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Anolyte - Innovative Method for the Treatment of Skin Infections in Dogs

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Abstract
Skin affections of dogs causing serious problems, some of these affections tolerate the antibiotic treatments. This study was carried out on police service dogs at different regions and some clinics in Egypt. 30 affected dogs (10 suffering of MRSA, 10 of *Trichophyton mentagrophytes* & *verrucosum* and 10 of *Microsporum canis*) were selected to carry out the field study to identify new antimicrobial agents. Results revealed that, Anolyte (strong degree of ionization, and when oxidation) and Silver nanoparticles (AgNPs) were induced high curing rate of skin affection in dogs.

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1. Introduction

Bacteria and fungi are invading the skin of animals causing serious problems. It is varied in degree and type of diseases producing multiple signs. The signs may classify into primary and secondary according to the layer of skin infected.

Dermatophytosis is a fungal skin infection caused by dermatophytes (*Microsporum canis, Microsporum gypsum, or Trichophyton* fungi) that affects humans and animals. Infected animals release infective spores in the environment which will then contaminate other animals or humans.

In one hand, some strains of bacteria (*such as Staphylococcus spp.*) causing pyoderma and much type of skin inflammations, some of these infections tolerate the antibiotic treatments as MRSA (Methicillin-Resistance *Staphylococcus aureus*).

On the other hand, dermatophytes (*Microsporum canis, Microsporum gypsum, or Trichophyton* fungi) that affects humans and animals, in spite infected animals usually develop immunity so the infection will spontaneously disappear after a few weeks to months, some of these infections tolerate the fungicidal treatments (Merlino et al., 2002; Songer and Post, 2005; Walther et al., 2008; Leonard and Markeya, 2008; Epstein et al., 2009).

Dermatophytosis in dogs reveals in different forms and can often mimic other skin diseases: eczema, dermatitis, pyoderma, dermatophilosis and mange. Diagnosis is based on clinical signs and often additional laboratory tests are needed for a final diagnosis. Lesions are examined with a Wood's lamp (ultraviolet light source), *Microsporum canis* fluoresces a greenish color.

Wide variety of dermatophytes have been isolated from animals, but few zoophilic species are responsible for the majority of the cases, *Microsporum canis, Trichophyton mentagrophytes, Trichophyton equinum* and Trichophyton verrucosum, and also the geophilic species *Microsporum gypseum* (Chermette et al., 2008).

The use of super-oxidized solutions as wound care products is a cutting-edge concept. The

moistening effect and the minimum toxicity found with the use of this super-oxidized solution makes it a good choice for wound care management. Preliminary results suggest that this non-antibiotic technology appears to offer a broad new paradigm for the prevention and treatment of acute and chronic wounds (Dalla Paola et al., 2005).

Bacteria and fungi in animals are causing serious skin problems; some of them tolerate the antibiotic and the fungicidal treatments.

Therefore, it is necessary to discover novel strategies and identify new antimicrobial agents to develop the next generation of agents to control microbial infections.

2. Experimental

2.1 Animals
This study was carried out on police service dogs at different regions and some clinics in Egypt. 30 affected dogs (10 of them suffering of MRSA, 10 of *Trichophyton mentagrophytes & verrucosum* and 10 of *Microsporum canis*) were selected to carry out the field study.

Skin samples (scraping and swaps) were taken from suspected skin lesions and direct to microscopical examination, tested by Wood's lamp and then cultured for the isolation of pathogens.

2.2 Isolation and Identification of bacteria from skin lesions in dogs
All skin swabs were inoculated onto the following media: Standard nutrient agar and Gassner agar. All plates were investigated twice, first after 18 h and 36 h of incubation at 37° C. *Staphylococcal* isolates were identified by morphology on agar plates, Gram stain appearance, catalase test, tube coagulase positive test and their ability to ferment mannitol anaerobically (Merlino 2002; Songer & Post 2005).

2.3 Isolation and identification of skin fungal lesions (*Dermatophytosis*)
Skin scraping is a common procedure performed to demonstrate the evidence of fungal infection in skin, hairs and nails. It can be done on an outpatient basis and the results are available within 1-2 h (Gupta et al., 2005). The skin samples were taken from the edge of the lesions with a surgical blade. Scrapings were taken very superficially to avoid bleeding. Samples were collected on a black piece of paper to see the skin scrapings in a dark background and some hairs were taken by plucking them off with forceps (Mackenzie, 1963).

Direct microscopic examination of the above specimen was done to detect fungal spores or hyphae. Initial examination is with low power magnification (x10) and low intensity of light with lowering of the condenser. At higher magnification (x40), fungal spores vary from 2-10 mm in diameter (Thirumurthy et al., 2002).

2.3.1 Wood's lamp skin examination
Wood's lamp (emitted UV light at a wavelength of 330 - 365 nm) was used in a dark room to examine hairs for certain dermatophytes by shining the light directly on the sample. *Microsporum canis* showed a yellowish-green fluorescence due to the pteridine secreted by these fungi (Mackenzie 1963).

2.3.2 Fungal culture
Skin swaps cultured for about 4 to 7 days at 25 - 28° C by using Sabaroud dextrose agar (Mackenzie, 1963).

2.4. Treatments
2.4.1 Anolyte
Anolyte (1\500) [pH 2.5-3.5, ORP>1150mV, C_{active} ~500mg/l]. It contains various mixed oxidants predominantly hypochlorous acid and sodium hypochlorite ($HClO$, ClO_2, $HClO_3$, $HClO_4$, H_2O_2, O_2, ClO^-, ClO_2^-, ClO_3^-, O^-, HO_2^-, OH^- - working substances, pH from 2.0 to 8.5, 1\500 = 2 mg /L active chlorine, 1\1000 = 1mg /L active chlorine).

2.4.2. AgNPs
The synthesis of Ag citrate was done according to the literature procedure (Kamat et al., 1998). Briefly, the synthesis involves the following materials and methods: 25 ml of 0.005 M stock solution of silver nitrate in water was diluted to 125 ml and heated until it begins to boil. Then5 ml of 1% sodium citrate solution was added; heating continued until the color was pale yellow. The solution was cooled to room temperature.

All diseased dogs were classified into 3 groups (10 animals each). The first group suffered from MRSA-affection, second group; *Microsporum canis* affection and third group; *Trichophyton mentagrophytes* & *verrucosum* affection. All groups were received local washing of the skin lesion with 1\500 Anolyte (2 mg of active chlorine) followed by topic application of AgNPs.

The treatment applied once daily and for 7 successive days. Clinical cure was defined as the disappearance of clinical signs which were observed on day before treatment and confirmed by negative bacteriological or mycotic isolation of pathogens.

3. Results and Discussion

Emerging infectious diseases and the increase in incidence of drug and antimicrobial resistance among pathogenic bacteria have made the search for new antimicrobials inevitable. In the current situation, one of the most promising and novel therapeutic agents are the nanoparticles (Rai and Bai, 2011).

Table 1: The efficiency of Anolyte (1\500) and AgNPs treatment applied to skin affections in dogs

Lines of treatment		Animals	
1st Group[1]	10	9	90
2nd Group[2]	10	10	100
3rd Group[3]	10	10	100

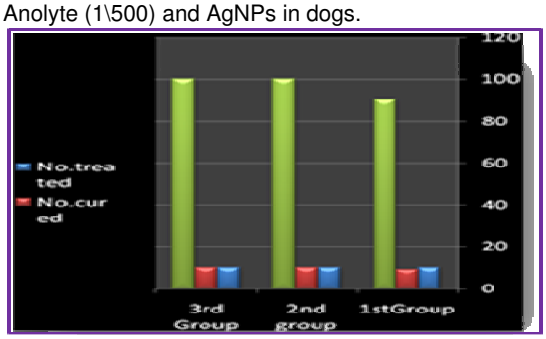
1: MRSA affection, 2: *Microsporum* affection, 3: *Trichophyton* affection

The cured cases were; 90 % for the first group, 100 % for the second, and the third one together with improvement of local clinical signs. Anolyte (1\500) and AgNPs were induced high curing rate after successive 7 days local treatment.

The unique physiochemical properties of the nanoparticles combined with the growth inhibitory capacity against microbes has led to the upsurge in the research on nanoparticles and their potential application as antimicrobials. The mechanism of antibacterial effect of silver nanoparticles has been reported in the literature (Sondi and Salopek-Sondi, 2004), which suggests that the particles are bactericidal and fungicidal.

Several possible modes of action are discussed in the literature on nano-Ag effects on bacteria and fungi. These are (1) membrane disruption through direct attachment of the nanoparticle to the bacterial membrane, (2) cellular invasion and enzyme disruption by nanoparticles, (3) changes in cell membrane permeability (4) interference with cellular S-containing compounds, and (5) intracellular ROS accumulation (Hwang et al., 2008; Kim et al., 2009; Lok et al., 2006; Morones et al., 2005; Pal et al., 2007; Panáček et al., 2006; Sondi and Salopek-Sondi, 2004). That several of these events might act together to result in cell death is probable, but the specific processes and interactions required for toxicity have not been fully confirmed.

Figure 1: The cure % of skin affection treated with Anolyte (1\500) and AgNPs in dogs.



Although the present study did not examine the exact mechanism of action, we believe that the bactericidal and the fungicidal effect of Anolyte against bacteria and fungi were due to the combined action of hydrogen

Figure 2: Plate (1) Typical Fungal skin infections in dogs face showing circular lesion detected by scraping and fungal culture. Left: before treatment; right: after treatment

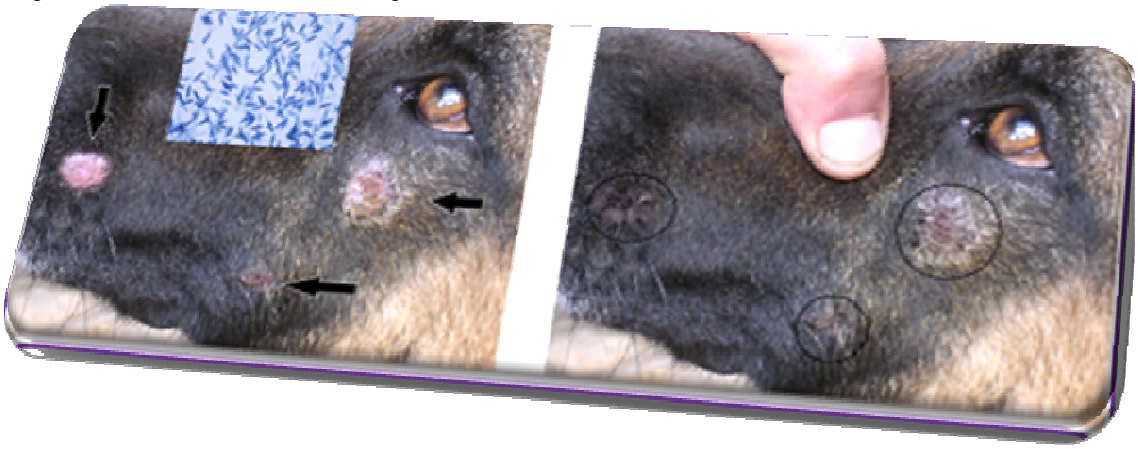


Figure 2: Plate (2): Typical Fungal skin infections in dogs face showing circular lesion detected by scraping and fungal culture. Left: before treatment; right: after treatment

ion concentration, oxidation-reduction potential and dissolved chlorine. Anolyte is a strong acid, but it is different to hydrochloric acid or sulphuric acid. These acids have a strong degree of ionization, and when oxidation occurs, H^+ is used and new H+ is generated (Iwasawa et al., 1993). In case of Anolyte, no new H^+ is generated because it is produced by electrolyses only of the saline solution. Thus the full-strength solution is not corrosive to skin and organic material.

Conclusion

Bacteria and fungi are invading the skin of animals causing serious problems. It is varied in degree and type of diseases producing multiple signs. Anolyte (1\500) and AgNPs were induced high curing rate of skin affection in dogs. Emerging infectious diseases and the increase in incidence of drug and antimicrobial resistance among pathogens have made the search for new antimicrobials inevitable.

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