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In field evaluation of impact on clinical signs of an inactivated autogenous vaccine against *Pasteurella multocida* in rabbits

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ABSTRACT

In Italy, the use of autogenous inactivated vaccines prepared with the bacterial strains isolated from affected animals is authorized by the Ministry of Health in farms where bacterial diseases occur frequently. The autogenous vaccine performed using *Pasteurella multocida* is frequently used in rabbit farms, but the feedback of its application is not available. Therefore, the aim of this study is to give information about the impact on the clinical signs of a bivalent autogenous vaccine in rabbits of a genetic centre. The vaccine was prepared using two *P. multocida* strains belonging to serogroups A and F, equipped with virulence genes and responsible for cyclical outbreak of pasteurellosis in the farm. The vaccine was administered with a first injection, followed by another one after 15 days, then another one four months after the first injection, and then continuing with a further injection every six months to all rabbits. Clinical conditions and mortality rates were monitored for two years after the first vaccination. The improvement in clinical condition and the decrease of the mortality rate were significant especially in the first year post-vaccine. In addition, the number of animals removed due to the disease decreased greatly. Based on the finding of *P. multocida* strains belonging to serogroup D and serogroup A equipped with different virulence-gene patterns from those previously found, we suggest that the vaccine was unable to prevent the introduction and spreading of new strains among the rabbits.

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1. Introduction

Pasteurellosis is one of the most common diseases of wild and domestic animals, including rabbits [1–3]. *Pasteurella multocida* (*P. multocida*) is commonly endemic in rabbitries and is a major cause of significant economic losses in commercial farms. It is so prevalent that the World Organization for Animal Health (WOAH) has classified it as a high-impact pathogen for livestock [4–8]. Infections caused by *P. multocida* are highly epizootic and can lead to a mortality rate of 50% when rabbits are co-infected with other agents [9]. *Pasteurella* species are often associated with both chronic and acute infections, resulting in a significant morbidity [10]. As part of the commensal microbiota in the oral, nasopharyngeal, and the upper respiratory tract of rabbits, this microorganism may act as a primary or secondary pathogen in the respiratory diseases [11]. The pathogenicity of *P. multocida* may be increased in immunodeficient or stressed hosts [12]. Co-infection with other respiratory pathogens, particularly *Bordetella bronchiseptica*, markedly increases the colonization by *P. multocida* [13,14]. Furthermore, nutritional imbalances, suboptimal management and environmental changes can predispose rabbits to the disease. These stressors, along with potential chemical injuries, such as prolonged mucosal exposure to ammonia, can increase the susceptibility of rabbits to *P. multocida* infection [15], and play a crucial role in the microbial transmission and disease severity [16]. Clinical forms of pasteurellosis range from asymptomatic or mild inflammation of the upper respiratory tract to acute or fatal pneumonia. Pathologic signs include rhinitis with purulent nasal discharge, pneumonia, otitis, pyometra, orchitis, abscesses, and septicaemia [5,17]. Transmission occurs mainly by aerosol, particularly when chronic infection develops in the nasal cavities, paranasal sinuses, middle ears, lacrimal and thoracic ducts of the lymphatic system, and lungs [18–20]. Adult rabbits may become carriers of *P. multocida*. Generally, the infection starts after birth and the prevalence of colonization increases until five months of age [21].

P. multocida is currently classified into five groups (A, B, D, E, and F), based on capsular antigens, and 16 somatic serotypes (1 to 16), based on lipopolysaccharide antigens, using serological test [22–27]. Capsular type A is most commonly associated with pasteurellosis in rabbits, while capsular types D and F are less often involved in the disease [2,28]. Type B has been detected

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in rabbitries in India [29] and in rabbits with septicaemia in Italy [28], although it is primarily associated with haemorrhagic septicaemia in cattle and calves [30]. Although the capsule is already a virulence determinant [31], the pathogenicity of *P. multocida* is associated with other virulence factors (VFs). Genes encoding haemoglobin-binding protein (*hgbB*), transferrin-binding protein (*tbpA*), filamentous haemagglutinin (*pfhA*), and dermonecrotic toxin (*toxA*) have been proposed as virulence markers [11]. A correlation between VFs and capsular types has been reported [11]. The *pfhA* gene is commonly associated with serovars A, B, E, and F. An association between *hgbB* and capsular type D has also been found in *P. multocida* isolates from rabbit respiratory disease [32].

Recently, additional virulence genes have been identified, such as *ptfA*, *fimA*, and *tadD* (encoding for fimbriae, adhesion and colonization factors, respectively); *exbB*, *exbD*, *tonB*, *hgbA* (encoding for iron and protein acquisition); *sodA* and *sodC* (superoxide dismutase); *nanB* and *nanH* (neuraminidases); *ompA*, *ompH*, *omp87*, *plpB* (Outer Membran Proteins – OMPs) that may be involved in the pathogenicity of *P. multocida* [11,33–36]. Conversely, *toxA* (encoding for dermonecrotic toxins) seems to be less relevant to the pathogenicity of *P. multocida* in rabbits, as it has not been detected in strains isolated from rabbits [28,33,37].

The drugs of choice for the treatment of pasteurellosis are the fluoroquinolones, of which enrofloxacin is approved for use in rabbit flocks. However, enrofloxacin is not always able to eradicate *P. multocida* in rabbits, resulting in clinical improvement in some animals rather than complete recovery and chronic infections [38].

In addition, administering treatment to rabbit flocks through drinking water could result in variable water intake by different animals, leading to under-dosing especially in sick ones. Repeated use of antibiotics could also increase the risk of antibiotic resistance onset. Therefore, preventive measures such as proper ventilation, improved environmental hygiene in the management of rabbitries, and the use of vaccines [39] may be more effective methods to control infection.

Several years ago, vaccines against *P. multocida*, including inactivated whole bacterial vaccines, were tested in rabbits [12,40]. Only a few studies on seroconversion induced by these vaccines are available [41]. Currently, autogenous inactivated vaccines are used to control the disease under field conditions. This type of vaccine is prepared using the strain/strains isolated from diseased/dead animals in the farm. Autogenous vaccines are widely used in Italian rabbit farms for the control of the bacterial diseases, particularly pasteurellosis, when bacterial diseases become recurrent in the flocks. Although a commercial bivalent vaccine against *Pasteurella multocida* and *Bordetella bronchiseptica* is licenced for use in rabbit farms, the farmers usually prefer to use the autogenous vaccine aiming to obtain a more specific protection of the animals. The Ministry of Health with the Ministerial Decision No 287 of 17 March 1994 authorized only selected laboratories (Experimental Zooprophylactic Institutes) to produce autogenous vaccines. The use of this type of vaccine is only authorized in farms with a specific bacterial infectious disease and is subjected to a detailed veterinary request. This means that it is not possible to assess how well the vaccine works. Therefore, the aim of this study was to assess the effect of an autogenous vaccine containing *P. multocida* strains of serogroups A and F in field conditions.

2. Material and methods

2.1. Farm

The study was carried out in the genetic centre of the Italian Rabbit Breeders Association (ANCI), in Volturara Appula, FG, southern Italy, authorized by the Ministry of Agricultural, Food and Forestry Policies (MIPAF) for the maintenance of rabbit breeding farms. The genetic centre is located 1000 m above sea level. Pure Italian White, Silver and Spotted breeds were selected and reared in the genetic centre. Four thousand and twenty rabbits were does. A total of 30250 rabbits were housed in 10 sheds in the farm when this study was started. A climate control system provided with pad cooling ensured appropriate environmental parameters, with a relative humidity of about 65% and temperatures between 20 and 25°C inside the halls. Ventilation was controlled at a maximum speed of 0.3 m/sec. A closed cycle production system was used in the farm. The animal density was 2 rabbits per square metre. Each breeder was housed in WRSA (World Rabbit Science Association) type cages, 70 cm long and 45 cm wide. The cages were used as dual-purpose cages (DP). In fact, at the time of weaning, the doe is moved into another cage while the kits remain in their cage for the full weaning period. In addition, moving animals from one cage to another or from one shed to another frequently occurred according to management needs. For example, the does that were not pregnant after the artificial insemination were moved in other rows of cages or to a different shed in order to be inserted in the next group of does to be inseminated. Finally, dead or discarded rabbits were constantly replaced by new weaned rabbits. Commercial food (Mignini&Petrini s.p.a.) was fed *ad libitum*.

2.2. Health conditions of the farm

A syndrome associated with frequent use of antibiotics occurred cyclically in the herd. Purulent conjunctivitis and rhinitis were the most common clinical signs observed over a five-year period. Coughing, sneezing,

increased respiration, vulvar discharge, and torticollis were also observed. Mortality due to the syndrome was mainly caused by respiratory injuries. In addition, during the year prior to vaccination, 13.2% of the rabbits were removed from the breeding group due to severe respiratory symptoms or reproductive disorders such as infertility and abortion, resulting in a loss of 17.9% of does that were replaced by new ones for breeding. Necropsies and laboratory investigations were performed at the Department of Veterinary Medicine in Bari, Italy. Purulent pneumonia, metritis and otitis were observed. *P. multocida* was consistently isolated on blood agar (Triptic Soy agar, Basingstoke, UK, supplemented with 5% sheep blood) and identified from tissue samples (lung, uterus and ear) as described in session 2.3. High economic losses due to mortality, discarded does and antibiotic use were reported, suggesting the use of a vaccination strategy.

2.3. Investigation on Pasteurella multocida strains

Thirty strains of *P. multocida* isolated from lesions (pneumonia and metritis) of 30 rabbits were typed for serogroup and virulence-associated genes, before the vaccine preparation. A multiplex PCR assay was used for species identification and capsular antigen typing [42]. Genes encoding virulence factors associated with the pathogenicity of *P. multocida* [11,33,43] were investigated using three multiplex PCR protocols for detection of *toxA*, *tbpA*, *fim4*, *sodA*, *pfha* (protocol A), *exbB*, *hgbB*, *nanB*, *tadD*, *plpB*, *sodC*(protocol B), and *oma87*, *fimA*, *nanH*, *fur* (protocol C), as previously described [37]. Briefly, the PCR mixture for Multiplex-PCRs consisted of 12.5 µL of 1X Platinum Mastermix (Thermo Scientific, Milan, Italy), 0.5 µL of each primer pair (50 pmol/µL primary concentration), 2 µL of sample DNA, and ultra-pure nuclease-free water (Thermo Scientific, Milan, Italy) to a final volume of 25 µL. Cycling conditions were as follows: 95°C for 5 min, then 35 cycles, each performed at 95°C for 30 s followed by 55°C for 30 s and then 72°C for 1 min and 10 s, with a final extension at 72°C for 10 min. The PCR products were loaded for electrophoresis on a 1.5% agarose gel stained with ethidium bromide, and the amplified PCR product was visualized using the Gel Doc-It image analyser (UVP, Upland, CA, USA).

Fourteen *P. multocida* strains isolated from 14 rabbits at the end of the second year after vaccination were also typed.

2.4. Vaccine preparation and vaccination schedule

Two strains of *P. multocida* belonging to serogroups A and F, respectively, and endowed with 10 virulence genes (*pfhA*, *sodC*, *soda*, *exbB*, *oma87*, *fur, fim4, nanB, nanH, fimA*) were selected to prepare the inactivated bivalent vaccine (AF). The bacterial strains were cultured no more than three times and tested for capsule persistence using Anthony's stain [44] prior to use. Vaccine production was carried out by the Istituto Zooprofilattico Sperimentale (IZS) in Foggia, Italy. The bacterial suspension was prepared in saline solution (0.9%) using strain A and strain F (1:1) until a final bacterial density of 1.5×10^9 cells/ml was inactivated with formalin (0.4%). Then, the bacterial suspension was diluted with 0.9% NaCl saline to achieve the desired cell density and fall within the maximum levels of formalin content allowed by the European Pharmacopoeia (0.05% of the final product).

The assessment of inactivation was performed by inoculating the bacterial suspension on Petri plates containing Blood Agar (Oxoid) that were incubated at 37°C for 48 h under aerobic conditions. The vaccine was adjuvated with aluminium hydroxide (25%).

One mL of vaccine AF was administered twice at 15-day intervals, then injected after four months from the first injection, and then repeated at six-month intervals to all rabbits. Rabbits were handled and held by an experienced operator during each procedure.

2.5. Statistical analysis

The data of mortality, symptomatology and removal from breeding sector were analysed by univariate statistical analysis (Pearson's chi-square test and Fisher's exact test for independence). The odds ratio (OR) and 95% confidence interval (CI95%) were also calculated. A p-value of < 0.05 was considered statistically significant. Statistical analyses were performed using spss 13 software for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Outcome of vaccination in the rabbits of the farm

A significant reduction in mortality due to respiratory and reproductive lesions was found in the first and second years post-vaccine administration compared with the pre-vaccine year (Table 1). During the same reporting periods, vaccination resulted in a significant reduction in the number of deaths due to respiratory and reproductive lesions other than in removals from the breeding sector.

Concerning the total of rabbits, the lower mortality rate was accompanied by a significant decrease in the incidence of rhinitis, conjunctivitis, torticollis, and vulvar discharge symptoms (Table 2). A similar decreasing trend in the incidence of all symptoms was also observed in the group of does, although only for conjunctivitis a statistically significant outcome was found two years after vaccination.

Table 1. Mortality recorded before and after vaccine use in all rabbits and mortality and loss of does removed from the breeding sector.

	Reporting period	Deaths from respiratory lesions (%)	OR	$CI_{95\%}$	p-value	Deaths from reproductive lesions (%)	OR	$CI_{95\%}$	p-value	Females removed from breeding sector (%)	OR	$CI_{95\%}$	p-value	Total (deaths + loss of does due to pasteurellosis) (%)	OR	$CI_{95\%}$	p-value
Does (4,020*)	Pre-vaccine year	100 (2.48)	Reference		0.009	90 (2.23)	Reference		0.007	530 (13.2)	Reference		<0.001	720 (17.9)	Reference		<0.001
	1-year post-vaccine	77 (1.9)	0.77	0.57–1.03		65 (1.6)	0.72	0.52–0.99		420 (10.44)	0.77	0.67–0.88		562 (13.9)	0.74	0.66–0.84	
	2-year post-vaccine	62 (1.54)	0.61	0.45–0.85		54 (1.34)	0.59	0.42–0.84		380 (9.45)	0.69	0.6–0.79		496 (12.3)	0.65	0.57–0.73	
All the other rabbits (26,230*)	Pre-vaccine year	436 (1.66)	Reference		<0.001	333 (1.26)	Reference		<0.001	-				769 (2.93)	Reference		<0.001
	1-year post-vaccine	310 (1.18)	0.71	0.62–0.83		174 (0.66)	0.52	0.43–0.62		-				484 (1.84)	0.62	0.55–0.7	
	2-year post-vaccine	285 (1.08)	0.65	0.56–0.76		132 (0.5)	0.39	0.32–0.48		-				417 (1.58)	0.53	0.47–0.6	
Total of rabbits (30,250*)	Pre-vaccine year	536 (1.77)	Reference		<0.001	423 (1.39)	Reference		<0.001	530 (1.75)	Reference		0.039	1489 (4.9)	Reference		<0.001
	1-year post-vaccine	387 (1.27)	0.72	0.63–0.82		239 (0.79)	0.56	0.48–0.66		420 (1.38)	0.79	0.69–0.9		1046 (3.45)	0.69	0.64–0.75	
	2-year post-vaccine	347 (1.14)	0.64	0.56–0.74		186 (0.61)	0.44	0.37–0.52		380 (1.25)	0.71	0.62–0.81		907 (2.9)	0.6	0.55–0.65	

OR: Odds ratio, $CI_{95\%}$: 95% Confidence Interval. Reference group is the pre-vaccine year.
*Other rabbits were introduced to replace the dead ones removed.

3.2. Laboratory investigation on P. multocida isolates

Eighteen strains of *P. multocida* identified from pneumonia and two strains identified from metritis belonged to serogroup A, while six and four strains identified from pneumonia and metritis, respectively, belonged to serogroup F (Table 3). Among the total of strains which were serotyped before the use of vaccine, 23 and 5 strains equipped with 2 different patterns of 10 virulence genes, respectively, were found. Also, two strains belonging to serogroup A were equipped with 9 virulence genes. Among the 14 strains detected and investigated at the end of the second-year post-vaccine use, serogroups A and D were detected in both pneumonia and metritis while serogroup F was no longer identified. Eight strains belonging to serogroup A were equipped with the same patterns of virulence genes already identified before the use of vaccine. Conversely, two and four strains were equipped with two different patterns not previously detected.

4. Discussion

The vaccination strategy has been shown to be effective in reducing the mortality rate and in improving the health status of rabbits, confirming that the inactivated vaccine is useful in mitigating the usual injuries associated with pasteurellosis [45]. The endemic infections are the cause of economic losses due to mortality and decrease of performances. Even if optimizing the environment of the rabbitry and culling and remotion of symptomatic animals remain useful control measures, the vaccination seems to be able to limit more effectively the infection and disease under field conditions [46]. Accordingly, as observed by the veterinarian of the rabbit genetic centre ANCI, the health conditions of the animals began to improve about four months after vaccination. The severity of the clinical manifestation of infection decreased, particularly in the first-year post-vaccination, with a continuous reduction observed in the second year after vaccination. Similarly, a decrease in mortality due to respiratory and uterine complications was found throughout the study period, with a more pronounced decrease recorded after the first post-vaccinal year. The number of females removed from the breeding sector due to infertility or severe clinical symptoms gradually decreased during the first- and second-year post-vaccine.

Previous studies have evidenced that a double dose of killed *P. multocida* vaccine increases the lymphocytes and monocytes of vaccinated rabbits leading to an improvement of the immune response [45]. In addition, the killed vaccine induces an increase in the phagocytic activity of the immune cells [47,48]. Accordingly, vaccinated rabbits showed very mild changes in spleen compared with non-vaccinated rabbit in which severe lesions and depletion of lymphoid tissue were found after challenge with a virulent *P. multocida* strain [45].

In contrast, an increase in ALT and AST levels suggesting a hepatic irritating effect associated with the use of formalized killed *P. multocida* vaccine was found in different studies [45,49]. Nevertheless, based on the serum urea and creatinine levels detected in challenged vaccinated and non-vaccinated rabbits, the vaccine seems to be able to protect against renal damaging induced by virulent *P. multocida* [45].

The effects of the killed vaccine are depending also on the kind of adjuvant used to prepare the vaccine other than the age of vaccinated hosts and the administration dose [50]. Oil adjuvants are usually responsible for moderate to serious lesions in the injection site in rabbit [51,52], although recent studies highlighted the safety of some vegetable oil adjuvants [53]. Therefore, as all the autovaccines usually used in rabbit farm, the vaccine monitored in this study was adjuvated with aluminium hydroxide inducing a potential less prolonged immune response than oily adjuvants. Accordingly, the vaccine was administrated twice a year, at six-month intervals.

Two years post-vaccine administration, *P. multocida* strains from serogroup F were no longer detected. The whole-cell lysate used in the vaccine contains a great amount of lipopolysaccharides, which are outer membrane components of the bacteria and the primary somatic markers responsible for immune response against *P. multocida* [54,55]. Therefore, although this finding might be coincidental, it could be related to the possible ability of the vaccine to protect the rabbits against *P. multocida* belonging to capsular type F. Further investigation extended over a longer period of monitoring of the vaccination plan could be useful to better assess this issue. On the other hand, as expected, the vaccine was unable to prevent the spreading of *P. multocida* among rabbits. In fact, strains of *P. multocida* belonging to serogroup A, that was more widespread among rabbits than F before the vaccine, were still identified in rabbit lesions two years post-vaccine administration. Also, based on the typing results, the detection of capsular type A strains with different virulence gene patterns than those identified before vaccine use suggests the introduction of new type A strains as well as the circulation of type A strains already detected into the rabbit flocks. These findings could be linked to the introduction of new rabbits to replace the discarded ones. In fact, although they belonged to the same farm, they often came from a different shed where different epidemiological conditions might have occurred. Moreover, other sources of bacteria introduction

Table 2. Symptoms observed in rabbits before and after vaccine use.

	Reporting period	Rhinitis (%)	OR	$CI_{95\%}$	p-value	Conjunctivitis (%)	OR	$CI_{95\%}$	p-value	Torticollis (%)	OR	$CI_{95\%}$	p-value	Vulvar discharge (%)	OR	$CI_{95\%}$	p-value
Does (4020*)	Pre-vaccine year	65 (1.6%)	Reference			59 (1.47%)	Reference		0.01	31 (0.77%)	Reference		0.30	40 (0.99%)	Reference		0.10
	1-year post-vaccine	50 (1.2%)	0.77	0.53–1.11	0.30	42 (1.04%)	0.71	0.48–1.06		25 (0.6%)	0.81	0.47–1.37		32 (0.79%)	0.80	0.50–1.27	
	2-year post-vaccine	52 (1.29%)	0.80	0.55–1.15		31 (0.77%)	0.52	0.34–0.81		20 (0.49%)	0.64	0.37–1.13		23 (0.5)	0.57	0.34–0.96	
All the other rabbits (26230*)	Pre-vaccine year	180 (0.68%)	Reference		<0.001	130 (0.49%)	Reference		<0.001	131 (0.5%)	Reference		<0.001	186 (0.7%)	Reference		<0.001
	1-year post-vaccine	119 (0.45%)	0.66	0.52–0.83		65 (0.25%)	0.5	0.37–0.67		87 (0.33%)	0.66	0.51–0.87		103 (0.39%)	0.55	0.43–0.7	
	2-year post-vaccine	102 (0.38%)	0.56	0.44–0.72		51 (0.19%)	0.39	0.28–0.54		72 (0.27%)	0.55	0.41–0.73		90 (0.34%)	0.48	0.37–0.62	
Total of rabbits (30250*)	Pre-vaccine year	245 (0.8%)	Reference		<0.001	189 (0.62%)	Reference		<0.001	162 (0.5%)	Reference		<0.001	226 (0.7%)	Reference		<0.001
	1-year post-vaccine	169 (0.55%)	0.69	0.57–0.84		107 (0.35%)	0.56	0.45–0.72		112 (0.37%)	0.69	0.54–0.88		134 (0.44%)	0.59	0.48–0.73	
	2-year post-vaccine	154 (0.5%)	0.63	0.51–0.77		82 (0.27%)	0.43	0.33–0.56		92 (0.3%)	0.57	0.44–0.73		113 (0.37%)	0.5	0.4–0.62	

OR: Odds ratio, $CI_{95\%}$: 95% Confidence Interval. Reference group is the pre-vaccine year.
*Other rabbits were introduced to replace the dead ones removed.

Table 3. Typing of *P. multocida* strains identified before and after vaccine use.

			Patterns of virulence genes				
Reporting Period	Lesion	Serogroup (N° of strains belonging to)	pfhA-sodC-sodA-exbB-oma87-fur-fim4-nanB-nanH-fimA	pfhA-sodC-sodA-tadD-exbB-oma87-fim4-nanB-nanH-fimA	sodC-sodA-exbB-oma87-fur-fim4-nanB-nanH-fimA	pfhA-sodC-oma87-fim4-fimA-nanB-nanH	sodC-oma87- -fim4-fimA- hgbB nanB-nanH
Pre-vaccine use	Pneumonia	A (18)	14	2	2	-	-
		D (0)	-	-	-	-	-
		F (6)	5	1	-	-	-
	Metritis	A (2)	-	2	-	-	-
		D (0)	-	-	-	-	-
		F (4)	4	-	-	-	-
Post vaccine use	Pneumonia	A (8)	4	1		1	2
		D (1)	-	-	-	-	1
		F (0)	-	-	-	-	-
	Metritis	A (4)		3		1	-
		D (1)	-	-	-	-	1
		F (0)	-	-	-	-	-

cannot be excluded because, although *P. multocida* is considered a fragile organism, it is able to survive in liquids and aerosol for relatively long periods [56].

In addition, strains belonging to serogroup D were found as responsible for disease two years post-vaccine administration. It is possible that those strains already present in the farm were not identified before using the vaccine simply due to the limited size of the sample that we used.

Anyway, although the whole-cell lysate prepared using strains belonging to some/certain serogroups could also potentially induce cross-reactions with other serogroups [46,57], lipopolysaccharides may induce specific reactions and antibodies which react poorly, or not at all, with the lipopolysaccharides of other serogroups [58]. Nonetheless, our findings underline the need for consistent monitoring of the serogroups spread in rabbit populations prior to the formulation of each new vaccine batch for the farm and for improving the biosecurity measures to minimize the risk of introduction of new strains.

In conclusion, a more frequent use of vaccines is particularly advisable in rabbit farms, which are the species with the highest antimicrobial consumption among food-producing animals [59]. In this study, the on-farm vaccination strategy improved the clinical condition of the rabbits, with benefits in terms of reducing economic losses due to mortality, breeder rejections and antibiotic treatments. Nevertheless, the use of the vaccine did not lead to the eradication of pasteurellosis in two years, and it is likely that more time will be needed to achieve this result. In addition, based on the detection of *P. multocida* strains belonging to serogroup D in rabbits at the end of the second year post-vaccine, the vaccine seems to be not able to prevent the introduction of new serotypes. Therefore, when using an autogenous vaccine, it is very important to monitor the serotypes present in the rabbit population prior to the formulation of each new vaccine batch for the farm to ensure maximum efficacy.

In addition, the influence of the management and environmental conditions, which can affect the cortisol levels of reared rabbits [60], on the immune response to the vaccine should be investigated to better define the vaccination plan and maximize vaccine efficacy.

Anyway, our results provide new information on the use of a vaccine for the prevention of pasteurellosis in rabbits, which may also be useful in companion animals. Rabbits are widely kept as pets and the use of vaccines against *P. multocida* should be routine due to the incidence of infection and its impact on rabbit health [37]. Currently, vaccination against *P. multocida* is rarely used in companion animals, and the disease control is achieved with antimicrobials.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethical approval statement

The study was conducted according to the guidelines of the Ethics Committee of Department of Veterinary Medicine of University of Bari "Aldo Moro", Italy (Approval number n. 20/2021).

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