IMPACT OF DISINFECTANT WATER TREATMENT FOR PIGS, POULTRY AND RABBITS ON THE STABILITY OF ANTIBIOTICS

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ABSTRACT

In farms, drinking water is frequently treated with a disinfectant to improve its bacteriological quality. This water can also be used to administer collective treatments for animals. However, the verification of compatibility between biocides and medicines is not required in the Marketing Authorization dossiers. This study aims to evaluate the impact of biocides on the stability of antibiotics. Ten veterinary medicinal products (VMPs) containing doxycycline, amoxicillin, sulphonamides-trimethoprim, tiamulin and colistin were tested with two biocides (hydrogen peroxide-H_2O_2 at 50 ppm and sodium hypochlorite at 0.5 ppm of active chlorine) in two standardized waters, a soft one (6 ° f, pH = 6) and a hard one (35 ° f, pH = 8). Then, VMPs containing amoxicillin, tiamulin and doxycycline were diluted with H_2O_2 in water from a well rich in iron and manganese. Antibiotics were dosed by UV-Liquid Chromatography at different defined times in a stock solution and in a 1:20 diluted solution to simulate an administration by a dosing pump or a tank. For each analysis, the stability of a substance was considered insufficient if its average concentration was more than 10% lower than that of the control sample without biocide and if the difference was significant (T-test, $p < 0.05$). Hydrogen peroxide impacted the stability of both amoxicillin VMPs in the hard water, only one amoxicillin VMP in the soft water and one doxycycline VMP in the well water. Chlorine degraded colistin in soft water and all VMPs in hard water except sulphonamides. This study confirms the impact of disinfectants on the stability of some antibiotics in the water and demonstrates the multifactorial and complex nature of this stability.

Keywords: antibiotics, biocides, sodium hypochlorite, hydrogen peroxide, water quality

INTRODUCTION

In farms, drinking water is frequently treated with a disinfectant biocide to improve its bacteriological quality. Water is also used as a drug support for the collective treatment of animals. However, compatibility verification between biocides and medicinal products is not currently required in Marketing Authorisation (MA) dossiers. Disinfecting biocides are widely known to react with organic chemicals in water (Postigo and Richardson, 2014). Their presence in water may induce the degradation of many pharmaceutical compounds during disinfection processes in drinking water production (Mompelat *et al.* 2009, Acero *et al.* 2010, Chamberlain and Adams, 2006). Thus the possibility and level of degradation of veterinary medicinal products (VMPs) administered in drinking water (notably antibiotics) when preparing medicated solutions on farms because of contact with usual biocides need to be assessed. The impact on the dose effectively ingested by the target species and the risk of antimicrobial resistance may not be negligible.

This study aims to assess or confirm the impact of water disinfecting biocides (including hydrogen peroxide) on the stability of seven usual antibiotics, in order to complete the results previously obtained by Hémonic *et al.* 2017, which demonstrated that all the six tested antibiotics remained stable in contact with sodium hypochlorite whereas they had all been degraded, to a greater or lesser degree, in the electrolysed water.

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MATERIALS AND METHODS

The study was carried out with standard laboratory water according to the European guideline (EMEA, 2005).

Seven active substances were selected on basis of sales volumes in 2015 (Anses-ANMV, 2016) their use in drinking water in at least two of three food producing animal sectors considered and their interest in veterinary medicine: doxycycline, amoxicillin, sulfadiazine and sulfadimethoxine combined with trimethoprim, tiamulin and colistin. For each active substance, two veterinary medicinal products (VMP1 and VMP2) were tested, except for sulfadiazine and sulfadimethoxine where only one medicinal product was tested. The choice of VMPs was made in order to study various formulations (liquid, powder) and excipients with a good representativeness of the different MA holders concerned and the animal production sectors studied.

The antibiotics have been diluted in water according to the dosage defined in the Summary of Product Characteristics (SPC) of each VMP.

Two biocides were selected according to usual husbandry practices: hydrogen peroxide (H_2O_2) at 50 ppm, stabilized with orthophosphoric acid and sodium hypochlorite (NaClO) at 0.5 ppm active chlorine.

Antibiotics and biocides were diluted in two standard types of water: soft acidic water (pH = 6, hardness = 6 °f) and hard basic water (pH = 8, hardness = 35 °f).

The first test compared the concentration of antibiotics in a concentrated stock solution, with and without biocide. The antibiotics were measured at the time the stock solution was prepared (T0), six hours later (T6) and 24 hours later (T24). The 24 hours is the maximum recommended storage time of the drug solution in the stock solution tank of a dosing pump (EMA/CVMP/QWP/540/03-rev01). The second test compared the concentration of antibiotics in 1:20 dilution, with and without biocide, to simulate administration by a dosing pump set at 5%. Antibiotic dosing was carried out at the time of dilution of the stock solution (T0') and six hours later (T6'), to represent the time of water circulation in the pipes between the dosing pump and the drinkers. Dilution and dosing also took place at T24' and T30'. T24' corresponds to the time when the stock solution, already kept for 24 hours in the tank, is diluted to one-twentieth to be distributed in the pipes. Then the antibiotic is dosed six hours later at T30'. This last case therefore represents a situation of supposed maximum degradation of the active substance. The analyses were carried out by Liquid Chromatography coupled with a UV detector. At each deadline, all the assays were carried out in duplicate. The stability of the VMPs was assessed by comparing the mean concentrations [Cmean] of the active substance(s) present in the samples with the biocide to the mean concentration in the control samples without biocide:

Stability in % = 100 x (Antibiotic mean concentration in samples with biocide)/(Antibiotic mean concentration in samples without biocide)

The stability of an active substance that lost significantly (t-test, $p<0.05$) more than 10 % of its concentration compared to the control sample was considered as being significantly degraded by the presence of biocide in the drinking water. This threshold of 10 % loss corresponds to the maximum acceptable in marketing authorisation dossiers (EMA/CVMP/846/99-rev01).

RESULTS AND DISCUSSION

Amoxicillin was the only antibiotic sensitive to the action of hydrogen peroxide (Table 1), confirming the result of Hémonic *et al.* ,2017. This sensitivity presented a "VMP-dependent" effect: VMP1 was more resistant than VMP2 to this oxidative action, probably due to a difference in excipients.

Chlorination only impacted the two colistin specialties in soft water (between 73% and 83 % of average substance remaining in diluted solutions), whereas in hard water it impacted the eight VMPs containing amoxicillin, doxycycline, colistin and tiamulin (between 24% and 90% of average substances remaining during the 30 hours experiment period). This effect is certainly related to the three times higher concentration of total chlorine in hard and basic water (pH = 8; 35°f) than in soft and acidic water (pH = 6; 6°f) needed to add in solutions in order to obtain for both types (*i.e.* acidic and basic solution) solutions with 0.5 ppm of active chlorine (i.e. HOCl). Indeed, depending on the pH of the solution, hypochlorous acid may undergo more or less partial dissociation to produce hypochlorite ions (ClO^-). Hypochlorous acid is much more chemically active than hypochlorite ions.

Table 1: Percentage recovery of average active substance concentrations of the veterinary medicinal products (VMPs) tested in water with biocide compared to the average concentration in water without biocide(use of standard laboratory water)

				Doxycylin		Amoxicillin		Sulfadiazine -Trimethoprim VMP 1		Sulfadimethoxine -Trimethoprim VMP 2		Tiamulin		Colistin	
				VMP 1	VMP 2	VMP 1	VMP 2	SDA	TMP	SDX	TMP	VMP 1	VMP 2	VMP 1	VMP 2
H_2O_2 at 50 ppm	Soft water (pH=6, 6°f)	Stock concentrated solution	T0	97,1	94,9	97,6	103,1	96,8	98,1	92,9	91,9	101,9	106	98,8	98,7
			T6	100,2	102,9	99,1	**72,4**	ND	ND	105	98,2	101,6	98,7	97,7	100,6
			T24	96,7	103,7	101,2	**67,2**	97,4	98,3	85,4	86	100,6	99,1	101,1	100,4
		Diluted (1:20) solution (id pipes)	T0'	95	105,9	97,4	99,6	100,7	101,9	99,1	99,5	100,9	102	95,3	98,8
			T6'	94,8	99,5	96,5	**51,5**	99,3	99,7	88,9	87,4	100,1	107	97,1	98,5
			T24'	97	97,3	96,9	**66,7**	100,4	100,3	99,7	100,9	99,1	98	101,3	100,4
			T30'	97,2	97,2	93,6	**42,7**	99,4	100,7	95,7	93,4	98,4	101,5	103,7	100,2
	Hard water (pH=8, 35°f)	Stock concentrated solution	T0	103,1	100,3	96,6	95,7	96,8	95,2	101,2	100,6	101,8	98,4	98	99,7
			T6	100	99,6	95,4	**68,2**	98,2	98,1	99,8	93,2	101,4	100,3	99,9	100,6
			T24	101,7	101,5	**88**	**70,2**	100,2	100,3	99,7	99,6	101,5	99,2	100,6	99,7
		Diluted (1:20) solution (id pipes)	T0'	99,5	100,7	99,1	94,8	92,8	91,2	88,6	89,3	96,3	100,5	100,4	99,6
			T6'	99,3	98,7	**66,1**	**33,9**	100	101,3	101,4	101,7	93,6	101,3	100,3	98,6
			T24'	98,7	99,7	**88,2**	**65,4**	98,9	99	97,6	97,5	99	107,9	100,3	99,7
			T30'	104	99,6	**61,4**	**27,2**	100,2	101,2	98,7	97,6	100	101,5	100,9	99,5
HOCl at 50 ppm of active chlorine	Soft water (pH=6, 6°f)	Stock concentrated solution	T0	98,6	96,3	95,3	101,1	100,6	101,1	93,6	91,7	97,8	99,3	104,2	98,8
			T6	98,9	93,1	100,1	95,3	102,9	102,7	91,8	92,1	99	96,7	96,1	100,1
			T24	97,6	87,9	100,3	93,6	101,9	100,7	100,8	98	100,2	98	98,8	99,1
		Diluted (1:20) solution (id pipes)	T0'	96	95,5	94,7	98,6	96,9	96,6	102,5	103,7	91,1	92	87,8	**74,9**
			T6'	98,3	94,6	95,2	98,6	98,2	97,2	84,8	84,6	97	91,7	**82,7**	**73,3**
			T24'	93,9	94,6	97,2	96,5	98,8	100	99,6	100,8	98,1	92,8	**81,5**	**73,7**
			T30'	95,5	94,1	101,1	97,4	96,6	97,1	97,2	95,2	95,7	93,8	**79,9**	**73,5**
	Hard water (pH=8, 35°f)	Stock concentrated solution	T0	99,4	91,8	105,6	99,1	97,9	98	106,7	106,4	99,3	98,2	101,3	**89,7**
			T6	99,1	97	101,7	98,3	94,7	94,5	110,2	99,1	99,6	100	99,2	**89,8**
			T24	99,6	98	104,3	103,2	93,5	94,4	101,5	101,2	100,5	98,1	ND	**89,7**
		Diluted (1:20) solution (id pipes)	T0'	**87,7**	**86,5**	88,4	90,1	94,6	98	99,6	99,9	90,8	**63,4**	**84,3**	**24,3**
			T6'	**87,8**	**83,6**	**86,3**	**87,5**	100,2	103,5	91,2	92,3	91,9	**63,2**	**75,5**	**29,6**
			T24'	**88,9**	85,9	91,9	91,1	88,5	105,2	98,6	102,9	90,8	**68,9**	**65,5**	**35,4**
			T30'	**88,5**	84,5	90,7	**89,8**	101	104,6	93,9	104,1	**85,1**	**70,2**	**64,5**	**33,6**

Coloured boxes mean that the antibiotic has lost more than 10% of its concentration compared to the control sample without biocide and that this difference is significant (p < 0.05). For example, a result of 67 % means that the antibiotic is only 67 % of the expected concentration compared to the control sample: it has lost 33 % of its concentration in the presence of the biocide.

The fraction of chlorine present as hypochlorous acid decreases with increasing pH, i.e. active chlorine (HOCl) is not very active and preponderant for pH > 7.5 and it is very active and preponderant for pH between 4 and 7.5. To obtain 0.5 ppm of active chlorine in the basic water samples, sodium hypochlorite therefore had to be incorporated at a level three times higher than in acidic water. However, the addition of the VMPs in the basic water caused a decrease in pH and therefore an increase in the level of active chlorine to levels much higher than 0.5 ppm, leading to increased degradation of the antibiotics in basic and hard water compared to acidic and soft water. In livestock farming, this problem should not theoretically occur: sodium hypochlorite is not the recommended method for disinfecting basic and hard water, precisely because it is necessary to incorporate doses that are much too high to have a result equivalent to that obtained in acidic water. Nevertheless, regardless of the type of water used in livestock farming, vigilance and controls will be necessary to ensure that the level of active chlorine does not exceed the recommendation of 0.5 ppm to avoid impacting the stability of the antibiotics. This threshold of 0.5 ppm may be exceeded in particular in the event of error in the rate of chlorine incorporated, during water electrolysis or during water

acidification (voluntary incorporation of organic acids into the water circuit to prevent digestive problems or use of certain medicines that lower the pH).
Furthermore, the degradation of 17 to 27% of colistin in diluted solution in chlorinated fresh water is a result that differs from the conclusions of the study by Hémonic *et al.*, 2017, which didn't detect colistin degradation. Nevertheless, the protocol was different because the antibiotic dosage in the diluted solution was carried out only once at a time equivalent to T1'. In this case, VMP1 was significantly degraded over the 6-hour interval after dilution (T6') but may still be stable after 1 hour (T1' assay not performed), which would then be consistent with the result of Hémonic *et al.* 2017. Another possible explanation is a difference between the VMPs studied: the "VMP-dependent" effect is well demonstrated here with VMP2 which was slightly more sensitive to chlorine than VMP1. However, as the VMPs were anonymmed in both studies, it is not possible to verify whether they were identical or different.

In the end, only the two sulphonamide-trimethoprim VMP remained stable whatever the conditions tested (type of water, nature of the biocide, concentration of active substance). In particular, they were the only ones that were not affected by the effect of chlorine in hard water. One possible explanation is that their dilution did not cause any decrease in the pH of the solution, unlike the other VMPs.

CONCLUSION

This study confirms the impact of disinfectants on the stability of certain antibiotics and VMPs in water and demonstrates the multifactorial and complex nature of this stability. Hydrogen peroxide impacted the stability of two amoxicillin VMPs in hard water and one amoxicillin VMP in soft water. Chlorine degraded colistin in soft water and all VMPs in hard water except for sulphonamides. The work carried out in this project will enable the French authorities to make recommendations in the framework of the new European regulations to define measures to ensure the safe and effective use of orally administered medicines in the presence of biocides. These will be general recommendations because only two VMPs per active substance were tested in this study, so the results obtained cannot be extrapolated to all VMPs containing the same active substance. Then, in the long term, the objective is that the verification of compatibility between the main biocides and VMPs should be required in marketing authorisation dossiers, both for antibiotics, but also for other VMPs administered to animals through the drinking water (anti-inflammatories, antiparasitic agents, etc.).

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