Alanine Amino Transferase (ALT), alkaline phosphatase (ALP), Creatine Phosphokinase (CPK) and bilirubin were in normal range as well as the Packed Cell Volume (PCV) and Red Blood Cell (RBC) counts. The infected cats were treated with oxytetracycline at 10 mg/kg dose intramuscularly (Geosol[®] flacon, Vetas) or oral oxytetracycline at 10 mg/kg dose (Neoterramycine[®] pow. Pfizer) for 15 days. After either above treatment blood smear preparations revealed negative for the *Rickettsia* (Akkan *et al.*, 2005).

Enrofloxacin has been recommended by some (Winter, 1993) for the treatment of haemobartonellosis at a dose of 10 mg/kg per os daily for at least 14 days. The anemia induced by *H. felis* is thought to be, in part, immune-mediated so glucocorticoids may be indicated (Van-Steenhouse *et al.*, 1993).

Homeopathy has been used in animals to treat a multitude of conditions (Mathie *et al.*, 2012); However, its use remains a controversial subject for many veterinarians and scientists despite studies claiming to show its effectiveness (Searcy *et al.*, 1995; Guajardo-Bernal *et al.*, 1996; Albrecht and Schütte, 1999). There

are 2 main areas of contention among skeptics who feel that the laws of homeopathy conflict with those of conventional medicine, physics and chemistry. First, conventional science cannot now offer any mechanism to explain how ultra-dilute solutions (in which it is unlikely that there is a single molecule of the original solute) can produce a specific beneficial therapeutic effect. Second, there is an absence of sound scientific studies that can show whether homeopathy actually produces a specific clinical benefit (Cucherat *et al.*, 2000).

Homeopathic prescriptions are generally based on the symptoms of disease and each characteristic of the patient, in this case the animal. In principle the homeopathic preparation of *E. coli* can be used for all types of coliform bacteria infection (Macleod, 1994).

Some homeopathic drugs has given the use to combat different diseases in animals. The importance of homeopathic drugs and their effective sustainable use other than in humans is explained well by Naveen (2005).

In a study by Naphade *et al.* (2010), the homeopathic medicine *Mercurius corrosivus* was tried against the experimental caecal coccidiosis in broiler chicks as a treatment of control (Naphade *et al.*, 2010).

Many experiments in the homeopathic field have failed to prove an effect of the treatment. Reasons for that could lie in the method of medicine testing as applied in regular medical science, which partly contradicts with the homeopathic philosophy (Hektoen, 2005).

CONCLUSION

In the current study, the group which was under the treatment by China, followed a very proper development process and from the speed treatment point of view, it was equaled to the chemical treatment. Three weeks treatment was enough time for this treatment. The only difference was that the China not only cleaned the clinical symptoms, but also, cleared the blood contamination and left no symptoms from contamination or the disease. This group samples experienced a complete treatment.

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