

# Results of Bacterial Culture and Sensitivity Testing From Nasolacrimal Duct Flushes in One Hundred and Three Both Healthy and Clinically Ill Pet Rabbits (*Oryctolagus Cuniculus*)

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## ABSTRACT

This study attempts to describe the bacterial nature and sensitivities of aerobic cultures from nasolacrimal duct (NLD) flushes in both healthy and clinically ill rabbits presenting nasal and ocular discharge to help effective treatment. The records of 83 pet rabbits presenting clinical signs (Clinical

Signs Group: CSG) and of 20 control pet rabbits with no clinical signs (NCSG : Non Clinical Signs Group) were evaluated.

The percentage of culture yielding no bacteria in control healthy rabbits group (25.9% of records) and in the group of rabbits with clinical signs (30% of records) is higher than expected in that the NLD environment is not sterile. Numerous bacterial organisms were isolated (26). The CSG didn't show any different bacteria than those found in the NCSG. Organisms were

**Table I:** Repartition of the 85 records of the CSG, according to age and clinical signs

ND: Nasal discharge

OD: Ocular discharge

ND/OD: Nasal and ocular discharge

	Age (year)	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	Total
Clinical signs														
ND		11	8	3	4	3	4	0	0	0	0	1	0	34
OD		3	5	4	5	8	7	2	1	1	1	0	0	37
ND/OD		2	1	1	2	3	0	2	1	0	0	1	1	14
<b>Total</b>		<b>16</b>	<b>14</b>	<b>8</b>	<b>11</b>	<b>14</b>	<b>11</b>	<b>4</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>1</b>	<b>85</b>

categorized as to potential pathogenicity, and typical site of isolation in four categories: *Pasteurella* species, common bacteria of the GI tract, bacteria usually present on skin and mucosa and ubiquitous bacteria from the environment. The commensal GI tract bacteria could have colonized the NLD or may have been collected, while the fluid was passing from the nares over the upper lips, since rabbits ingest their cecotrophs. Ubiquitous bacteria could have been present in the NLD, but could also probably be present on the skin or come from an external contamination during sampling. Among the CSG, *Pasteurella multocida* was the most commonly isolated microorganism (34.8% of the total number of bacterial isolates), with a significant difference in juvenile rabbits. There was no significant difference between the percentage of *Pasteurella multocida* cultured in the NCSG and in the CSG. *Enterobacter cloacae* (10.1%) and *Pseudomonas aeruginosa* (8.7%) were consistent findings that can behave as opportunistic pathogens of clinical relevance. There was no significant difference in percentage of the four different bacterial categories among the groups showing various clinical signs. The sensitivity tests were consistent with the typical sensitivities of the bacteria that were isolated.

Based on the majority of organisms cultured in the present study and their sensitivity panels, empiric choices of antibiotics include sulfonamides or quinolones.

## BACKGROUNDS

Nasal and ocular discharge are two of the most common clinical signs for rabbits presenting to the veterinarian.<sup>1</sup> Nasal discharge may be due to rhinitis, and/or may originate from the nasolacrimal duct. Primary disease of the nasolacrimal duct may include infection and inflammation. Chronic infection may result in stenosis.<sup>2,3</sup> Ocular discharge may be a part of rhinitis and upper respiratory disease complex; epiphora can also occur with obstructive disease of the nasolacrimal duct. Cannulation and flushing of the nasolacrimal duct is a common diagnostic procedure which can both alleviate the signs of disease and provide samples for bacterial culture.<sup>4</sup> Topical or parenteral antibiotic therapy is often used as therapy. This study attempts to describe the bacterial nature and sensitivities in both healthy and clinically ill rabbits to help guide effective treatment. To our knowledge, this is the first report of this type of data in a large group of pet rabbits.

Among the rabbits presenting clinical signs, *Pasteurella multocida* was the most commonly isolated microorganism, with a significant difference in juvenile rabbits. *Pseudomonas aeruginosa* and *Enterobacter cloacae* were consistent findings which can behave as opportunistic pathogens of clinical relevance.

## MATERIALS AND METHODS

The records of all pet rabbits brought to the Advetia Veterinary Hospital (Paris, France) between the years 2007 and 2012 were

**Table II:** No growth samples in all groups

NCSG: Non clinical sign group

CSG: Clinical sign group

ND: Nasal discharge

OD: Ocular discharge

ND/OD: Nasal and ocular discharge

Group	Total Rabbits	No Growth	Percent No Growth
NCSG	20	6	30
CSG	85	22	25.8
CSG-ND	34	8	23.5
CSG-OD	39	12	30.7
CSG-ND/OD	12	2	16.6

evaluated. Records were included in this study if the rabbit had a history of ocular discharge (OD) or nasal discharge (ND), and if a bacteriological assessment of the NLD flush was performed. Ocular and nasal discharge were identified as serous, mucoid, or purulent. Out of 2783 individual rabbits presented (1,530 males and 1,253 females), 85 records, including 83 individual rabbits (two of them were seen twice) fulfilled the above criteria. There were 54 males (six neutered) and 29 females (20 neutered) ranging in age from 4 months to 11 years of age. Age distribution is presented in Table I. This group of rabbits was called the CSG (Clinical Signs Group). This group was further divided into three subgroups:

- Rabbits presenting with nasal discharge only (Nasal Discharge Group: NDG, 34 records)
- Rabbits presenting ocular discharge only (Ocular Discharge Group: ODG, 37 records)
- Rabbit presenting both clinical signs (Nasal plus Ocular Discharge Group: ND/ODG, 14 records).

In addition, in order to determine the normal NLD flush flora, bacteriological sampling of NLD flushes were performed in 20 different individual pet rabbits: nine males (five intact, 4 neutered) and 11 females (five intact, six neutered), ranging in age from 1 to 10 years. These rabbits had no history of ocular and/or nasal discharge or

rhinitis and appeared to be clinically healthy. This control group was called NCSG (Non Clinical Signs Group).

In both groups, patients receiving any form of antibiotics therapy (ie, topical or parenteral) were withdrawn at least 2 weeks prior to the study. Most of the rabbits were conscious and manually restrained, but six rabbits in the CSG and nine rabbits in the control group were anesthetized for another procedure or because they did not tolerate the restraint. The rabbit nares and upper lips were cleaned with sterile saline for 15 seconds and operator hands were washed with a hydroalcoholic solution. One to two drops of an ocular anesthetic (tetracaine 1% eye drop unidose, TVM Lab, Lempdes, TVM, France), was instilled in both eyes 5 to 10 minutes before the flush was performed.

These drops were free of any preservative that could inhibit further bacterial growth. A sterile metallic irrigating cannula (19 G) was introduced in the lacrimal punctum and 2 to 4 ml of sterile saline was flushed. If the duct was fully or partially patent, the instilled fluid and contents of the duct exited through the nares and were collected in a sterile tube. A sterile swab was dipped in the fluid and sent to a veterinary bacteriological laboratory the same day in a medium suitable for aerobic culture. Aerobic culture, identification, and antibiotic sensitivity were performed by the same laboratory. Twenty-four samples were

**Table III:** Bacterial culture results from NLD flushes in NCSG and CSG  
 NCSG: Non clinical sign group  
 CSG: Clinical sign group

Bacterial organism	NCSG		CSG							
	Number of times cultured	Percentage (%) of total	ND + OD + ND/OD		ND		OD		ND/OD	
			Number of times cultured	% of total	Number of times cultured	% of total	Number of times cultured	% of total	Number of times cultured	% of total
<i>Pasteurella multocida</i>	2	10.5	20	28.9	12	41.3	6	21.4	2	16.7
<i>Pasteurella sp.</i>	0	0	4	5.8	2	6.9	2	7.1	0	0
<i>Pantoea sp.</i>	6	31.6	0	0	0	0	0	0	0	0
<i>Enterobacter cloacae</i>	3	15.8	7	10.1	2	6.9	2	7.1	3	25
<i>Enterobacter aerogenes</i>	1	5.2	0	0	0	0	0	0	0	0
<i>Pseudomonas aeruginosa</i>	1	5.2	4	5.8	2	6.9	2	7.1	0	0
<i>Pseudomonas putida</i>	0	0	2	2.9	1	3.4	1	3.6	0	0
<i>Streptococcus sp.</i>	0	0	2	2.9	2	6.9	0	0	0	0
<i>Streptococcus Group D</i>	0	0	2	2.9	0	0	2	7.1	0	0
<i>Streptococcus Group G</i>	0	0	1	1.4	0	0	1	3.6	0	0
<i>Staphylococcus coagulase negative</i>	0	0	4	5.8	2	6.9	1	3.6	1	8.3
<i>Staphylococcus xylosus</i>	0	0	1	1.4	0	0	0	0	1	8.3
<i>Bordetella bronchiseptica</i>	0	0	4	5.8	1	3.4	2	7.1	1	8.3
<i>Moraxella sp.</i>	0	0	4	5.8	2	6.9	0	0	2	18.2
<i>Escherichia coli</i>	3	15.8	3	4.3	0	0	2	7.1	1	8.3
<i>Bacillus sp.</i>	0	0	1	1.4	0	0	1	3.6	0	0
<i>Haemophilus sp.</i>	0	0	1	1.4	1	3.4	0	0	0	0
<i>Aeromonas sobria</i>	0	0	1	1.4	1	3.4	0	0	0	8.3
<i>Stenotrophomonas maltophilia</i>	0	0	1	1.4	0	0	1	3.6	0	0
<i>Serratia liquefaciens</i>	0	0	1	1.4	0	0	1	3.6	0	0
<i>Providencia sp.</i>	0	0	1	1.4	1	3.4	0	0	0	0
<i>Providencia rettgeri</i>	0	0	1	1.4	0	0	1	3.6	0	0
<i>Acinetobacter baumannii</i>	1	5.2	2	2.9	0	0	2	7.1	0	0
<i>Acinetobacter sp.</i>	1	5.2	0	0	0	0	0	0	0	0
<i>Delftia acidovorans</i>	0	0	2	2.9	0	0	1	3.6	1	8.3
<i>Rhizobium radiobacter</i>	1	5.2	0	0	0	0	0	0	0	0
Total number of cultures	19	100	69	100	29	100	28	100	12	100

collected on one side (rabbits with unilateral OD), and 61 samples were collected on both sides (rabbits with ND or bilateral OD). All control rabbits had both nasal lacrimal ducts cultured.

Sensitivity tests were performed on the cultures that grew from the CSG. Among the CSG, the percentage of a specific bacteria

found among the total number of bacterial isolate was then correlated to subgroups of rabbit records presenting different clinical signs. (NDG, ODG, and ND/ODG). Sensitivity tests were not performed on the cultures of NCSG.

Statistical analysis was performed with two-sided Fisher's exact tests, applied to 2x2

contingency tables.

## RESULTS

Even though there were slightly more males (3.5%: 54 cases among 1,530 males) than females (2.3%: 29 cases among 1,253 females) that had clinical disease, the difference is not significant ( $p=0.07$ ).

No growth was seen in 22/85 (25.9%) of the CSG and 6/20 (30.0%) of the NCSG (Table II). The difference between the two groups was not significant ( $p=0.78$ ). Amongst the three different subgroups of the CSG rabbits (NDG, ODG, and ND/ODG), there was no significant difference in no growth of the samples when each subgroup is compared to each other.

Cultures yielding bacterial organisms are presented in Table III. Most of them yielded only one species of bacteria, but cultures from six of the rabbits with clinical signs and five of the healthy rabbits grew a mixed population of bacterial organisms.

### Nature of the Isolated Bacteria

Numerous bacterial organisms were isolated (26), including *Pasteurella multocida* which can be pathogenic in rabbits. Organisms were categorized as to potential pathogenicity, and typical site of isolation:

- *Pasteurella* species: *Pasteurella multocida* and *Pasteurella* sp.
- Common bacteria of the GI tract : Gram-negative enterobacteriaceae (in descending order *Enterobacter cloacae*, *Pantoea* sp., *Escherichia coli*, *Serratia liquefaciens*, *Enterobacter aerogenes*, *Providencia* sp. and *Providencia rettgeri*) and Gram-positive cocci bacteria (*Streptococcus* sp. and Group D *streptococci*), being part with enterobacteria to the predominant microflora in the lower gastrointestinal tract of the rabbit.<sup>5</sup>
- Bacteria usually present on skin and mucosa: Group G and non groupable *Streptococcus*, coagulase-negative *Staphylococcus*, *Staphylococcus xylosum*, *Bordetella bronchiseptica*, *Moraxella* sp., *Haemophilus*.<sup>6,7,8,9,10</sup>

- Ubiquitous bacteria from the environment: *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Acinetobacter baumannii*, *Acinetobacter* sp, *Delftia acidovorans*, *Stenotrophomonas maltophilia*, *Aeromonas sobria*, *Rhizobium radiobacter* and *Bacillus* sp. These Gram-negative bacteria are widely distributed in moist environments, such as soil, water, plants and healthy skin and mucosa of humans and animals<sup>11,12,13,14,15,16,17</sup>.

### Isolated Bacteria from the NCSG

From NLD flushes of the 20 animals with no clinical signs, 68.5% of total isolated organisms were common GI tract bacteria, 21.0% were ubiquitous bacteria from the environment, and 10.5% were *Pasteurella multocida*.

### Isolated Bacteria from the CSG

In this group, *Pasteurella* was the most commonly isolated genus with a percentage of 34.8% of the total number of bacterial isolates. Common GI tract rods represented 21.7%, ubiquitous environmental bacteria was 18.8%, and commensal bacteria of the skin and mucosa represented 24.7% of the total.

There was no significant difference between the percentage of *Pasteurella multocida* cultured in the NCSG (10.5%: 2/19), and in the CSG (29%: 20/69) ( $p=0.1376$ ), between the percentage of common GI tract bacteria in the NCSG (36.8%: 7/19) and in the CSG (78.9%: 15/69) ( $p=0.2318$ ), and between the percentage of environmental bacteria in the NCSG (21.1%: 4/19) and in the CSG (68.4%: 13/69) ( $p=1$ ). There was a significant difference for the group of the bacteria of skin and mucosa, which are absent in the NCSG (0%: 0/19) and present in the CSG (24.6%: 17/69) ( $p=0.01815$ ).

*P. multocida* was the most frequently isolated bacteria in CSG, more frequently found in young rabbits (Table IV). When rabbits were grouped into three age classifications [juvenile (from 0 to 1 year), young adults (from 1 to 3 years) and adults (> 3 years)], juveniles were more likely to

**Table IV:** Repartition of records of positive cultures of *Pasteurella multocida* according to groups of age and clinical signs of the CSG

ND: Nasal discharge

OD: Ocular discharge

ND/OD: Nasal and ocular discharge

	Age (year)	0-1	1-3	>3
ND		7	4	1
OD		1	0	5
ND /OD		1	0	1
Total number of positive cultures of <i>P. multocida</i> among clinical cases of rabbits		9	4	7
Total number of clinical cases of rabbits		16	22	47

demonstrate *P. multocida* in NLD flushes. There was a significant difference between 0-1 year and 1-3 year old ( $p=0.01993$ ), and no significant difference between 1-3 years and > 3 years old ( $p=0.7345$ ) rabbits.

#### Presence of Bacteria in Relation to Clinical Signs

There was no significant difference in percentage of the four different bacterial categories (*Pasteurella*, GI tract bacteria, skin and mucosa bacteria and environmental bacteria) among the groups showing various clinical signs (NDG, ODG and ND/ODG) (Table V).

#### Antibiotic Sensitivity Testing

Sensitivity tests were performed on the 69 cultures from the CSG. The results are presented in Table VI.

*Pasteurella sp.* were sensitive to most of the antibiotics frequently used in rabbits (quinolones, trimethoprim sulfa, and injectable cephalosporins and penicillins). *Streptococcus sp.* were sensitive to cephalosporins and penicillins. *Enterobacter sp.* are sensitive to trimethoprim sulfa and quinolones. *Pseudomonas sp.* were only sensitive to quinolones.

#### DISCUSSION

This study attempts to demonstrate which bacteria may be present in rabbits with nasal or ocular disease, two very common conditions in pet rabbits. Furthermore, healthy rabbits underwent the same procedure in an

attempt to describe the bacterial population found in rabbits without clinical signs of the respiratory or ocular tissues.

The CSG didn't show any different bacteria than those found in the NCSG. The most commonly isolated germs from the CSG were *Pasteurella multocida*, which were found more frequently in young rabbits. The second more frequent bacteria isolated in the CSG was *Enterobacter cloacae*. The bacteria isolated in the CSG seem to act as opportunistic pathogens (due to the lack of strain identification tests, it was impossible to conclude if the strains of *Pasteurella multocida* were commensal or pathogenic).

#### Percentage of Absence of Growth of Cultures (Negative Animals)

The percentage of culture yielding no bacteria (negative results) in both groups (25.9% of records in the CSG and 30% in the NCSG) was higher than expected in that the NLD environment is not sterile. The lack of organism growth could be laboratory related as cultures were done under the laboratory routine conditions, meaning no culture was done for organisms which could be found in the respiratory tract and that would require specialized media to grow and identify, such as mycoplasma and anaerobes. It could be also due to a failure in the collection method (as too high a dilution of the sample with the saline) or a failure in correctly preserving the sample. It is unlikely that previous antibiotic administration could have inhibited

**Table V:** Comparison of frequencies of groups of bacterial isolates between groups of rabbits showing different clinical signs (NDG, ODG and ND/ODG), and p-values of corresponding two-sided Fisher's exact tests

NDG : Nasal discharge group

ODG : Ocular discharge group

ND/ODG : Nasal and ocular discharge group

	<i>Pasteurella</i> genus	GI tract bacteria	Environmental ubiquitous bacteria	Commensal bacteria of skin and mucosa
ND versus OD	14/29 versus 8/28 p=0.1755	5/29 versus 8/28 p=0.3578	4/29 versus 8/28 p=0.207	8/29 versus 4/28 p=0.3313
ND versus ND/OD	14/29 versus 2/12 p=0.08361	5/29 versus 4/12 p=0.4077	4/29 versus 1/12 p=1	8/29 versus 5/12 p=0.4686
OD versus ND /OD	8/28 versus 2/12 p=0.6927	8/28 versus 4/12 p=1	8/28 versus 1/12 p=0.2332	4/28 versus 5/12 p=0.09678

bacterial growth, as all rabbits in the study were given at least a two week “wash-out” period in which no antibiotics were administered for any reason. None of the rabbits of the study received any injection of long-acting antibiotics. Antibiotics administered to the rabbits before the study were oral enrofloxacin, marbofloxacin, trimethprim-sulfa, and injectable procaine penicillin G. None of the rabbits received an injection of long-action antibiotic. Elimination half-life of oral quinolones in rabbits is around 8 hours for oral marbofloxacin,<sup>18</sup> and about 2.4 hours for oral enrofloxacin.<sup>19</sup> Achievement of therapeutic blood concentrations of penicillin G after intramuscular injection in rabbits needs drug to be administered at 8 hours intervals,<sup>20</sup> It is unlikely that owners would have been giving antibiotics without divulging this information because it was explained prior to taking the sample that the culture and identification would be useless if the rabbit was on antibiotics at the time. No bacterial growth could also be related to an absence of bacteria in the sample. The nonbacterial causes of ND and OD in rabbits can be local irritation or viral infection in case of myxomatosis. However, none of the rabbits of either group presented with other clinical signs were consistent with myxomatosis infection. Nonbacterial OD can also be the result of a mechanical obstruction (intraluminal concretion or compression

by a tooth root). A previous study about swab specimens of the ocular discharge or NLD flushing of eight rabbits presenting dacryocystis, placed in a commercially available transport device, and then sent to a bacteriological laboratory showed no bacterial growth in two samples.<sup>21</sup> Though the number of individuals is low in this study, the percentage of negative animals (25%) is consistent with what was found in the present report.

### Nature of the Isolated Bacteria

#### *Pasteurella* Species

*Pasteurella multocida* is considered an opportunist or secondary pathogen which can be found in the respiratory tract of healthy and diseased animals.<sup>22</sup> In rabbit colonies, it can emerge as a major pathogen which can produce several disease processes in the rabbit.<sup>23,24</sup> Many pet rabbits harbor *P. multocida*, and they can resist infection, develop the disease or become asymptomatic carriers.<sup>22</sup> Only some strains of *P. multocida* are pathogenic. Characterization of *P. multocida* isolates need phenotypical or genotypical identification using biochemical tests (such as production of ornithine decarboxylase: ODC) and repetitive extragenic palindromic polymerase chain reaction (REP-PCR).<sup>23</sup> In our study, these tests were not performed by the laboratory, which limits our ability to make a definitive conclusion about the pathogenicity of the isolated *Pasteurella*

**Table VI: Antibiotic sensitivity testing of the 69 cultures of the CSG**

AM: Ampicillin, P: Penicillin G, AMX: Amoxicillin, AMC: Amoxicillin Clavulanic Acid, CEF: Ceftriaxone, CL: Cefalexin, CF: Cefovecin, FA: Fusidic acid, L: Lincocin, G: Gentamycin, DO: Doxycycline hyclate, SXT: Trimethoprim sulfate, ENR: Enrofloxacin, PRA: Pradofloxacin, MAR: Marbofloxacin, FRA: Framycetin, UB: Flumequin, PB: Polymixin B, E: Erythromycin  
 R: Resistant, S: Sensitive, I: Intermediate, Blank: Non tested

	AM	P	AMX	AMC	CEF	CL	CF	FA	L	G	DO	SXT	ENR	PRA	MAR	FRA	UB	PB	E
<b>Culture Number</b> <i>Pasteurella multocida</i>																			
1			R	S	S	S	S	S	S	R	S	S	S					R	
2		S	S	S	S	S	S	I	R	S	S	S	S					S	
3		R	S	S	S	S	S	R	R	S	S	S	S	S		S		S	
4		S	S	S	S	S	S	R	R	S	S	S	S		S			S	
5		S	S	S	S	S	S	R	R	S	S	S	S		S			S	
6		S	S	S	S	S	S	R	R	S	S	S	S		S	S		S	
7		R	S	S	S	S	S	R	R	S	S	S	S		S	S		S	
8		R	R	R	S	S	S				S	S	S		S			R	
9	S		S	S		S	S	R	R	S	S	S	S		S	S		S	
10	S		S	S		S	S	R	R	S	S	S	S		S	S		S	
11	S		S	S		S	S	R	R	S	S	S				S		S	
12	S		S	S		S	R	R	R	S	S	S	S		S	S	S	S	
13	S		S	S		S	S	R	R	S	S	S	S		S	S		S	
14	S		S	S		S	S	I	R	S	S	S	S		S	S	S	S	
15	S		S	S		S	S	R	R	S	S	S	S		S	S	S	S	
16	S		S	S		S	S	R	R	S	S	S	S		S	S	S	S	
17	S		S	S		S	S	R	R	S	S	S	S		S	S	S	S	
18	S		S	S		S	S	R	R	S	S	R	S		S	S	S	S	
19	S		S	S		S	S	R	R	S	S	S	S		S	S	S	S	
20	S		S	S		S	S	R	R	S	S	I	R		S	S	S	S	
<b>Total Percent sensitive</b>	<b>100</b>	<b>50</b>	<b>95</b>	<b>95</b>	<b>100</b>	<b>100</b>	<b>95</b>	<b>5.2</b>	<b>0</b>	<b>100</b>	<b>100</b>	<b>95</b>	<b>94.7</b>		<b>100</b>	<b>100</b>	<b>100</b>	<b>90</b>	
<b>Culture Number</b> <i>Pasteurella sp.</i>																			
21		R	S	S	S	S	R	R	R	S	S	S	S		S			R	R
22	S		S	S		S	S	R	R	S	S	S	S		S	S		S	
23		R	S	S	S	S	S	R	R	S	S	S		S	S	S		S	
24		R	S	S	S	S	S	R	R	S	S	S	S		S			S	
<b>Total Percent sensitive</b>	<b>100</b>	<b>0</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>75</b>	<b>0</b>	<b>0</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>0</b>	<b>75</b>	
<b>Culture Number</b> <i>Enterobacter cloacae</i>																			
25		R	R	R	S	R	R	R	R	S	I	S	S		S	S		R	R
26	R		R	R		R	S	R	R	S	R	S	S		S	S		R	
27	R		R	R		R	S	R	R	S	R	S	S		S	S	S	S	
28	R		R	R		R	S	R	R	S	R	S	S		S	S	S	S	
29	R		R	R		R	R	R	R	R	R	R	R		R	R	R	S	
30	R		R	R		R	S	R	R	S	I	S			S	S	S	S	
31	R		R	R		R		R	R	S	I	S	S		S	S	S	S	
<b>Total Percent sensitive</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>100</b>	<b>0</b>	<b>66.6</b>	<b>0</b>	<b>0</b>	<b>85.7</b>	<b>0</b>	<b>85.7</b>	<b>83.3</b>		<b>85.7</b>	<b>85.7</b>	<b>80</b>	<b>71.4</b>	



	AM	P	AMX	AMC	CEF	CL	CF	FA	L	G	DO	SXT	ENR	PRA	MAR	FRA	UB	PB	E
<b>Culture Number</b> <i>Pseudomonas aeruginosa</i>																			
32		R	R	R	R	R	R	R	R	I	R	R	S	S		S		S	R
33	R		R	R		R	R	R	R	S	R		S		S	S	R	S	
34	R		R	R		R	R	R	R	S	R	R	S		S	S	R	S	
35	R		R	R		R	R	R	R	S	R	R	S		S	S	R	S	
Total Percent sensitive	0	0	0	0	0	0	0	0	0	75	0	0	100	100	100	100	0	100	0
<b>Culture number</b> <i>Pseudomonas putida</i>																			
36	R		R	I		R	R	R	R	S	R	R	S		S	S	R	S	
37	R		R	R		R	R	R	R	S	I	R	S		S	S		S	
Total Percent sensitive	0		0	0		0	0	0	0	100	0	0	100		100	100	0	100	
<b>Culture number</b> <i>Streptococcus Group D</i>																			
38	S		S	S	I	I	I	R	R	R	I	R	I		I	I		R	R
39	S		S	S		R	R	R	R		R	R	I		I			R	
Total Percent sensitive	100		100	100	0	0	0	0	0	0	0	0	0		0			0	0
<b>Culture number</b> <i>Streptococcus sp.</i>																			
40		S	S	S	S	S	S	R	R	S	S	I	S		S	S		R	R
41	S		S	S	S		S	I	R		S	R	I		I		R	R	
Total Percent sensitive	100	100	100	100	100	100	100	0	0	100	100	0	50		50	100	0	0	0
<b>Culture number</b> <i>Streptococcus Group G</i>																			
42	S		S	S		S	S	R	S		S	S	S		S		R	R	
<b>Culture number</b> <i>Staphylococcus coagulase negative</i>																			
43		R	S	S	S	S	I	R	R	S	S	S		S	S	S		R	I
44	S		S	S		S	S	S	S	S	S	S	S		S	S	S	S	
45	R		R	R		R	R	R	R	I		R	R		R	I	R	R	
46	S		S	S		S	S	R	S	S	R	S	S		S	S	R	R	
Total Percent sensitive	66.6	0	75	75	100	75	50	75	50	75	66.6	75	66.6	100	75	75	33.3	25	
<b>Culture number</b> <i>Staphylococcus xylosus</i>																			
47	S		S	S		S	S	S	R	S	S	S	S		S	S	I	R	
<b>Culture number</b> <i>Bordetella bronchiseptica</i>																			
48	R		I	S		R	R	R	R	S	S	S	S		S	S		S	
49	S		S	S		R	R	I	R	S	S	S	S		S	S	S	S	
50	S		S	S		R	R	R	R	S	S	S	S		S	S	I	S	
51	R		R	S		R	R	R	R	S	S	S	S		S	S	I	S	
Total Percent sensitive	50		50	100		0	0	0	0	100	100	100	100		100	100	33.3	100	
<b>Culture number</b> <i>Moraxella sp.</i>																			
52		S	S	S	S	S	S	R	R	S	S	I	R		S	S		R	R
53		R	S	S	S	S		R	R	S	S	S	R		R			R	R
54		S	S	S		S	S	S	S	S	R	S	S		S	S	R	S	
55		S	S	S	S	S	S	I	R	S	S	S	R	R		S		S	R
Total Percent sensitive		75	100	100	100	100	100	25	25	100	75	75	25	0	66.6	100	0	50	0

	AM	P	AMX	AMC	CEF	CL	CF	FA	L	G	DO	SXT	ENR	PRA	MAR	FRA	UB	PB	E
<b>Culture number</b> <i>Escherichia coli</i>																			
56		R	R	R	R	R	R	R	R	R	R	R	R	R	S	S		S	R
57	R		R	R		R	S	R	R	S	S	S	S		S	S		S	
58	R		R	I		S	S	R	R	S	R	R	R		S	S		R	
Total Percent sensitive	0	0	0	0	0	33.3	66.6	0	0	66.6	33.3	33.3	50	0	100	100		66.6	0
<b>Culture number</b> <i>Acinetobacter baumannii</i>																			
59		R	I	I	R	R	R	R	R	S	S	S	S		S			S	
60		R	S	S	S	S	S	S	R	S	S	S	S	S		S		S	R
Total Percent sensitive		0	50	50	50	50	50	50	0	100	100	100	100	100	100	100		100	0
<b>Culture number</b> <i>Delftia acidovorans</i>																			
61	R		R	S		S	S	R	R	R	S	R	I		S	R		S	
62	R		R	R		R	S	R	R	R	S	R	I		S	R		S	
Total Percent sensitive	0		0	50		50	100	0	0	0	100	0	0		100	0		100	
<b>Culture number</b> <i>Bacillus sp.</i>																			
63		R	R	I	R	R	R	S	S	S	S	S	S	S		S		R	
<b>Culture number</b> <i>Haemophilus sp.</i>																			
64	S		S	S		R	R	R	R	S	R	I	S		S	S	R	S	
<b>Culture number</b> <i>Aeromonas sobria</i>																			
65	S		S	S		S	S	R	R	S	S	S	S		S	S	S	S	
<b>Culture number</b> <i>Stenotrophomonas maltophilia</i>																			
66		R	R	R	R	R	R	R	R	S	S	S	S	S		S		S	
<b>Culture number</b> <i>Serratia liquefaciens</i>																			
67	R		R	R		R	S	R	R	S	S	S	S		S	S		S	
<b>Culture number</b> <i>Providencia sp.</i>																			
68		R	S	S	R	R	R	R	R	S	S	R	S	S		S		S	
<b>Culture number</b> <i>Providencia rettgeri</i>																			
69	R		R	R		R	S	R	R	R	R	S	S		S	R	S	R	

species.

### Digestive GI Tract Bacteria

These commensal GI tract bacteria could have colonized the NLD or may have been collected while the fluid was passing from the nares over the upper lips, since rabbits ingest their cecotrophs.

### Common Commensal Bacterias of Skin and Mucosa

Group G and non groupable streptococci are commensals of the rhinopharynx.<sup>6</sup> Coagulase-negative *staphylococci* and *Staphylococcus xylosus* are part of the normal flora of

human and animal skin and mucosa.<sup>7</sup> They can occasionally be found in the digestive and respiratory tract of the hosts.<sup>25</sup> *S. xylosus* typically is described as a nonpathogenic common inhabitant of rodent skin. Infections in which *S. xylosus* was isolated and considered to be the primary pathogen contributing to the death have been reported. They were characterized by abscesses and granulomas in soft tissue laboratory mice.<sup>8</sup>

*Bordetella bronchiseptica* is a small, Gram-negative, rod shaped bacterium that are common inhabitants of the respiratory tract of rabbits.<sup>9</sup> It can cause a mild rhinitis

but its presence is usually asymptomatic. It is suspected to be a predisposing factor for the development of *P. multocida* infection.<sup>22</sup>

*Moraxella sp.*, a Gram-negative bacterium, is a well-represented member of the nasal flora of rabbits.<sup>9</sup> *Moraxella bovis* is the cause of the infectious bovine keratoconjunctivitis.

*Haemophilus sp.* is a Gram-negative bacteria belonging to the *Pasteurellaceae* family. *Haemophilus sp.* was reported to infect 16.8% of the rats in a laboratory colony during a routine quality control inspection.<sup>26</sup> *Haemophilus somnus* is associated with respiratory disease in American bison, domestic sheep, and cattle.<sup>27</sup>

### **Ubiquitous Bacteria from the Environment**

These bacteria can survive on moist and dry surfaces, including a hospital environment. *Pseudomonas aeruginosa* is an environmentally stable organism that causes infections in animals whose immune systems or host defences are impaired.<sup>11</sup> *Pseudomonas aeruginosa* has been associated with moist dermatitis of the dewlap in the rabbit.<sup>28</sup>

*Acinetobacter baumannii* forms opportunistic nosocomial infection in humans which may cause severe clinical disease.<sup>29,30</sup> *Delftia acidovorans* is an aerobic, non-fermentative, Gram-negative rod, classified in the *Pseudomonas* rRNA homology Group III, found ubiquitously in soil and water.<sup>31,32</sup> In humans, confirmed isolation from clinical infections is rare, and has been documented mostly in immunocompromised patients. Two cases in humans of respiratory empyema have been reported on immunocompetent patients.<sup>31,32</sup>

Initially classified as *Pseudomonas maltophilia*, *Stenotrophomonas maltophilia* is ubiquitous in aqueous environments, soil, and plants. It has been isolated from human and rabbit feces.<sup>33</sup> In human immunocompromised patients, *S. maltophilia* can lead to nosocomial infections, colonizing endotracheal tubes, and urinary or IV catheters.<sup>34</sup>

*Aeromonas sobria* is a Gram-negative

bacteria which belongs to the *Aeromonas* genus. This gender morphologically resembles to members of the family *Enterobacteriaceae*. These facultative anaerobic rods are commonly found in fresh and coastal water. They are naturally found in the intestines of healthy fish, where they are usually considered secondary or opportunistic bacteria.<sup>15</sup> In humans, *Aeromonas sobria* infection has been associated to gastroenteritis and wound infection.<sup>35</sup>

*Rhizobium radiobacter* is a Gram-negative uncommon opportunistic pathogen present in soil. It has been associated with human systemic diseases, including peritonitis, urinary tract infection, cellulitis, and myositis.<sup>16</sup> *Bacillus sp.* is an ubiquitous bacteria, producing highly resistant endospores.<sup>17</sup>

These ubiquitous bacteria may have been present in the NLD, but it is impossible to exclude the fact that they may also have been present on the skin or come from an external contamination during sampling.

### **Isolated Germs in the No Clinical Signs Group (NCSG)**

The most commonly isolated germs from the NCSG were GI tract Gram-negative bacteria. To our knowledge, there is no other data available about NLD flush culture in healthy rabbits. A recent survey of conjunctival flora from 70 normal rabbits<sup>36</sup> demonstrated the following organisms: DNase-negative *Staphylococcus sp.*, *Micrococcus sp.* and *Bacillus sp.* Other less frequently seen organisms included *Stomatococcus sp.*, *Neisseria sp.*, *Pasteurella sp.*, *Corynebacterium sp.*, *Streptococcus sp.* and *Moraxella sp.*

A study performed on 15 specific-pathogen-free New Zealand White rabbits (13 showing no clinical signs and 2 showing epiphora) consisted of bacterial culture of conjunctival scrapings and specimens from the NLD.<sup>37</sup> The organisms most commonly isolated from the conjunctiva were *Moraxella sp.*, *Oligella urethralis*, *Staphylococcus aureus*, coagulase-negative *Staphylococcus sp.*, and *Streptococcus viridans*. The organisms most commonly isolated from the

nasolacrimal duct flush fluid were *Moraxella sp.*, *Streptococcus viridians*, and *Neisseria sp.* All these bacteria belonged to the commensal flora of skin and mucosa. There was no apparent contribution of microorganisms to the development of epiphora.

### Comparison of Nature and Percentage of Isolated Bacteria Between NCSG and CSG

The present study showed no significant difference between the species of bacteria found in the CSG versus the NCSG. This is consistent with previous surveys.<sup>36</sup> The bacteria isolated in the CSG act as opportunistic pathogens.

*Pasteurella multocida* was the most frequently found bacteria in the CSG in our study (29% of the total number of bacterial cultures), mainly on young rabbits. The second most frequently isolated bacteria was *Enterobacter cloacae* (10.1%). The presence of *Enterobacter cloacae* can be secondary to contamination of the sample during the passage on the lips of the rabbits, but it may also play a role in infections as it has also been cultured from the surgical site of two other rabbits which were not recorded in the study (personal observation). The first sample was taken from a tympanic bulla in a case of otitis media and the second one from the nasal cavity in a case of a rhinostomy in purulent rhinitis. *Pseudomonas* was the third most common genus in this study as it was found in CSG in 8.7% of the cultures.

A study based on nasal samples of 121 pet rabbits with respiratory disease showed the following percentages among isolates: *Pasteurella multocida* (54.8%), *Bordetella bronchiseptica* (52.2%), *Pseudomonas sp.* (27.9%), and *Staphylococcus sp.* (17.4%), with common mixed infections.<sup>38</sup> Compared to this study, the results in the CSG reveal that *Pasteurella multocida* is also the most frequent bacteria isolated, though in a lower percentage. *Pseudomonas sp.* shows also a lower percentage and *Bordetella bronchiseptica* a much lower percentage. There was no evidence of *Enterobacter cloacae* and other Gram-negative enterobacteriaceae.

Different environments and methodologies could explain this discrepancy.

A previous retrospective study about bacteriological results of swab specimens of purulent ocular discharge or NLD flushing of eight rabbits with dacryocystitis did not reveal any *Pasteurella sp.* but *E. coli* in two rabbits, *Staphylococcus sp.* in two other rabbits, *Staphylococcus intermedius*, *Streptococcus sp.* and a Gram-negative cocci, in one rabbit respectively<sup>21</sup>.

### Sensitivity Test

The sensitivity tests in the present study were consistent with the typical sensitivities of the bacteria that were isolated.<sup>39</sup>

Antibiotic therapy were discontinued at least 2 weeks prior to the study with the majority (over 80%) of the rabbits at some point had been previously treated with antibiotics. This could explain why one colony of *Escherichia coli* and one colony of *Streptococcus sp.* were found on the Extended Spectrum Beta-Lactamase panel to be multi-drug resistant.

Based on the majority of organisms cultured in the present study and their sensitivity panels, empiric choices of antibiotics include sulfonamides, or quinolones. The two limitations of trimethoprim sulfonamides in rabbits are the lack of effectiveness against anaerobic infections<sup>40</sup> and the fact that they are inactivated in purulent material due to freely available para-aminobenzoic acid from dead neutrophils.<sup>41</sup> Most quinolones are also ineffective against anaerobes and there is always the concern that overuse of this class of antibiotics can lead to drug resistance<sup>42</sup>.

### CONCLUSION

Knowledge of the expected normal and pathologic bacterial flora of the NLD flush helps to better treat rabbits with clinical signs of rhinitis and ocular discharge. Among the Clinical Sign Group, *Pasteurella multocida* is the most commonly isolated microorganism, with a significant difference in juvenile rabbits. *Pseudomonas aeruginosa* and *Enterobacter cloacae* are a consistent

finding and can behave as opportunistic pathogens of clinical relevance. This way of sampling is prone to external contamination. Further study including anaerobes and mycoplasma cultures would be of interest.

### COMPETING INTERESTS

The authors declare that they have no competing interests.

### AUTHORS' CONTRIBUTIONS

JFQ conceived the study, provided clinical cases, analysed and interpreted the data and drafted the manuscript

AL has been involved in drafting the manuscript

LG provided many clinical cases

CM performed the bacterial cultures

AA performed the statistical analysis

KR has been involved in critically revising the manuscript and proof read it in English

All authors read and approved the final manuscript

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