

Approaches to the Treatment of Diseases of Distal Limb Parts in Cattle

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Abstract

Mortellaro's disease affects up to 25% of Russia's cattle, which causes significant economic damage to dairy products. The indicators of the therapeutic efficacy of existing drugs vary, and obsolete agents and schemes are used for treatment. A drug was developed and tested using the technology of liposomal silver nanoparticles (Ag) for treatment of Mortellaro's disease in dairy cows. By comparison and grouping method, the effectiveness of 5 drug samples at different stages of development of infectious and pathological processes in 5 experimental groups of cows was compared with 2 control groups: Group 1 received no treatment, Group 2 was treated with Solka Hoofgel. It was established that sample 2 of the developed preparation with Ag content of 15 ml showed 92-96% of the therapeutic efficacy in the treatment of Mortellaro's disease, which is higher than that of the analog preparation by 25%. The drug provides the appearance of granulation tissue in the affected areas as early as the fifth day after application to the damaged surface of the interdigital space and the epithelization of the damaged areas after 7 days of treatment.

Key-words: Mortellaro Disease, Ag Nanoparticles, Cows.

1. Actuality of the Topic

Mortellaro's disease is widespread in many countries of Europe and Russia. This disease, not being contagious, nevertheless can affect up to 70% of the herd (Cheli R. e., Mortellaro C., 1974). The economic damage caused by infectious diseases of the distal limbs only in the world's dairy cattle industry is estimated at hundreds of millions of dollars per year (Losinger W., 2006). Damage to other ungulates is difficult to assess due to lack of data.

Researchers around the world do not abandon the attempts to invent a highly effective agent for the prevention and treatment of Mortellaro's disease (Hernandez J. et al., 1999; Koziy, 2005; Holzhauser M. et al., 2008; Toholj B. et al., 2012). At the moment, there is no unequivocal opinion on the existence of a universally recognized tool and scheme for the prevention and treatment of this disease, as the data on the effectiveness of existing drugs vary (Solka, Hoof Skin). However, most foreign drugs are not available for domestic dairy producers because of their high price.

Thus, the necessity emerged to develop a domestic therapeutic and prophylactic drug which can be used to combat diseases of the distal limb parts of cattle.

At the same time, with the development of nanotechnology and the use of nanomaterials, a large number of publications appear that describe the high efficiency of metals in the nanocrystalline state (Jose Ruben Morones et al., 2005). In the present study stabilized silver nanoparticles were used to prepare an antiseptic and wound healing drug that would possess a high therapeutic effect in treating cows suffering from Mortellaro's disease.

The work is aimed at the development and testing of a new effective therapeutic and prophylactic drug based on liposome silver nanoparticles (Ag) in the treatment of cows with Mortellaro's disease.

2. The Aim of the Work

The development and testing of a therapeutic and prophylactic drug to fight the development of Mortellaro's disease in cattle. For this, the following necessary tasks were performed:

- To develop and test a drug for the treatment of cows afflicted with Mortellaro's disease;
- To experimentally study the effectiveness of the developed drug series for treating cows with Mortellaro's disease using the method of comparison and grouping.

3. Materials and Methods

The work was performed on the basis of the Department of Infectious and Invasive Pathology, Laboratory of Infectious and Invasive Pathologies, Development and Approbation of Veterinary Preparations at Belgorod State Agricultural University named after V. Gorin and at LLC 'M9' (Samara).

Scientific and industrial experiments were carried out on the basis of the dairy cattle farm LLC 'Zelenaya Dolina' of the Yakovlevsky District of the Belgorod Region.

The study of the peculiarities of the clinical manifestation of the Mortellaro's disease was carried out directly in the conditions of dairy farming. Identification of the causative agent (*Treponema* sp.) was carried out by bacteriological and microscopic methods. Clinical evaluation of lesions intensity was carried out according to the classification proposed by Döpfer D. et al. (1997). This classification describes the degree of disease manifestation.

M1 – small-sized changes with a red or white-red surface, exudation is possible. The epithelium is either preserved or damaged;

M2 – 'classic ulceration', red or white-red lesions with a diameter of more than 1 cm. On the surface, granulation or proliferative tissue is present (in large quantities or filamentary).

M3 – healing lesions with a black scaboid surface.

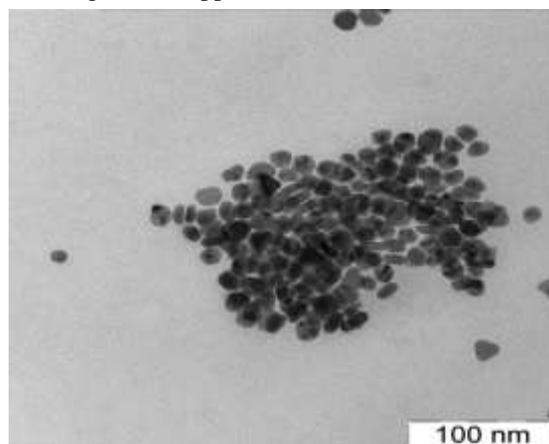
M4 – skin changes that raise suspicion of the disease, as well as a proliferative form (D. Döpfer et al. [1997]).

To prepare 5 test samples of the drug, nanoscale silver particles were prepared, obtained by chemical reduction of silver nitrate in a liquid solvent by sol-gel method with transfer of the obtained nanoparticles to the liposome fraction. Samples of the drug were prepared on a gel basis with nanosized liposomal silver particles distributed in its volume; the structure of the particles was specially modified to ensure high efficiency on skin and keratinized tissues. Special molecular coating on the surface of nanoparticles allows them to gain a foothold in the deep layers of the skin and cornified layer, subcutaneous tissue, thereby ensuring a strong and lasting effect on the conditionally pathogenic and pathogenic microflora in short exposure.

The main difference between the developed product and existing analogues is the presence of metal in the crystalline form. These very small crystals very effectively penetrate deep layers of the skin and subcutaneous tissue exactly where the inflammation site is located and effectively influence the pathogen, inactivating it.

The average size of silver nanoparticles is $16 \text{ nm} \pm 5$ (transmission electron microscopy) (Fig. 1).

Fig.1 - Silver Nano Particles Sized $16 \text{ nm} \pm 5$ (Transmission Electron Microscopy), Obtained in Laboratory of Infectious and Invasive Pathologies and Approbation at BSAU named after V. Gorin



Samples of the drug to compare the therapeutic efficacy of different volumes of silver nanoparticles were prepared by the following calculation:

- Sample 1: 995.0 mlgel + 5.0 mlsilver;
- Sample 2: 985.0 mlgel + 15.0 mlsilver;
- Sample 3: 975.0 mlgel + 25.0 mlsilver;
- Sample 4: 970.0 mlgel + 30.0 mlsilver;
- Sample 5: 960.0 mlgel + 40.0 mlsilver.

The object of study was Holstein Friesian cows of European selection of the first and second lactation periods with different stages of the disease of the distal limbs (Mortellaro's disease), 170 cows in total, of which 7 groups were formed:

Control Group 1 (n = 10), no treatment received;

Control Group 2 (n = 10), hoof gel 'Solkahoofgel' used;

Test Group 1 (n = 30), 'Sample 1' of the preparation used;

Test Group 2 (n = 30), 'Sample 2' of the preparation used;

Test Group 3 (n = 30), 'Sample 3' of the preparation used;

Test Group 4 (n = 30), 'Sample 4' of the preparation used;

Test Group 5 (n = 30), 'Sample 5' of the preparation used;

In Control Group 2, after trimming the hoofs for the treatment of Mortellaro's disease, SolkaHoofgel was used (KantersSpecialProducts B.V., the Netherlands). In the animals of the

experimental groups (3, 4, 5, 6, 7) of dairy cows with lesions of the interdigital space due to Mortellaro's disease after trimming and preliminary treatment of hoofs (mechanical cleaning, washing with running water, drying) the drug samples were applied to the affected surface of the wound with a brush, then covered with gauze bandage, closing the wound with parchment paper before applying the gauze.

After 3, 5, 7 days, the examination of the affected area was performed and the therapeutic efficacy of the drug samples was evaluated according to the degree of healing.

4. Research Results

As a result of the drug tests, changes in the condition of the limbs of 7 groups of cows affected by Mortellaro's disease were studied. The condition of curability and clinical signs were analysed in experimental animals before treatment and then 3, 5, 7 days after the start and the end of treatment.

In the animals of Control Group 1 during the study period the condition of the affected surface of the interdigital space did not change in 3 cows. The affected area increased from 3 mm or more to 6 mm and 8 mm. In 6 cows, the pathological changes increased, which was evident in the visual examination of the limbs (the diameter of the lesions was increased), and the intensity of the lesions caused pain in the animals of the given group and the appearance of edema, that is, the transition to a more acute stage of the pathological process of digital dermatitis (Table 1).

Table 1 - Dynamics of the Pathological Process in Control Group 1

	The degree of distal limb impact by Döpfer D., (1994) prior to the experiment / 7 days later, n heads								Infected animals in the group, n heads		
	M1		M2		M3		M4		prior to the experiment	7 days later	±
Number of animals, n	4	3	3	5	2	0	1	2	10	10	±0
Affected area diameter, mm	48	39	123	208	50	0	23	52	244	351	+107

Analyzing the data of Table 1, it should be noted that in the animals of Control Group 1 the pathological process was aggravated. Thus, at the beginning of the experiment, among 10 heads of cattle 4 animals had M1 lesion, and in 7 days their number decreased to 3, since the lesions in 1

animal on Day 7 were already classified as M2 stage. The number of animals with M2 lesion increased from 3 to 5 heads, and the number of animals with M4 lesion increased from 1 to 2 heads. The total diameter of lesions increased by 107 mm – a 143% increase compared to the beginning of the experiment. These data clearly demonstrate that without treatment, Mortellaro’s disease is rapidly progressing.

Control Group 2 was treated with Solka Hoofgel (Table 2). It should be noted that in this group a significant therapeutic effect of the drug was observed.

Table 2 - Dynamics of the Pathological Process in Control Group 2

	The degree of distal limb impact by Döpfer D., (1994) prior to the experiment / 7 days later								Summary indicators		
	M1		M2		M3		M4		prior to the experiment	7 days later	±
Number of animals, n	3	3	5	1	1	2	1	1	10	7	- 7
Affected area diameter, mm	85	24	258	40	35	54	31	17	409	135	- 274

In one cow, the healing process worsened at the 3rd examination in comparison with the 2nd. On the third day after the gel application, a scab formed on the affected area, which is a necessary condition and a sign of the healing process. Full-scale scab on the third day was formed in 2 animals, but after 7 days, when the scab peeled off, repeated signs of M1 and M2 stage damage were observed on the skin. In 2 animals, the scab was formed partially, that is, not covering the entire lesion area; this caused partial healing in 1 cow (M1 stage) and the persistence of the initial stage of the lesion until the end of the study (M4). In 2 animals no full-scale scab was formed on the 3rd day (by this time, the changes on the lesions surface can be characterized as necrosis and a mild degree of maceration, giving the surface a grey color). In this case, the scab formed a few days later and persisted until the third examination. In three animals with the initial stage of lesions (M1) the drug cured the disease completely. Based on quantitative data of the total diameter of lesions before and after the experiment, the therapeutic efficacy of the preparation ‘SolkaHoofgel’ was 67%.

In Test Group 1 of dairy cows to treat interdigital space lesions Sample 1 was used with the following content of silver nanoparticles: 5 ml/1 L (Table 3).

Table 3 - Dynamics of the Pathological Process in Test Group 1

	The degree of distal limb impact by Döpfer D., (1994) prior to the experiment / 7 days later								Summary indicators		
	M1		M2		M3		M4		prior to the experiment	7 days later	±
Number of animals, n	6	3	12	1	7	2	5	1	30	7	- 23
Affected area diameter, mm	93	44	720	63	158	42	136	35	1107	184	- 923

Analyzing the data of Table 3, it should be noted that the developed drug cured 23 animals completely; in 6 cows, the pathological changes entered less intensive stages: from M2 to M3 in 4 cows, from M4 to M3 in 2 cows, from M1 to M3 in 1 cow. Persistence of the initial stage of the lesion until the end of the study (M4) was observed in 1 cow. The therapeutic efficiency of the sample was 83%.

The use 'Lipometall' in Sample 2 provides higher indices of therapeutic effectiveness in comparison with Sample 1 (Table 4).

Table 4 - Dynamics of the Pathological Process in Test Group 2

	The degree of distal limb impact by Döpfer D., (1994) prior to the experiment / 7 days later								Summary indicators		
	M1		M2		M3		M4		prior to the experiment	7 days later	±
Number of animals, n	10	4	14	0	4	0	2	0	30	2	- 28
Affected area diameter, mm	174	84	1080	0	163	0	62	0	1479	84	- 1395

From M4 stage of interdigital space lesions 1 cow was transferred to stage M1; 1 other animal with M2 was transferred to M1. The remaining animals (28 heads) were cured completely. As a result, the efficiency of the sample was 92-96%.

For Test Group 3, Sample 3 (Ag mass 25 ml) was used. The indices of therapeutic effectiveness turned out to be at the level of Sample 2 (Table 5).

Table 5 - Dynamics of the Pathological Process in Test Group 3

	The degree of distal limb impact by Döpfer D., (1994) prior to the experiment / 7 days later								Summary indicators		
	M1		M2		M3		M4		prior to the experiment	7 days later	±
Number of animals, n	5	2	16	0	4	0	5	0	30	2	- 28
Affected area diameter, mm	42	51	740	0	39	0	112	0	933	51	- 882

As a result of applying Sample 3, 28 cows with M1, M2, M3, M4 lesions were cured. One animal with M4 lesions was transferred to M1 stage; in one other animal with M2 lesion the intensity of the process decreased to M1 stage. The sample showed an efficacy of 93-95%.

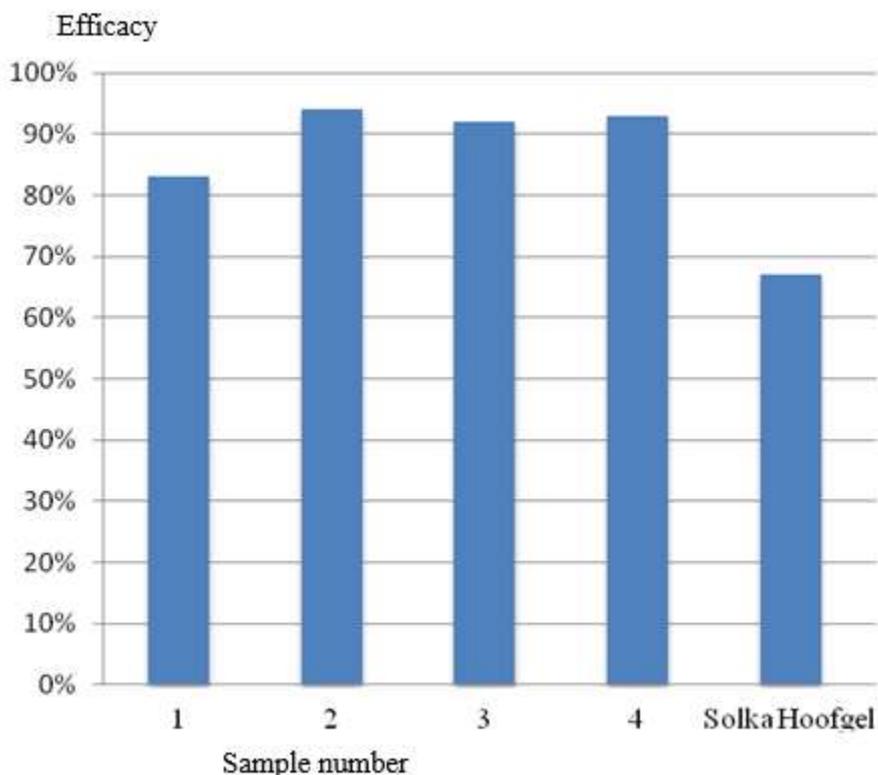
In the study of Test Group 4, Sample 4 (Ag 35 ml) was used, and lesions were also completely cured in more than 90% of cows (Table 6).

Table 6 - Dynamics of the Pathological Process in Test Group 4

	The degree of distal limb impact by Döpfer D., (1994) prior to the experiment / 7 days later								Summary indicators		
	M1		M2		M3		M4		prior to the experiment	7 days later	±
Number of animals, n	9	2	12	0	4	0	5	0	30	2	- 28
Affected area diameter, mm	182	41	1003	0	89	0	174	0	1448	41	- 1407

The therapeutic efficacy of Sample 4 also varies between 93 and 97%. 28 heads of dairy cows were cured completely.

Graph 1 - Comparative Effectiveness of Preparation Samples and of Solka Hoofgel



5. Conclusion

Thus, the efficacy of the drug samples in the comparative aspect of silver nanoparticles proportion in gel compared with 'SolkaHoofgel' (Chart 1) was studied for the prevention and treatment of Mortellaro's disease and showed a 85-96% efficacy. As a result, Sample No. 2 (Ag 15,0 mg) showed a maximum efficacy of 92% -96% with a minimum amount of Ag nanoparticles in the treatment of digital dermatitis, providing a high bactericidal effect on pathogens, manifested by significant therapeutic properties expressed by the appearance of granulation tissue in the affected areas on the fifth day and epithelization of lesions after 7 days of treatment.

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