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(54) Title: TEAT DIPPING AGENT

(57) Abstract

Use of a composition comprising chitosan in combination with a polysaccharide selected from heparin, heparan sulphate and dextran sulphate, as an active component in a solvent, for the manufacture of a teat dipping solution for lactating animals, particularly cows. The invention also relates to a process for prophylactic or wound healing treatment of lactating animals, particularly cows, against mastitis.

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TEAT DIPPING AGENT

Technical area

The present invention relates to the area teat dipping agents for lactating animals. More in particular it relates to a teat dipping agent based on components which are new within this technical area. This new teat dipping agent possesses several advantages in relation to known teat dipping agents.

Background of the invention

Mastitis is an inflammatory reaction of udder tissue and is the most common and most costly disease among lactating cows over the world. The inflammation is a reaction of the lactating tissues on the presence of infectious microorganisms. A large number of different bacteria have been identified as mastitis pathogens. They have been divided into four different groups, contagious, environmental, opportunistic and other bacteria. The majority of the mastitis infections are caused by *S. aureus*. Another contagious mastitis pathogen is *Streptococcus agalactiae*. Among the environmental bacteria there are other streptococci and the coliform bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae*.

A large number of different disinfectants (most frequently chlorohexidine or iodophors) are used for dipping the teats immediately after milking in order to prevent bacteria from penetrating into the teat canal and further to lactating tissues. These disinfectants have a killing effect in direct contact between disinfectant and bacterium. In spite of routine use of these agents a number of bacteria escaped the killing effect, i.e. the known agents are not sufficiently effective, which can be due to insufficient amount of active components and the fact that the agents do not reach sufficient contact with the infected sites. It is also known that the effect of these

agents fades out very quickly and that renewed contamination of the teats takes place shortly after the treatment. Small wounds and skin tissues on the teats can act as reservoirs for certain bacteria, and live stock with infected teat wounds often show higher mastitis frequencies than other live stock. Furthermore, iodine and chlorohexidin can result in taste changes of the milk and relatively small quantities of iodine and chlorohexidin in milk can cause problems in the manufacture of dairy products.

Description of the invention

The present invention thus relates to a new teat dipping composition for lactating animals, which composition represents a substantial contribution to the technology within the area since it has been found to show improvement in several respects compared to existing teat dipping compositions.

More in particular, it has, in accordance with the invention, surprisingly been found that a combination of chitosan and a group of polysaccharides functions quite well as active components in such teat dipping composition. Several different effects and advantages have been observed. Quite generally, it means that the composition according to the invention shows higher activity than known teat dipping agents of the type chlorohexidin and iodophors. An effect in this context is that the new teat dipping composition forms a long time barrier at the teat canal opening and prevents bacteria from penetrating into the teat canal. Furthermore, the composition has a growth inhibiting effect on bacteria and a healing effect on skin wounds further strengthening the protection. This means that the composition can be used for a pure bacteria barrier or bacteriostatic effect, by which follows that it can be used as a prophylactic agent against mastitis. In addition, the composition assists in the healing of wounds and skin fissures. Furthermore, the composition can be given such consistency that it is easy to

apply to the teats, for example by dipping or spraying, at the same time as it imparts a softening effect on the teats.

It can be added in this context with regard to prior art that a composition similar in composition is know per se from for example WO96/02260. However, this known composition has not been referred to or remotely indicated as a teat dipping composition, much less is there in the prior art any indication about the fact that it in such context even shows higher activity than known teat dipping agents of the type chlorohexidin and iodophors.

The expression teat dipping in connection with the present invention shall be interpreted broadly and in accordance with the terminology used within this technical area. Thus, the composition is not only intended for a dipping of the teats in same but it can, of course, also be applied in another way, such as by spraying, but still fall within the recognised term teat dipping composition or agent.

A first object of the invention is, with other words, to provide a teat dipping composition intended to be used for its bacteria barrier effect or bacteriostatic effect.

Another object of the invention is to provide a composition which, when applied, results in a wound healing effect.

Yet an object of the invention is to provide a composition which, when applied, results in a prophylactic action against mastitis.

Another object of the invention is to provide a composition, which has such consistency that it is extremely well suited for dipping or spray application on the teats of lactating animals, at the same time as it thereby imparts a softening effect.

A further object of the invention is to provide a composition which assists in a faster and qualitatively improved healing of wounds on the teats.

Other objects and advantages of the invention will be clear from the more detailed description thereof below.

Detailed description of the invention

More in particular, the invention relates to use of a composition comprising chitosan in combination with a polysaccharide selected from heparin, heparan sulphate and dextran sulphate, as an active component in a solvent, for the manufacture of a teat dipping solution for lactating animals, particularly cows.

The composition according to the invention is useful for all kinds of lactating animals but, of course, in the first place cows. It is further presented in the form of a solution of the active component in a solvent, said solution with regard to consistency or viscosity generally being adapted to the method of application in a conventional manner.

From the above it is clear that the invention primarily is based on the specific combination of chitosan and the indicated polysaccharide and that primarily it is not restricted to any specific ratio between said components. The ratio between the components and the total quantity of same in the solution are therefore, in other words, to be determined by the expert in each individual case depending on the effect desired.

However, in accordance with the invention it is primarily a question of using the stated components in such quantities that the solution when applied results in a bacteria barrier effect or bacteriostatic effect.

Yet another embodiment means that the composition comprises chitosan and the stated polysaccharide in such quantities that the solution when applied results in prophylactic effect against mastitis.

Although the invention is not restricted to any specific total concentration of the active components it is generally suitable that the total concentration of chitosan plus polysaccharide in the solution is within the

range 0.2-10, particularly 0.2-5, for example 0.25-3 percent by weight, based on the total weight of the solution, where the upper limit is mainly dictated by economic factors.

According to a preferred embodiment of the invention the weight ratio chitosan:polysaccharide in the composition according to the invention lies within the range 100:1 - 10:1, particularly 60:1 - 15:1.

With regard to the component chitosan it is constituted by a polysaccharide consisting of 1.4-β-bound D-glucose amine units. Said polysaccharide is linear and the properties can vary according to the degree of N-acetylation. In the nature all amino groups are acetylated and the polysaccharide is then called chitin corresponding to the shell of inter alia insects and shellfish. Chitosan is manufactured by N-deacetylation of chitin. Commercially, chitosan is recovered from crab and shrimpshells which are waste products from the fishing industry. When treating chitin with alkali, usually sodium hydroxide, N-deacetylation takes place, i.e. acetamido groups are converted into amino groups. By controlling the conditions for this alkali treatment chitosans of varying degree of N-acetylation can be manufactured.

In connection with the invention said chitosan, according to a preferred embodiment, has an N-acetylation degree of at most about 90%. In particular, said N-acetylation degree is at most about 50%, in some cases at most about 25%.

Accordingly, the polysaccharide used in the teat dipping composition according to the invention is selected from heparin, heparansulphate and dextransulphate, and these are commercially available on the market from several manufacturers. Within the framework of the invention are, furthermore, partially hydrolysed forms of these polysaccharides as long as they essentially maintain the activity desired in the context. The expression

polysaccharide shall, in other words, be interpreted broadly in connection with the invention. The particularly preferred polysaccharide is, however, dextransulphate since it shows very good activity at the same time as it is considerably cheaper than the other polysaccharides.

As a carrier or matrix the composition according to the invention contains a conventional one, usually an aqueous carrier, for example pure water.

The solution is imparted suitable consistency or viscosity suitable for the application method used and this can be obtained according to conventional technology. As exemples of viscosity controlling agents for use in the composition according to the invention, there may be mentioned hemicellulose types, for example arabinoxylanes and glucomannanes; plant gum materials, for example guar gum and johannistree gums; cellulose types and derivatives thereof, for example methyl cellulose, ethyl cellulose, hydroxyethyl cellulose or carboxymethyl cellulose; starch and starch derivatives, for example hydroxyethyl starch or crosslinked starch; microbial polysaccharides, for example xantan rubber, curdlan, pullulan or dextran; and glycerol. Particularly preferred viscosity controlling agents are, however, different types of cellulose and derivatives thereof, particularly methyl cellulose, and glycerol.

Since the composition described above can be used as a barrier-forming teat dipping agent for lactating animals without any therapeutic effect another aspect of the invention relates to the use of the composition as defined above as a barrier-forming or bacteriostatic teat dipping agent for lactating animals, particularly cows.

Since the composition, furthermore, can be utilised to counteract mastitis according to another aspect of the invention it represents the use of said composition for the manufacture of a teat dipping agent having prophylac-

tic action against mastitis of lactating animals, particularly cows.

A further aspect of the invention is represented by a method for prophylactic treatment of lactating animals, particularly cows, against mastitis, where on the teats of said animals a composition according to the above definition in a quantity effective for preventing the generation of mastitis is applied.

The composition according to the invention can be prepared by dissolving the active components and, optionally, additives, such as for example viscosity controlling agents, in the selected solvent in the desired concentration. An advantageous method in connection with the composition according to the invention resides in dissolving chitosan and polysaccharide separately in a suitable quantity of solvent, for example about half each of the quantity solvent totally used. The additives, for example the viscosity controlling agents, are thereby dissolved together with the polysaccharide before both solutions of chitosan and polysaccharide are admixed to a common solution which is carefully admixed, for example in a mixer, to a final teat dipping solution.

Examples

The invention will now be further illustrated by the following non-limiting examples.

Example 1

General procedure

Vertical agar plates are covered with chitosan-dextransulphate solution before they are dipped in concentrated bacteria solutions and after said dipping. Chitosan-dextransulphate is compared with commercial iodine solution used as a teat dipping agent, and the agar plates are treated in exactly the same manner with iodine solution. The agar plates are incubated in a heating cabinet over night and the number of bacteria colonies

are calculated and recorded as cfu/mL of the bacteria solution. Controls are untreated agar plates.

Materials and reagents

E-coli, isolated from mastitis infection
(Mastitlaboratoriet, SVA)
S.aureus, isolated from mastitis infection
(Mastitlaboratoriet, SVA)
Strept. agalactiae, Strept. dysgalactiae and Strept. uberis, isolated from mastitis infection
(Mastitlaboratoriet, SVA)
Soy broth, Tryptic soy broth, Difco 0370-01-1
Verical agar plates, Uricult Orion UF009

Sterile bench, Kojair, LAF 7022
Photometer, Shimadzu UV-1201
Shake incubator, Orbit Environ-Shaker, Lab-Line Instruments Inc.
Heat cabinet, Memmert

NaCl, Riedel-de Haën 31434
Chitosan, Seacure 343, Pronova Biopolymer
Acetic acid, Riedel-de Haën
NaOH
Methyl cellulose, Sigma M-0387
Glycerol, Johnson Matthey Electronics 13797
Dextran sulphate, Pharmacia
Iodine solution, Dipal, Alfa Laval 997515-80

Procedure

E.coli and S.aureus and three different streptococci (Strept. agalactiae, Strept. dysgalactiae and Strept. uberis) are sterile transferred (sterile bench) with loop from storage agar to separate sterilized trypton soy broth and incubated over night at 37°C in a shake incubator. The following morning the bacteria are washed three

times with a physiological salt solution. The optical density at 650 nm was measured with a photometer, and by means of standard curves the bacteria density was adjusted.

Chitosan-dextran sulphate solution

1 g chitosan was dissolved in 50 mL sterile water, and the pH was adjusted to 5.8. 0.5 g glycerol and 0.1 g methylcellulose and 0.02 g dextransulphate were dissolved in 50 mL sterile water. Equal amounts of chitosan solution and glycerol + methyl cellulose + detransulphate solution were mixed in a mixer.

Iodine solution

The base solution was diluted according to the instruction 6 times with water for giving a solution for use.

Bacteria tests

(i) Bacterial growth under chitosan-dextran sulphate film.

Each bacterial type was treated separately according to the following:

Three agar plates were lowered into the bacteria solution, and two of the agar plates were then dipped three times in each of the above solutions. One agar plate was left untreated.

(ii) Bactierial growth on top of chitosan-dextran sulphate film.

Each type of bacterium was treated separately according to the following:

Two agar plates were dipped three times in each of the above solutions. One agar plate was left untreated. All three agar plates were then immediately quenched in the bacteria solution.

The agar plates were incubated over night in a heat cabinet at 37°C. Then the plates and the number of colonies were inspected and registered.

Results

Table 1

Bacterial growth under chitosan-dextran sulphate film

Patogene	CFU/mL		
	Control	Chitosan-dextran sulphate	Iodine
E.coli	10 000 000	0	0
S.aureus	1 000 000	0	0
Streptococci	10 000 000	0	0

Table 2

Bacterial growth on top of chitosan-dextran sulphate film

Patogen	CFU/mL		
	Controll	Chitosan dextran sulphate	Iodine
E.coli	10 000 000	0	10 000 000
S.aureus	1 000 000	0	1 000 000
Streptococci	10 0000 000	0	10 000 000

The results are presented in Figures 1 and 2.

Example 2

Materials and methods

6 cows of an age of 3-5 years were involved in the study. Five of the cows were of SRB-rase (Svensk Rödbrokig Boskap) and the sixth was a crossing between the SRB and SLB (Svensk Låglands Boskap). The animals were acquired from one and the same stock and were bought 12 days before initiating the experiments. The cows were stabled and a maintenance ration of fodder was composed similar to the maintenance ration of fodder that the cows had had in their original stock. The maintenance ration of fodder consisted of hay, grass ensilage, rich food and

concentrate. The cows were collared in long stalls with rubber mat and cutter shavings as a litter bed.

Five cows were in the first lactation and one cow was in its third lactation. The stage of lactation varied between the second to the seventh lactation month. The yield of milk in the start of the experiments varied between 28-48 kgs ECM. The udder health was documented to be good before the start of the experiments, with negative cultivation tests and CMT-values of 1 and 2. However, coagulase negative staphylococci grew in one udder section (forward left) on one cow, together with increased cell contents (CMT 3), in view of which this udder section was excluded from the experiment.

A teat dipping solution containing (percent by weight) 1% chitosan, 0.02 dextran sulphate, 0.1% methyl cellulose and 0.5% glycerol was prepared.

Milking was carried out at 07^{30} and at 15^{30}. Milk samples were taken directly after each milking situation for bacterial growth and analysis of the cell contents.

The cell contents were determined with a fluorooptoelectronic method (Fossomatic 90, A/S N. Foss Electric Denmark). The bacteriological analysis was carried out by smearing 10 µl of the milk sample on to a bovine blood agar plate with esculine added and was incubated at 37°C for two days. Recordings were made after 24 and 48 hours and any mastitis pathogenic bacteria were isolated and identified. In connection with bacterial growth also routine CMT-analysis was made (California Mastit Test).

The study was carried out in three experimental rounds. In the first experimental round the animals were divided up so that three cows with together 12 udder sections were teat-dipped diagonally right forward and left rear, and three cows were teat-dipped reversed diagonally, i.e. left forward and right rear (see Table 5). The other 11 udder sections were left untreated as controls.

To the second experimental round four of the cows having 10 udder sections not infected in the first experiment were used. The udder sections were randomly distributed so that five were treated with the new teat dipping agent and five constituted controls. Two cows contributed with 3 udder sections each and two udder sections each and the two others with two udder sections each, see Table 5.

For the third experimental round four of the cows were used with seven udder sections which had not become infected during the previous experimental rounds. All 7 udder sections were treated with commercial teat dipping agent containing iodine. Two cows contributed with one udder section each and the two others with 2 and 3 udder sections, respectively, each, see Table 8.

In order to make the udder sections more sensitive to infection during the experiment keratin was removed from the teat canal on all udder sections. This was done with a darning-needle (John James & Sons, Ltd, Studley, Warwickshire, size 16). Keratin was removed from all teat canals after each morning milking.

In the first experimental round the keratin removal was terminated day 8. For two cows which up to day 7 had not become infected keratin removal was carried out both morning and night day 7 and 8.

In the second experimental round the keratin removal was terminated day 6, here double keratin removals did not have to be performed in order to infect the cows.

In the third experimental round the keratin removal was terminated day 8. Keratin removal was carried out both morning and night day 7 and 8.

Ten minutes after the teat dipping procedure all teats were dipped for 10 seconds in a bacteria suspension, containing about 1-7 x 10^7 CFU/ml of a ß-hemolysing *Streptococcus agalactiae* strain (S-B8).

The criterium for infection of an udder section was two subsequent milk samples having bacterial growth or

increased cell contents. A cow with symptom for acute clinical mastitis was treated with antibiotic and increased milking frequency.

Experimental schedule

Morning milking

1. After milking the teats were cleaned with 70% ethanol. The ethanol was allowed to evaporate and milk samples were then taken in test tubes from each udder quarter.
2. The teats were then carefully washed with 70% ethanol. A sterile darning-needle was inserted into the teat canal, rotated and then withdrawn. All teats were treated in this manner.
3. The pre-determined teats were immediately after point 2 dipped with teat dipping agent. The control teats were left untreated.
4. After 10 minutes all teats were dipped for 10 seconds in the bacterium suspension with $1\text{-}7 \times 10^7$ CFU/ml of ß-hemolysing *Streptococcus agalactiae*.

Night milking

1. After milking the teats were cleaned with 70% ethanol. The ethanol was allowed to evaporate and milk samples were then taken in test tubes from each udder quarter.
2. The predetermined teats were dipped immediately after point 1 with teat dipping agent. The control teats were left untreated.
3. After 10 minutes all teats were dipped in bacterium suspension for 10 seconds.

Statistic evaluation has been made by means of χ^2-analysis according to the following formula.

$$\sum \frac{(O-E)^2}{E}$$

and 95% confidence interval was calculated by means of the formula

$$(p_1 - p_2) \pm 1,96 \times se(p_1 - p_2)$$

wherein $se(p_1 - p_2)$ has been calculated as

$$\sqrt{\frac{p_1(1-p_1)}{n_1} + \frac{p_2(1-p_2)}{n_2}}$$

se = standard error
n_1 = number of infected non-treated udder sections
n_2 = number of infected treated udder sections
p_1 = number of non-treated udder sections/n_1
p_2 = number of treated udder sections/n_2

Results

In total 14 of the 33 udder sections were infected during the two experimental rounds, of these were 12 not teat dipped and two were teat dipped. All cows in experimental rounds 1 and 2 became infected. In experimental round 3 two of the cows became infected.

The number of days between start of experiments and up to infection of udder sections varied between 2 and 9 days.

Table 3

The number of infected udder sections after treatment with teat dipping agent compared with no teat dipping (control). Experimental rounds 1 and 2 are assembled to one table.

	Treated	Control	Total
Infected	2	12	14
Not in-fected	14	5	19
Total	16	17	33

Table 4

The number of infected udder sections after treatment with commercial teat dipping agent. Experimental round 3.

	Treated
Infected	2
Not infec	5
Total	7

Experimental round 1

Of the control udder sections 7/12 were infected and of teat dipped udder sections 1/11 were infected.

Five cows were infected with *Streptococcus agalactiae* in one udder section, the udder sections infected were controls.

One cow (No. 335) was infected in 3 udder sections, 2 control udder sections and 1 teat dipped udder section. The udder section of cow 335 which was strongly infected and reacted with the highest cell contents in the milk was one control section, see tables 5 and 6.

Experimental round 2

Out of the control udder sections 5/5 were infected and out of teat dipped udder sections 1/5 were infected, see table 7.

Cow 334 was infected in all 3 udder sections partaking in the experiment, 2 udder sections were controls

and 1 udder section was treated. It was the treated udder section that became most infected and responded with a strong inflammatory reaction in the form of swelling, soreness and increased cell contents.

The other three cows in the experiment were infected in one control udder section each, see table 7.

Experimental round 3

Out of the 7 udder sections treated with commercial iodine teat dipping agent, 2 udder sections were infected, see Table 8.

The result of the χ^2-analysis for the assembled experimental results from rounds 1 and 2 gave an χ^2-value of 11.39, which corresponds to a probability value of < 0.1% at one degree of liberty. I.e., the probability to obtain such a deviating value as 11.39, if h_o (the zero hypothesis) starts from the assumption that there is no difference between treated and untreated group, is less than 0.1%.

When calculating 95% confidence interval for the difference in proportion ($p_1 - p_2$) between the two experimental groups the result was $0.581 \pm 1.96 \times 0.138$, i.e. between 0.311 and 0.851.

Only 7 udder sections were part of experimental round 3. These were constituted by udder sections resisting infection for the longest period of time and were therefore the result of a selection. In view of this no statistic evaluation of this experimental round was made.

The infection frequency of the different experimental rounds is summarized in Figure 3.

Discussion

All cows originated from the same live stock and had thereby the same immunological background. This was an advantage since one could expect that the cows would react in essentially the same manner during the readjust-

ment period to the new environment including new maintenance ration of fodder. This resulted, together with the maintenance ration of fodder that was similar to that the cows were used to, in a situation whereby the time for buying the cows to the start of the experiments could be kept short (by 10 days).

Streptococcus agalactiae was selected in order that the animals with high probability would not have been subjected to this bacterium earlier, it is easy to find in cultivation (β-hemolysing), it is strictly udder specific and it is documented sensitive to penicillin.

The cows which were infected in 1 udder section in experimental rounds 1 and 2 became so in the untreated udder section, whereas the cows receiving infection in teat dipped udder sections became simultaneously infected in untreated udder sections.

In order to increase the infection risk of two cows which in the first experimental round were not infected up to day 7, the removal of keratin was extended to two times a day. The rate of generation of teat canal keratin is high (12-36h) and probably varies between individuals in view of which they have different sensibility for similar challenges in this context. Also the quantity of keratin, the majority of the keratin and composition thereof can vary between individuals.

It can be noted that animals infected late in the first experiment were also infected late in the second experiment.

Table 5. Analysis results of cell contents, CMT and bacterial growth in cows Nos. 306, 331 and 333 from the first experiment round. Cows Nos. 306 and 331 were teat dipped on right rear and left forward teat, respectively, cow No. 333 was teat dipped on right forward and left rear teat, respectively.

	Day	23		24		25		26	
	Time of exp.	am	pm	am	pm	am	pm	am	pm
Cow no									
306	Cell cont. Hf	97	304	173	193	107	135	65	150
	•Hb	158	331	208	221	237	303	128	179
	Vb	207	216	158	220	125	1659	28872	20590
	•Vf	1099	2133	764	2996	2008	2679	1052	2812
	CMT Hf	2	3	2	2	2	2	1	1
	•Hb	2	2	2	2	1	2	1	1
	Vb	1	2	1	2	2	4	5	5
	•Vf	3	3	3	4	4	4	3	5
	Bact. Hf								
	growth •Hb								
	Vb				Sr				
	•Vf	S		S	S	Sr	Sr Sr	Sr Sr	E Sr Sr
331	Cell Hf	72	126	53	101	60	93	62	128
	cont. •Hb	41	60	49	68	46	66	54	82
	Vb	30	42	26	45	45	56	66	114
	•Vf	46	57	42	74	48	67	44	126
	CMT Hf	1	2	1	2	1	2	1	1
	•Hb	1	1	1	2	1	1	1	1
	Vb	1	1	1	1	1	2	1	1
	•Vf	1	2	2	3	2	2	1	1
	Bact. Hf								
	growth •Hb								
	Vb								
	•Vf								
333	Cell								
	cont •Hf	92	387	173	198	120	131	131	169
	Hb	95	386	194	181	155	175	110	158
	•Vb	169	654	204	251	195	283	120	179
	Vf	113	739	255	267	156	252	138	297
	CMT •Hf	2	3	3	3	2	2	1	1
	Hb	1	3	3	3	3	2	1	1
	•Vb	2	3	3	3	3	2	1	1
	Vf	1	3	3	3	2	2		1
	Bact. •Hf								
	growth Hb								
	•Vb								
	Vf								

am=morning Hf=right forward teat Vb=left rear teat
pm=afternoon Hb=right rear teat Vf=left forward teat
Cell cont=counted in 1000;CMT=California Mastitis Test;
Bact.growth,Sr=Streptococcus agalactiae,S=Staphylococcus spp.;E=Ethacilin;•=teat-dipped;N=Novocillin **Table 5 cont.**

Table 5 cont.

	Day	27		28		29		30	
	Time of exp.	am	pm	am	pm	am	pm	am	pm
Cow #									
306	Cell cont. Hf	107	138	80	172	102	261	119	205
	•Hb	193	245	124	143	108	220	149	217
	Vb	16023	14595	9944	10211	3527	7958	2625	4080
	•Vf	19707	14980	13471	13422	3839	6175	2724	2793
	CMT Hf	2	2	2	2	1	3	2	2
	•Hb	2	2	2	2	2	3	2	2
	Vb	5	5	5	5	4	5	3	4
	•Vf	5	5	5	5	4	4	3	4
	Bact. Hf growth •Hb								
	Vb	E		E					
	•Vf	Sr	Sr						
331	Cell cont. Hf	84	111	63	99	31	102	102	118
	•Hb	57	70	66	89	34	108	136	246
	Vb	194	159	126	194	12599	22497	23828	32556
	•Vf	47	82	50	91	38	84	96	167
	CMT Hf	2	1	1	2	1	2	1	1
	•Hb	1	1	1	1	1	2	2	1
	Vb	2	2	1	2	5	5	5	5
	•Vf	1	1	1	1	1	3	2	2
	Bact. Hf growth •Hb								
	Vb			Sr	Sr E		Sr N	Sr N	Sr
	•Vf								
333	Cell cont. •Hf	81	108	86	152	45	96	78	96
	Hb	53	104	54	105	48	114	112	133
	•Vb	129	149	80	143	79	130	255	160
	Vf	80	142	85	131	59	92	128	115
	CMT •Hf	1	2	1	2	1	1	1	1
	Hb	1	2	1	2	1	1	1	1
	•Vb	2	2	1	2	2	3	2	2
	Vf	2	2	1	1	1	1	2	1
	Bact. •Hf growth Hb								
	•Vb								
	Vf								

am=morning Hf=right forward teat Vb=left rear teat
pm=afternoon Hb=right rear teat Vf=left forward teat
Cell cont=counted in 1000;CMT=California Mastitis Test;
Bact.growth,Sr=Streptococcus agalactiae,S=Staphylococcus spp.;E=Ethacilin;•=teat-dipped;N=Novocillin

Table 5 cont.

Table 5 cont.

	Day	1		2	
	Time of exp.	am	pm	am	pm
Cow #					
306	Cell cont. Hf	88	192	36	100
	•Hb	116	173	153	214
	Vb	1379	2941	1608	1692
	•Vf	1402	1957	935	1410
	CMT Hf	1	2	1	1
	•Hb	2	1	1	1
	Vb	3	2	2	2
	•Vf	3	1	1	2
	Bact. Hf				
	growth •Hb				
	Vb				
	•Vf				Sr
331	Cell cont. Hf	85	118	50	77
	•Hb	139	166	58	64
	Vb	41565	25450	21898	13532
	•Vf	138	187	67	62
	CMT Hf	1	1	1	1
	•Hb	1	1	1	1
	Vb	5	5	5	5
	•Vf	1	1	1	1
	Bact. Hf				
	growth •Hb				
	Vb	N Sr	N Sr	Sr	E
	•Vf				
333	Cell cont. •Hf	48	78	69	125
	Hb	132	5099	39878	30290
	•Vb	122	242	165	305
	Vf	58	144	127	175
	CMT •Hf	1	1	1	1
	Hb	1	5	5	5
	•Vb	2	2	1	1
	Vf	1	1	1	1
	Bact. •Hf				
	growth Hb	Sr N	Sr N	Sr N	Sr
	•Vb				
	Vf				

am=morning Hf=right forward teat Vb=left rear teat
pm=afternoon Hb=right rear teat Vf=left forward teat
Cell cont=counted in 1000; CMT=California Mastitis Test;
Bact.growth,Sr=Streptococcus agalactiae,S=Staphylococcus
spp.;E=Ethacilin;•=teat-dipped;N=Novocillin

Table 6. Analysis results of cell contents, CMT and bacterial growth in cow Nos. 334, 335 and 338 from the first experiment round. Cow Nos. 306 and 335 were teat dipped on right forward and left rear teat, respectively, cow No. 338 was teat dipped on right rear and left forward teat, respectively.

	Day	23		24		25		26	
	Time of exp.	am	pm	am	pm	am	pm	am	pm
Cow #									
334	Cell •Hf	191	144	156	187	126	72	80	106
	cont. Hb	83	87	97	98	63	65	56	78
	•Vb	108	114	109	107	111	84	65	78
	Vf	117	164	130	143	16959	43705	44471	40411
	CMT •Hf	1	2	2	2	1	1	1	1
	Hb	1	3	2	2	1	1	1	1
	•Vb	1	3	2	2	1	1	1	1
	Vf	1	2	1	1	5	5	5	5
	Bact. •Hf								
	growth Hb							Sr	
	•Vb								
	Vf					E	Sr	Sr E	Sr
335	Cell •Hf	87	86	64	83	318	1029	4187	4733
	cont Hb	100	101	58	96	14956	35956	45703	37195
	•Vb	68	94	42	51	60	159	76	111
	Vf	80	75	38	67	688	1037	2204	2314
	CMT •Hf	1	2	1	1	3	4	5	5
	Hb	1	1	1	1	5	5	5	5
	•Vb	1	1	1	1	1	1	1	1
	Vf	1	1	1	1	3	4	4	4
	Bact. •Hf					Sr	Sr		
	growth Hb					Sr E	Sr	Sr E	Sr
	•Vb								
	Vf					Sr	Sr		
338	Cell Hf	33	83	82	74	33	58	26	69
	cont.. •Hb	193	130	77	118	49	65	21	74
	Vb	146	90	69	146	112	150	62	130
	•Vf	53	87	66	82	424	1685	2935	2005
	CMT Hf	1	1	1	1	1	1	1	1
	•Hb	1	1	1	1	1	1	1	1
	Vb	1	1	1	1	2	1	1	1
	•Vf	1	1	1	1	3	4	4	4
	Bact. Hf								
	growth •Hb								
	Vb								
	•Vf	S				S	S		

am=morning Hf=right forward teat Vb=left rear teat
pm=afternoon Hb=right rear teat Vf=left forward teat
Cell cont=counted in 1000;CMT=California Mastitis Test;Bact.growth,Sr=Streptococcus agalactiae, S=Staphylococcus spp.;E=Ethacilin;•=teat-dipped;N=Novocillin

Table 6 cont.

Table 6 cont.

	Day	27		28		29		30	
	Time of exp.	am	pm	am	pm	am	pm	am	pm
Cow #									
334	Cell •Hf	66	47	105	105	103	60	106	110
	cont. Hb	80	73	67	106	81	108	85	97
	•Vb	91	79	134	74	83	126	106	73
	Vf	35281	22651	23493	18960	16792	17835	8841	13602
	CMT •Hf	1	1	2	2	2	1	1	1
	Hb	1	1	1	1	1	1	1	1
	•Vb	2	1	1	1	1	1	1	1
	Vf	5	5	5	5	5	5	5	5
	Bact. •Hf								
	growth Hb								
	•Vb							S	
	Vf	Sr E	Sr						
335	Cell •Hf	3369	2980	2289	2139	1032	1398	749	1325
	cont Hb	32044	17631	9923	10081	6821	7330	4418	6865
	•Vb	97	99	56	80	50	68	60	77
	Vf	2061	2321	1353	2123	804	962	530	921
	CMT •Hf	4	5	4	3	3	3	3	3
	Hb	5	5	5	5	5	4	4	4
	•Vb	2	2	1	1	1	2	1	1
	Vf	3	5	3	3	3	3	3	3
	Bact. •Hf								
	growth Hb	Sr	E						
	•Vb								
	Vf								
338	Cell Hf	31	40	29	48	64	132	38	75
	cont. •Hb	97	73	43	80	68	99	128	153
	Vb	49	105	44	98	133	121	94	136
	•Vf	1445	1348	650	994	395	392	257	501
	CMT Hf	1	1	1	1	1	3	1	1
	•Hb	1	1	1	1	1	3	1	1
	Vb	1	1	1	1	2	1	2	2
	•Vf	3	3	2	3	2	2	3	2
	Bact. Hf								
	growth•Hb								
	Vb								S
	•Vf							S	

am=morning Hf=right forward teat Vb=left rear teat
pm=afternoon Hb=right rear teat Vf=left forward teat
Cell cont=counted in 1000;CMT=California Mastitis
Test;Bact.growth,Sr=Streptococcus agalactiae, S=Staphylococcus spp.;E=Ethacilin;•=teat-dipped;N=Novocillin

Table 6 cont.

Table 6 cont.

	Day	1		2	
	Time of exp.	am	pm	am	pm
Cow no					
334	Cell cont. •Hf	124	71	82	151
	Hb	78	49	63	88
	•Vb	81	105	65	75
	Vf	4894	6013	1821	6099
	CMT •Hf	1	1	1	1
	Hb	1	1	1	1
	•Vb	1	1	1	1
	Vf	4	3	3	3
	Bact. •Hf growth Hb				
	•Vb				
	Vf				
335	Cell cont. •Hf	675	964	483	10731
	Hb	3245	6150	2186	2855
	•Vb	73	82	66	70
	Vf	869	22227	19869	16789
	CMT •Hf	3	3	3	5
	Hb	4	4	3	3
	•Vb	1	2	1	1
	Vf	3	5	5	5
	Bact. •Hf growth Hb			Sr	Sr(2) E
	•Vb				
	Vf	Sr	Sr	Sr	Sr
338	Cell cont. Hf	88	8862	30894	17377
	•Hb	198	204	136	209
	Vb	91	138	352	299
	•Vf	387	692	566	621
	CMT Hf	1	5	5	5
	•Hb	1	1	1	1
	Vb	1	1	1	1
	•Vf	3	2	2	3
	Bact. Hf growth •Hb	Sr	Sr	Sr E	Sr
	Vb				
	•Vf	S			

am=morning Hf=right forward teat Vb=left rear teat
pm=afternoon Hb=right rear teat Vf=left forward teat
Cell cont=counted in 1000;CMT=California Mastitis Test;Bact.growth,Sr=Streptococcus agalactiae, S=Staphylococcus spp.;E=Ethacilin;•=teat-dipped;N=Novocillin

Table 7. Analysis results of cell contents, CMT and bacterial growth in cow Nos. 306, 331, 334 and 338 from the second experiment round. Cow No. 306 was teat dipped on right forward teat, 331 was teat dipped on right forward and right rear teat, respectively, 334 and 338 were teat dipped on right rear teat.

	Day	13		14		15		16	
	Time of exp.	am	pm	am	pm	am	pm	am	pm
Cow #									
306	Cell •Hf	89	160	141	235	91	131	129	143
	cont. Hb	78	131	127	123	31822	38666	39981	16350
	CMT •Hf	2	3	2	3	2	2	2	2
	Hb	2	3	1	2	3	5	5	5
	Bact. •Hf								
	growth Hb				E	Sr	Sr E	Sr	
331	Cell •Hf	58	112	76	72	69	97	46	88
	cont •Hb	75	109	92	74	73	75	69	1011
	Vf	50	67	65	55	51	39	33	48
	CMT •Hf	1	3	1	1	2	2	1	1
	•Hb	2	2	1	1	3	2	1	1
	Vf	1	1	1	2	1	1	1	1
	Bact. •Hf								
	growth •Hb								
	Vf								
334	Cell cont								
	. Hf	116	149	113	159	108	143	144	174
	•Hb	47	62	56	71	6870	26446	43123	36795
	Vb	47	62	86	333	2686	2550	1459	1561
	CMT Hf	1	1	1	2	2	3	1	1
	•Hb	1	1	1	1	4	5	5	5
	Vb	1	1	1	3	3	3	3	3
	Bact. Hf					Sr	Sr		
	growth •Hb				E	Sr	Sr E	Sr	Sr
	Vb				S	Sr		-	
338	Cell •Hb	50	135	29	64	44	56	28	34
	cont. Vb	32	88	26	35	26	57	18	43
	CMT •Hb	1	1	1	1	1	1	1	1
	Vb	1	1	1	1	1	1	1	1
	Bact. •Hb								
	growth Vb								

am=morning Hf=right forward teat Vb=left rear teat

pm=afternoon Cell cont=in 1000;CMT=California Mastitis Test; Bact.growth,Sr=Streptococcus agalactiae,S=Staphylococcus spp.;E=Ethacilin;•=teat-dipped;N=Novocillin **Table 7 cont.**

Table 7 cont.

	Day	17		18		19	
	Time of exp.	am	pm	am	pm	am	pm
Cow no							
306	Cell cont. Hf	108	95				
	•Hb	19969	12399				
	CMT Hf	2	2				
	•Hb	5	5				
	Bact. Hf						
	growth •Hb	E		E		E	
331	Cell						
	cont. •Hf	56	95	62	96	148	6
	•Hb	68	133	133	130	211	174
	Vf	41	50	375	26511	44130	35022
	CMT •Hf	2	2	2	1	2	1
	•Hb	2	2	1	2	2	3
	Vf	1	1	4	5	5	5
	Bact. •Hf						
	growth •Hb						
	Vf			Sr E	Sr	Sr E	Sr
334	Cell						
	cont. Hf	112					
	•Hb	27753					
	Vb	1816					
	CMT Hf	1					
	•Hb	5					
	Vb	3					
	Bact. Hf						
	growth •Hb	E		E		E	
	Vb						
338	Cell •Hb	33	47	39	56	110	145
	cont. Vb	21	47	35	9548	29784	25099
	CMT •Hb	1	1	1	1	3	3
	Vb	1	1	1	5	5	5
	Bact. •Hb						
	growth Vb			Sr E	Sr	Sr E	Sr

am=morning Hf=right forward teat Vb=left rear teat
pm=afternoon Hb=right rear teat Vf=left forward teat
Cell cont=counted in 1000;CMT=California Mastitis Test;
Bact.growth,Sr=Streptococcus agalactiae; S=Staphylococcus spp.;E=Ethacilin;•=teat-dipped;N=Novocillin

Table 8. Analysis results of cell contents, CMT and bacterial growth in cow Nos. 306, 331, 333 and 335 from the third experiment round with commercial teat dipping composition. Cow No. 306 was teat dipped on right forward teat, cow No. 331 on right forward and right rear teat, respectively, cow No. 333 on right forward, left rear teat and left forward teat, respectively, and cow No. 335 on left rear teat..

	Day	28		29		30		31	
	Time of exp.	am	pm	am	pm	am	pm	am	pm
Cow #									
306	Cell cont. Hf	96	144	107	155	57	132	114	114
	CMT Hf	1	1	1	1	1	1	1	1
	Bact. Hf growth								
331	Cell cont Hf	42	64	82	153	59	125	58	86
	Hb	51	67	86	109	86	113	74	91
	CMT Hf	2	1	1	1	1	1	1	2
	Hb	2	1	1	1	1	2	1	2
	Bact. Hf growth Hb								
333	Cell cont Hf	49	95	34	104	44	139	103	159
	Vb	73	150	85	131	73	115	91	114
	Vf	81	133	82	195	68	195	119	156
	CMT Hf	1	2	1	1	1	3	2	3
	Vb	2	2	3	1	3	2	1	3
	Vf	3	2	1	1	3	3	2	3
	Bact. Hf growth								
	Vb								
	Vf								
335	Cell Vb cont.	115	133	118	144	106	144	225	333
	CMT Vb	3	2	3	1	3	3	2	3
	Bact. Vb growth								

am=morning Hf=right forward teat Vb=left rear teat

pm=afternoon Hb-right rear teat Vf=left forward teat

Cell cont=counted in 1000;CMT=California Mastitis Test;Bact.growth,Sr=Streptococcus agalactiae,S=Staphylococcus spp.;E=Ethacilin;

Table 8 cont.

Table 8 cont.

	Day	1		2		3		4	
	Time of exp.	am	pm	pm	am	pm	am	pm	am
Cow #									
306	Cell cont. Hf	125	152	62	66	129	89	115	104
	CMT Hf	1	1	1	1	2	1	2	2
	Bact. Hf growth								Sr
331	Cell cont. Hf	53	158	72	88	248	129	224	121
	Hb	66	107	100	61	114	101	143	107
	CMT Hf	1	1	1	2	2	2	2	2
	Hb	1	1	1	2	2	2	3	2
	Bact. Hf growth Hb								
333	Cell cont. Hf	107	199	250	93	217	134	207	192
	Vb	.44	83	83	72	135	141	208	164
	Vf	106	184	167	146	263	172	278	198
	CMT Hf	1	2	2	2	2	3	3	2
	Vb	1	2	2	2	2	3	3	2
	Vf	2	3	2	2	3	3	1	2
	Bact. Hf growth								
	Vb								
	Vf								
335	Cell cont. Vb	265	456	316	434	605	251	280	240
	CMT Vb	2	3	2	2	3	3	1	2
	Bact. Vb growth								Sr

am=morning Hf=right forward teat Vb=left rear teat

pm=afternoon Hb-right rear teat Vf=left forward teat

Cell cont=counted in 1000;CMT=California Mastitis Test;Bact.growth,Sr=Streptococcus agalactiae,S=Staphylococcus spp.;E=Ethacilin;

Table 8 cont.

Table 8 cont.

	Day	5		6	
	Time of exp.	pm	am	pm	
Cow #					
306	Cell cont. Hf	12055	38455		
	CMT Hf	5	5		
	Bact. Hf growth	Sr	Sr		
331	Cell cont. Hf	208	148	271	143
	Hb	187	114	136	118
	CMT Hf	1	2	3	
	Hb	2	2	2	
	Bact. Hf growth Hb				
333	Cell cont. Hf	258	179	326	195
	Vb	288	118	147	81
	Vf	278	137	237	94
	CMT Hf	3	2	3	
	Vb	2	2	2	
	Vf	2	2	2	
	Bact. Hf growth Vb				
	Vf				
335	Cell cont. Vb	7997	23298		
	CMT Vb	5	5		
	Bact. Vb growth	Sr	Sr		

am=morning Hf=right forward teat Vb=left rear teat

pm=afternoon Hb=right rear teat Vf=left forward teat

Cell cont=counted in 1000;CMT=California Mastitis Test;Bact.growth,Sr=Streptococcus agalactiae,S=Staphylococcus spp.;E=Ethacilin;

CLAIMS

1. Use of a composition comprising chitosan in combination with a polysaccharide selected from heparin, heparan sulphate and dextran sulphate, as an active component in a solvent, for the manufacture of a teat dipping solution for lactating animals, particularly cows,.

2. Use according to claim 1, characterized in that the composition comprises chitosan and said polysaccharide in such quantities that the solution when applied results in a bacterial barrier or bacteriostatic effect.

3. Use according to claim 1, characterized in that the composition comprises chitosan and polysaccharide in such quantities that the solution when applied results in a wound healing effect.

4. Use according to claim 1, characterized in that the composition comprises chitosan and polysaccharide in such quantities that the solution when applied results in a prophylactic effect against mastitis.

5. Use according to any preceding claim, characterized in that the total concentration of chitosan plus polysaccharide in the solution lies within the range 0.2-10, particularly 0.2-5, for example 0.25-3 percent by weight, based on the weight of the solution.

6. Use according to any preceding claim, characterized in that the weight ratio chitosan:polysaccharide lies within the range 100:1 - 10:1.

7. Use according to claim 6, characterized in that said weight ratio lies within the range 60:1 - 15:1.

8. Use according to any preceding claim, characterized in that the polysaccharide is dextran sulphate.

9. Use according to any preceding claim, characterized in that said chitosan has a degree of N-acetylation of at most about 90%.

10. Use according to claim 9, characterized in that said degree of N-acetylation is at most about 50%, preferably at most about 25%.

11. Use according to any preceding claim, characterized in that the solvent is water based, preferably water.

12. Use according to any preceding claim, characterized in that the solution contains a viscosity controlling agent selected from cellulose derivatives, particularly methyl cellulose, and glycerol, particularly in such quantities that the solution is applicable by dipping or spraying.

13. A process for prophylactic or wound healing treatment of lactating animals, particularly cows, against mastitis, where a composition comprising chitosan in combination with a polysaccharide selected from heparin, heparan sulphate and dextran sulphate, as an active component in a solvent, is applied on the teats of said animals in a quantity effective for preventing mastitis.

14. A process according to claim 13, characterized in that the composition is as defined in anyone of claims 5 - 12.

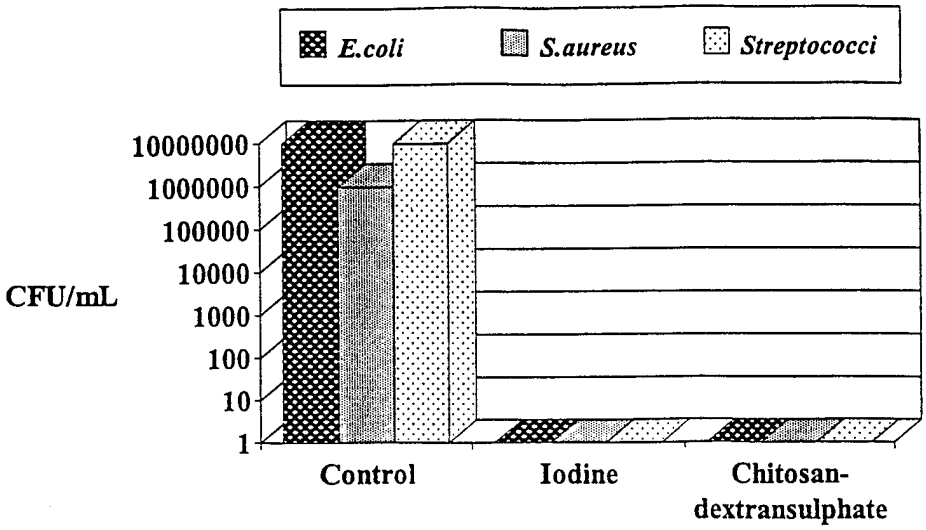


Figure 1. Bacterial growth beneath chitosan-dextran sulphate film
Control=Untreated agar plate
Chitosan-dextran sulphate=1.0% chitosan + 0.02% dextran sulphate + 0.1% methyl cellulose + 0.5% glycerol.
Iodine=Commercial dipping agent containing iodine
Streptococcus=Strept.agalactiae, Strept. dysgalactiae, Strept.uberis

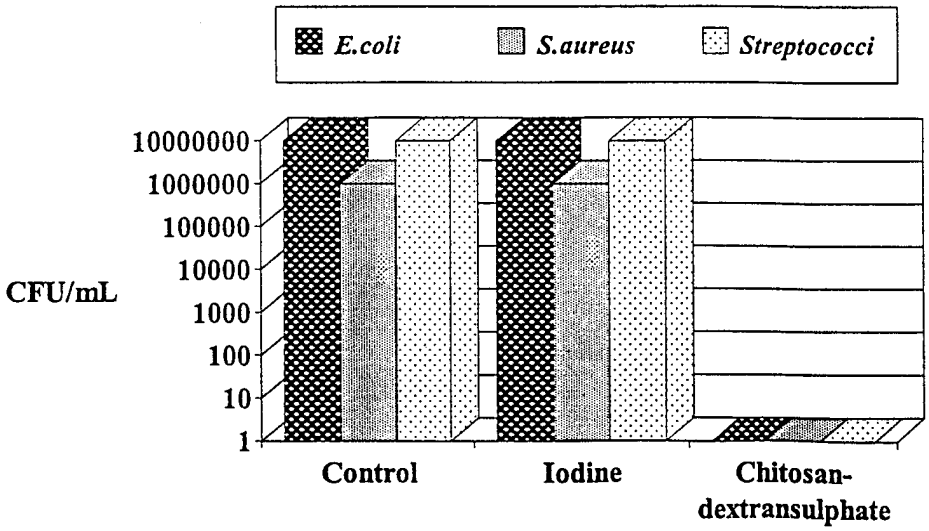


Figure 2. Bacterial growth on top of chitosan-dextran sulphate film
Control=Untreated agar plate
Chitosan-dextran sulphate=1.0% chitosan + 0.02% dextran sulphate + 0.1% methyl cellulose + 0.5% glycerol.
Iodine=Commercial dipping agent containing iodine
Streptococcus=Strept.agalactiae, Strept. dysgalactiae, Strept.uberis

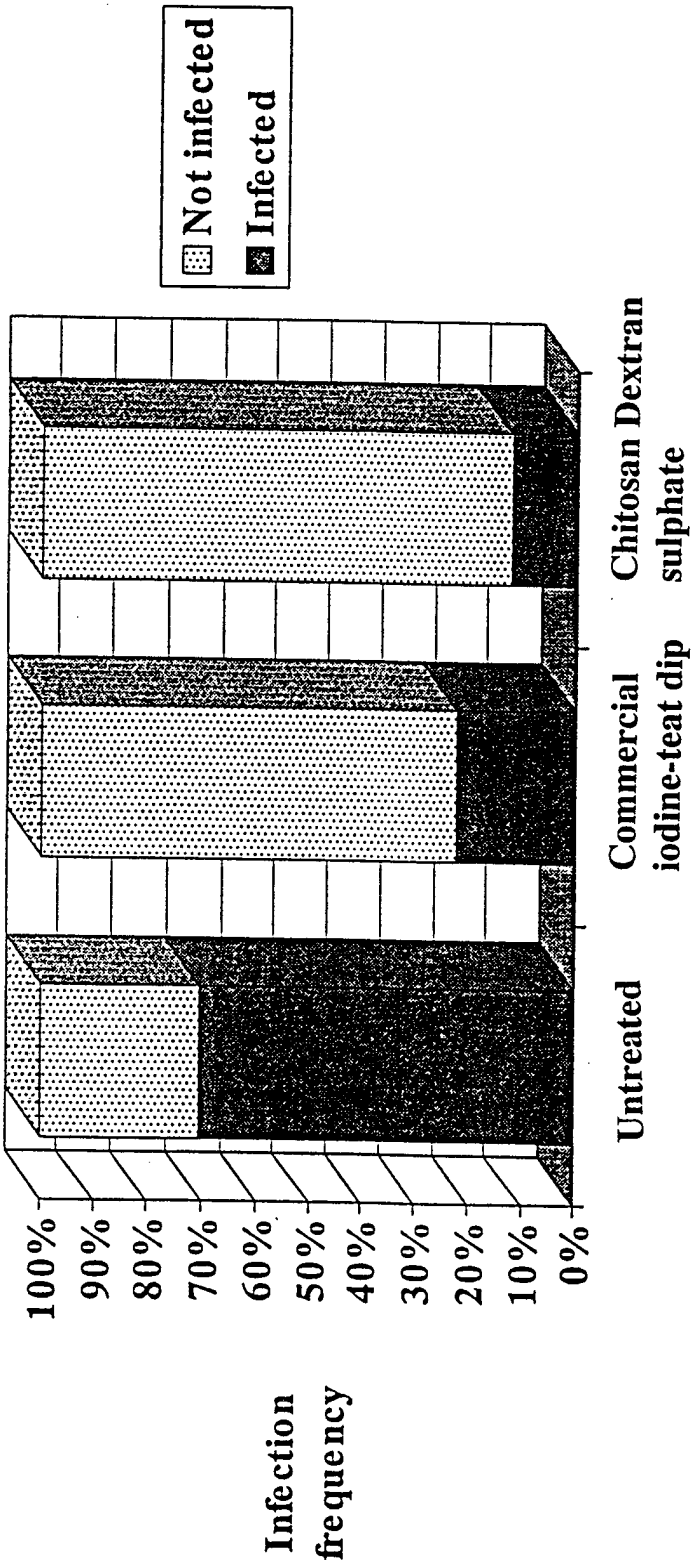


Figure 3. Infection frequency of untreated, chitosan-dextran sulphate treated and iodine treated udder parts.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/00776

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A01N 43/16, A61K 7/48, A61K 31/725, A61K 31/73
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A01N, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA, WPI, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9602260 A1 (MEDICARB AB), 1 February 1996 (01.02.96) --	1-12
A	WO 9530403 A1 (LARM, OLLE), 16 November 1995 (16.11.95), page 12, line 30 - page 14, line 33, the claims -- --------	1-12

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 July 1998	08-07-1998
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Gerd Strandell Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/00776

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 13,14
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

09/06/98

International application No.
PCT/SE 98/00776

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9602260 A1	01/02/96	AU 691322 B	14/05/98
		AU 3090295 A	16/02/96
		CA 2194976 A	01/02/96
		EP 0759760 A	05/03/97
		JP 10502665 T	10/03/98
		SE 9402531 A	20/01/96
WO 9530403 A1	16/11/95	AU 687560 B	26/02/98
		AU 2458595 A	29/11/95
		EP 0758223 A	19/02/97
		JP 9512818 T	22/12/97
		SE 9401540 A	05/11/95
		US 5711938 A	27/01/98