THE DISINFECTION OF URINE.¹

(*From the Clinical Laboratory of the Dresden Veterinary College.*)

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NOTWITHSTANDING the numerous works that have appeared within the last ten years on disinfection, only a very small number relate to the disinfection of excreta. In medical literature there are a few articles on the disinfection of human fæces (Liborius, Sternberg, and more recently V. Gerlóczy), but these refer only to the sterilising of excreta and cesspool contents, without special respect to particular species of pathogenic bacteria. No special articles of this nature are known to us in veterinary literature.

At the instigation of Professor Siedamgrotzky we instituted a series of disinfection experiments with stall urine, principally with respect to the following points:—

1. To ascertain the richness of different urines in micro-organisms.
2. Cultivation experiments with pathogenic micro-organisms in fresh non-sterilised urine.
3. Cultivation experiments with pathogenic micro-organisms in sterilised urine.
4. Disinfection experiments.
 - (a) Sterilisation of fresh urine.
 - (b) Disinfection of pure cultures of pathogenic micro-organisms in sterilised urine.

In our investigations we employed urine taken from the urine tanks of various milk establishments in and around Dresden. Partly we obtained pure cow urine, as from a Dresden milk-cure establishment, partly mixed urine, where the locality had only a common receptacle, and the sample contained also pigs' urine. We did not succeed in obtaining pure pigs' urine. The specific gravity of the urine, which always smelled more or less strongly of sulphuretted hydrogen, varied from 1·010 to 1·015 (ascertained with the urometer). The reaction in all cases was strongly alkaline.

The proportion of micro-organisms in the various samples was exceedingly high, as plate cultures showed.

With a view to the qualitative recognition of the germs in single samples of urine, 1 oese² of the urine to be examined was mixed as uniformly as possible with 5 ccm. of liquefied sterilised gelatine; from this dilution a second was prepared by transferring a drop of it to a fresh tube of gelatine, and in like manner a third. Each of the tubes was poured out on a plate, and all three were left together for three days in a moist chamber at summer temperature.

The number of species of micro-organisms that developed in the separate plates was strikingly low, and repeated itself with great regularity in the different kinds of urine. There constantly appeared: large non-liquefying (gelatine) diplococci, bacillus subtilis, liquefying small cocci, and a number of yellow and white torulæ. The proportion of moulds was not constant; in a few samples there were none, in others they were sparing, and they were never in striking number.

¹ Translated from the *Archiv für wissenschaft. und praktische Thierheilkunde*, 1890.

² [An oese is an inoculating needle (platinum wire) with a small loop turned on the free end of it.—Ed.]

The bacteriological information brought to light in connection with one sample was interesting. This came from a cow byre five weeks after the last emptying of the receptacle. Here in the plate cultures there appeared liquefying, intensely white, sharply circumscribed colonies, which were composed of small cocci. When transferred to stab-cultures an air bubble formed at the point of puncture, as in cultures of the staphylococcus pyogenes aureus and the micrococcus ascoformans. Subsequently liquefaction of the gelatine set in in the form of a blunt cone with the smaller end directed downwards. In the lowest part of the liquefied gelatine there formed a white, tough, mucus-like precipitate, composed of small cocci. When transferred to agar-agar the cocci grew with an intensely white colour. These cultures had a very close resemblance to those of the staphylococcus pyogenes albus; whether the two are identical, however, we leave undecided, although on inquiry at the place we ascertained that in the byre from which the urine was taken a cow had stood with a large abscess which continued to discharge pus after its opening.

The cultivation experiments with rothlauf bacilli and swine-plague bacteria in non-sterilised urine were attended with much difficulty. In natural conditions these pathogenic micro-organisms are brought to the urine tank with dung and urine in relatively large quantity, so that they can for a longer or shorter time successfully survive the struggle for existence with the budding and fission fungi present in the urine. In the limited conditions with test-tube experiments, the experiment can be carried out only by adding minimal quantities of the pathogenic microbes to the sample of urine, and after suitable periods ascertaining the development of the introduced organisms by means of plate cultures, in which the rothlauf colonies (*e.g.* by their characteristic mode of growth) are easily recognised.

There now exists the danger that the minimal quantities of the bacteria introduced may be overcome by the greater number of micro-organisms present, and that on control experiments with plates the rothlauf colonies may not be discoverable. Proportionally large quantities of the pathogenic bacteria are therefore necessary, in order to bring them to view in the control experiments. But there immediately arises the objection that the cultures appearing in the control experiments may have been directly derived from the bacteria inoculated—that the inoculated micro-organisms, without multiplying, to a certain extent persist in a latent stage after their introduction into the new nutritive medium (the urine), and that they first on re-transference to favourable soil (gelatine) again give evidence of their power of multiplication.

On these grounds we could not arrive at any precise results in this direction.

More simple and certain are cultivation experiments with pathogenic micro-organisms in sterilised urine. In these experiments there were employed rothlauf bacilli, bacteria of swine-plague, and the micrococcus ascoformans, the cultures of the latter having a short time previously been obtained in the clinique from a mykofibroma of the spermatic cord of a horse.

Sterilisation was effected by heating test-tubes with about 5 ccm. of urine for one hour on four successive days in a Koch's steam steriliser. On the day after the heating, as a proof, plate cultures were made, from which it appeared that the whole of the micro-

organisms had been destroyed, with the exception of an easily recognisable torula, which constantly appeared in all kinds of urine in greyish-white, non-liquefying colonies. The so sterilised urine tubes were prepared with minimal quantities of the above-mentioned micro-organisms, and left for three days at summer temperature. On the fourth day, after thorough shaking, a trace from each tube was transferred with a straight platinum needle to sterilised gelatine. In all of the gelatine tubes the cultures in question appeared abundantly in their characteristic form, with the exception of the cultures of the swine-plague bacteria, which sometimes showed only a trace of growth, but were never completely absent.

The experiments yielded proportionally the same result in the whole five samples of urine used.

After the growth of the rothlauf bacilli, swine-plague bacteria, and cocci of the mykofibroma had been recognised, at least in sterilised urine, the disinfection experiments were proceeded with.

The rothlauf bacilli and the bacteria of swine-plague have already been repeatedly examined here as regards their behaviour towards various disinfecting media. Caustic soda, freshly burned lime, sulphate of iron, chloride of lime, permanganate of potash, and tar were submitted to examination. Jaeger used soda lye of the specific gravity 1.084, prepared by dissolving crude caustic soda in hot water, according to the prescription for the disinfection procedure in infectious diseases of the domestic animals. In the second place he used solutions of chemically pure soda and potash in water, in the proportion of 1 : 100, and a similarly prepared potash solution 7.5 : 100, corresponding to the specific gravity 1.084. Jaeger's experiments showed that rothlauf bacilli were destroyed by these solutions of the specified strength. The soda solution was uncertain in its action on the bacteria of swine-plague in the strength of 1 : 100, but active, on the contrary, in stronger concentrations.

Lime also is a strong poison for these microbes, since the bacilli of swine rothlauf and swine-plague bacteria do not survive a single application of a mixture of lime and water 1 : 2.

In these experiments, which were also carried out by Jaeger, the micro-organisms were dried on silk threads, stretched on boards, painted with the lime wash, and then inoculated to susceptible animals. Sulphate of iron first destroyed these germs in a concentration of 1 : 3, certainly with almost momentary action. The experiments were carried out in the same way as the preceding.

For rothlauf bacilli and swine-plague bacteria chloride of lime proves especially fatal, since these were destroyed by a minute's action of a 1 : 100 milk of chloride of lime.

Bacteria of swine-plague dried on silk threads were destroyed by a single dipping of the threads into a 5 per cent. watery solution of permanganate of potash. Rothlauf bacilli were not certainly disinfected by the same medium. Also by a single painting with tar both species of bacteria were certainly destroyed.

The table on page 144 shows the above-mentioned results in a condensed form.

Some of the here cited media are very well suited for stall disinfection in these particular plagues; few, or rather only one—lime—could come into consideration in the case of urine; however, an

objection to this exists, since urine disinfected with lime would lose its manure, or rather its manurial value would be lost. In other words, there is brought about by the freshly burned lime a decomposition of the nitrogeous substances, and hence a slow but steady formation of ammonia, which passes into the atmospheric air, and is thus lost for plant nourishment. Caustic potash, chloride of lime, permanganate of potash, and tar could not come into consideration for the disinfection of urine, inasmuch as these media are in part too dear (permanganate of potash), in part they could act injuriously by their chemical properties (caustic soda and chloride of lime), and, thirdly, are inappropriate for the purpose named in consequence of their consistence (tar). The disinfectant strength of sulphate of iron has through numerous recent investigations been proved to be very slight, so that this medium was from the outset put aside. Sublimate also was put aside, and indeed on two grounds; first, it appeared hazardous to give to the public, even with the recommendation of the greatest care, such large quantities of sublimate as would obviously be required for the disinfection of urine; and secondly, it may be assumed that, owing to the strong alkalinity of the urine, and its richness in organic matters, the degree of concentration necessary for the desired action would require to be very high. This suspicion is corroborated by the since published observation of Gerlóczy, who could not disinfect cesspool contents even with a strength of 1 : 120 and forty-eight hours' exposure.

TABULAR REPRESENTATION OF THE RESULTS MENTIONED (p. 143).

<i>Disinfectant.</i>	<i>Minimum Exposure.</i>	<i>Minimum Concentration.</i>	<i>Result with Rothlauf Bacilli.</i>	<i>Result with Swine-Plague Bacteria.</i>
Potash Lye	3 ×	1'084 sp. g.	+	+
Lime	1 ×	1 : 2	+	+
Sulphate of Iron . .	1 ×	33⅓ p.c.	+	+
Chloride of Lime . .	1 M.	1 p.c.	+	+
Permanganate of Potash	1 ×	5 p.c.	—	+
Tar	1 ×	...	+	+

The mark × indicates so many times dipping into or painting with the disinfecting fluid.

On the suggestion of Professor Siedamgrotzky, we used in our disinfection experiments sulphate of copper. Although in recent disinfection experiments sulphate of copper has been most completely left without attention, it still in practice enjoys great confidence, especially by the owners of vineyards in Switzerland, the Tyrol, and Italy, and is used with great success for disinfection purposes against diseases attacking the vines.

Besides the sulphate of copper, we brought within the sphere of our investigations the mixture of crude carbolic acid and hydrochloric acid introduced by Laplace and found by Jaeger to be of high value.

In the sterilisation experiments it has already been seen that organisms of a very resistant kind were present in the urine. It was, therefore, to be assumed that relatively large quantities of the disinfecting medium would be required for the complete sterilising of the urine.

On this ground also we believed that we might neglect the otherwise customary principle—to effect the sterilisation by the shortest possible exposure to the action of the disinfecting medium. By longer exposure to the action of a low degree of concentration of the disinfectant, sterilisation can be effected with the same certainty as by the shortest possible exposure to the action of a correspondingly higher degree of concentration. This distinction from a pecuniary point of view deserves decided consideration, especially since it may be quite immaterial whether the urine, when once the disinfectant has been added to it, remains in the place a day longer or not.

In our experiments the effect of the disinfectant was ascertained after 24 hours' exposure to its action.

The disinfection experiments with non-sterilised urine were carried out on a large and a small scale.

1. Test-tubes containing about 5 ccm. of non-sterilised urine were treated with so much sulphate of copper solution that the following concentrations were produced :—

$\frac{1}{4}$ per cent., $\frac{1}{2}$ per cent., 1 per cent., 2 per cent., 3 per cent.,
4 per cent., 5 per cent.

Immediately after the addition of the sulphate of copper the previously brownish-yellow urine assumed an almost blackish-brown colour. Numerous dense blackish-brown flocculi were suspended in the fluid. After some hours these had collected at the bottom as a dark brownish-black deposit, over which stood a clear fluid, betraying by its bluish colour the presence of the sulphate of copper.

At the end of 24 hours, after previous shaking, 1 oese was taken out of each of the tubes with different proportions of copper, and transferred to sterile gelatine, which was then poured out on a plate. After four days the plates made from the tubes with 3, 4, and 5 per cent. of sulphate of copper showed themselves completely sterile, while the other plates, though only sparingly, exhibited well developed cultures of the bacillus subtilis, and some liquefying and non-liquefying species of torulæ.

The addition of 3 per cent. of sulphate of copper, therefore, suffices to kill in 24 hours all the micro-organisms present in the urine.

With Laplace's mixture (8 parts of a 50 % crude carbolic acid, 2 parts of crude hydrochloric acid, and 90 parts of water) experiments were made in the same way, and with the following concentrations :—
0.67 per cent., 1 per cent., 1.3 per cent., 1.5 per cent., 2 per cent., 3 per cent.

The control experiments made after 24 hours showed that complete sterility of the urine was first attained with 2 per cent. and 3 per cent. of the carbolic acid.

2. Experiments on a larger scale, and only with sulphate of copper, were carried out by preparing large glass vessels with 4 litres each of fresh non-sterilised urine. Solution of sulphate of copper sufficient to

produce strengths of 0·25, 0·5, and 1 per cent. was added. Here also the urine showed the same changes as in the experiments in test-tubes. The control experiments, made after 24 hours, showed that here also a complete sterilisation had not been effected with any of the concentrations used. This was first attained in an experiment made with 3 per cent. of sulphate of copper.

For practical purposes these experiments are of no importance, since they had not for their object any pathogenic organism of epizootic importance, but only the micro-organisms occurring in urine under ordinary circumstances.

However, the correspondence with the experiments of V. Gerlóczy is interesting. He was able to sterilise cesspool contents with sulphate of copper only in the strength of 1 : 35 (2·8 %) after 48 hours' exposure, while he was not successful with the reagents accredited by others as disinfectants of great intensity, since, as already mentioned, even sublimate of the strength of 1 : 120 (·833 %) failed. At the time of our experiments V. Gerlóczy's investigations were unknown to us.

The principal value of our experiments lay in the behaviour of our two media towards pure cultures of rothlauf bacilli and swine-plague bacteria in sterilised urine.

The above mentioned experiments have shown that rothlauf bacilli and swine-plague bacteria find a suitable soil in sterilised urine. About 5 ccm. of the different samples of urine were sterilised in test-tubes, by heating for one hour daily for four successive days in a Koch's steam steriliser. On the day after the last heating a trace of each pure culture was brought with a straight platinum needle into the test-tubes. The cultures were allowed to stand for three days at summer temperature, and on the fourth day, after thorough shaking, a trace from each tube was transferred with a straight platinum needle into sterilised gelatine. When this control experiment gave a positive result, enough of the disinfecting medium to produce the desired concentration was added to the urine cultures.

1. EXPERIMENTS WITH SULPHATE OF COPPER.

In the manner indicated, enough sulphate of copper was added to rothlauf and swine-plague cultures to produce the following proportions :—

0·25 per cent., 0·5 per cent., 1 per cent., 2 per cent.,
3 per cent., 4 per cent., 5 per cent.

The urine passed through the changes already described. At the end of 24 hours, after thorough shaking of each tube, control experiments were made by transferring 1 oese into sterile gelatine. It was thus shown that all the above concentrations sufficed to hinder the growth of the micro-organisms in question, and by means of plate cultures it was also shown that even the micro-organisms introduced into the urine had lost their power to develop when re-transferred to a suitable soil.

One quarter per cent of sulphate of copper added to urine is thus sufficient to destroy any rothlauf bacilli and swine-plague bacteria present in the latter. Since in veterinary police the important object to be attained is the complete destruction not of all the micro-organisms present in the infected urine, but only of the pathogenic

germs, the strength indicated is quite sufficient for the purposes of sanitary police.

2. EXPERIMENTS WITH CRUDE CARBOLIC ACID WITH THE ADDITION OF CRUDE HYDROCHLORIC ACID (Laplace's Mixture).

After preparation of pure cultures of rothlauf bacilli and swine-plague bacteria in sterilised urine, and when the control experiments had yielded a positive result, disinfection experiments with Laplace's mixture were repeated, with the following concentrations :—

0·25 per cent., 0·67 per cent., 1 per cent., 1·3 per cent., 1·5 per cent.,
2 per cent., 3 per cent.

Here, also, after 24 hours the whole of the experiments yielded the same results, the desired disinfection being obtained in all except with the $\frac{1}{4}$ % solution, in whose control gelatine tube a distinct growth of rothlauf bacilli in the characteristic bottle-brush form was observable after four days.

The whole of the experiments were frequently repeated, and always with the same result.

To this series of experiments there was added a parallel series which had for object to test in the same way the action of the same disinfectants on pure cultures of the micrococcus ascoformans in sterilised urine. The result was the same as in the experiments with rothlauf bacilli and swine-plague bacteria.

The cost of disinfecting urine with the above-named disinfectants is comparatively slight. For carbolic acid, whose price is the same as that of sulphate of copper, the conditions are rather less favourable than for the latter, since it must be used in stronger concentration ($\frac{2}{3}$ per cent.), and its use is made still more expensive through the necessary combination with raw hydrochloric acid. The cost of disinfection with Laplace's mixture will, therefore, amount to three times as much as with sulphate of copper. When sulphate of copper is employed in practice for disinfection, care must be taken that the place is secluded from all animals, especially from poultry, or otherwise poisoning must certainly be expected, as has already been observed in fowls from the use of sulphate of iron for the same purpose.

THE OCCURRENCE OF TUMOURS IN THE DOMESTICATED ANIMALS.

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(Continued from page 45.)

TUMOUR XI. *Carcinoma of the Penis of a Horse*.—This specimen was forwarded by Mr Burnett of Maybole, who furnishes the following history: "Some time ago I was called to see this pony (a gelding), and on examination I found that 'matter' was running from his prepuce. Having syringed out the cavity of the prepuce, I drew out the penis, and found that the extremity of the latter presented what