INFLUENCE OF DIFFERENT STRAINS AND WAYS OF INOCULATION ON THE RABBIT'S RESPONSE TO EXPERIMENTAL INFECTION WITH *PASTEURELLA MULTOCIDA*

Kulcsár G.[1], Fábián K.[1]*, Barna T.[1], Virág Gy.[2]

[1]Central Agric. Office, Directorate of Veterinary Medicinal Products, Szállás u. 8, 1107 Budapest, Hungary
[2]Research Institute for Animal Breeding and Nutrition, Isaszegi ut 200, 2100 Gödöllő, Hungary
*Corresponding author: fabianka@oai.hu

ABSTRACT

Extensively characterized *Pasteurella multocida* strains isolated from rabbits with different signs of *pasteurellosis* were used to study the response of rabbits triggered by aerogen, conjunctival, intradermal or intramuscular experimental infection. Lethality was small (5/45) in general and somewhat higher tendency (4/17) was found after conjunctival inoculation. Number of days with appearance of illness and fever within 14 days p.i., number of rabbits with at least one day occurrence of fever, *P. multocida* reisolation rate, and average rectal temperature were influenced both by the strain inoculated and by the route of inoculation. Strain 31 presented higher ($P<0.05$) values at more parameters (all had fever, 39.9°C average rectal temperature, 60% *P. multocida* reisolation rate) except number of days with illness and with fever. Intradermal and intramuscular inoculation caused doubled values compared to that caused by aerogen or conjuctival inoculations, except *P. multocida* reisolation rate. Feed intake and body weight gain was also different by the route of inoculation, rabbits inoculated intradermally or intramuscularly consuming less feed and loosing weight instead of gaining. Serum antibody level elevated 2-3 times in rabbits infected intramuscularly, and strain 1 was found to have the higher antigenecity ($P<0.05$).

Key words: Rabbit, *Pasteurella multocida*, Experimental infection, Challenge model.

INTRODUCTION

Rabbit *pasteurellosis* is still often enzootic on big farms (Coudert *et al*., 2006) and the most prevailing chronic forms cause important losses (Rosell *et al.*, 1991). Farm specific vaccines have been and are to be developed and their efficacy should be evaluated one by one in challenge experiments. At the same time when accepting the strong intention towards decreasing the number of animal experiments it should be recognized that challenge experiments are unavoidable in case of vaccination studies. The number of animals however should be kept at the possible lower number but in this way to keep a good experimental model is necessary. Making good design is not easy in case of rabbit because of the big individual differences in response given to experimental infections with *P. multocida* (Rideaud *et al*., 1999).

The purpose of this study was to compare the rabbit pathogenecity of five *P. multocida* (*P.m.*) strains isolated from rabbits with different signs of *pasteurellosis* and applied on four different ways in order to establish a strong model for challenge experiments.

MATERIALS AND METHODS

Parameters of inoculums used for the experimental infection

P.m. strains used for the infection (Table 1) have been isolated from rabbits with different signs of *pasteurellosis*. Strains maintained in lyophilized stock were grown on TSA plates. Overnight cultures were suspended in PBS and diluted to approximately 10^5 CFU/ml and 1 ml was inoculated into each rabbit, except the negative controls.

Table 1: Characteristics of strains used for inoculation

Strain	Mouse LD_{50}	Lesion of isolation	***capGene production: biovar***
1	1.1×10^3	pneumonia	*capA*+ : 6
5	7.7×10^5	subcutaneous abscess	*capA* - : n.t.
13	1.9×10^5	rhinitis	*capA*+ : 1
31	4×10^5	empyema	*capA* -: 6
32	5.2×10^5	torticollis	*capA*+ : n.t.

Table 2: Number of rabbits inoculated with different strains and by different routes

Inoculation way	Strain						
	1	5	13	31	32	-cont	Total
aerosol	0	0	3	3	0		6
id	3	3	0	0	3		9
im	2	2	3	3	2		12
orrkonj	3	3	3	3	3		15
-cont						3	3
Total	8	8	9	9	8	3	45

Experimental animals and design

Forty five 12 weeks old hybrid rabbits weighing 2860±41 g were used in this experiment (Table 2). All rabbits were screened three times for *P.m.* nasal carriage and once for serum antibodies by an ELISA (modified FlockCheck IDEXX *P.m.* Antibody Test Kit) previously and all were negative. The inoculation was performed by aerogen (aero.), conjunctival (conj.), intradermal (id.) and intramuscular (im.) routes. Negative control (-cont.) rabbits were kept without inoculation but otherwise within the same conditions. Rabbits were caged individually and groups infected with different strains were kept in different rooms. According to the route of the infection the rabbits were divided further into cage blocks to close off any possibility of the direct contact between these animals. Management, feeding and water supply were the same for all groups.

Clinical, pathological and microbiological observations

Rabbits were examined and rectal temperature was recorded daily to day 14 post infection (p.i.). Sneezing, depression and forced respiration were considered as disease signs. Body weight (gram) was controlled on the day of inoculation then on day 7 and 14 p.i. Feed consumption (gram) was measured weekly during two weeks p.i. Blood samples were taken before inoculation and at day 14 p.i. At day 14 p.i. all survived rabbits were euthanized humaneously then necropsied. Swabs were taken from the nose, pharynx, trachea and lung, heart chamber and abscesses or other lesions if present and spread onto SBA. *P.m.* was isolated and identified by standard microbiological methods.

Statistical Analysis

Powerful statistical processes in GenStat 8th software (VSN International Ltd., 2004*)* GLM module enabled for dealing with unbalanced designs were used for the detection of strain and inoculation way effects. Interaction of these factors was not studied. Feed intake, rectal temperature were averaged over rabbits and ELISA titers after inoculation were given as percentage of the basic value. The data were evaluated with GLM regression analysis. Weight gain on subsequent weeks was evaluated by REML variance component analysis. Occurrence of abnormally high body temperature and reisolation of *P.m.* from different organs on the level of single animal was considered as yes or not variable and evaluated with Bernoulli distribution. Number of days with presence of fever or illness and number of successful reisolations of *P.m.* compared to the number of total observations on the level of single rabbits were evaluated by modeling of binomial proportions. Results are presented as mean±s.e. for groups formed by the two factors.

RESULTS AND DISCUSSION

One rabbit infected id. with strain 1 died on day 2 p.i. and conjunctival inoculation with strain 1, 13 and 31 killed further 4 rabbits in all which died between day 7 and 14 p.i. showing a tendency (P<0.1) for higher lethality in case of conjunctival inoculation route. Both infective strains (Table 3) and inoculation routes (Table 4) influenced the prevalence of illness and fever given in number of days with positive observations, the proportion of feverish rabbits, *P.m.* reisolation rate and the average body temperature.

Table 3: Proportion of observed animals and days with occurrence of clinical signs of sickness and with fever, *P. multocida* reisolation rate by strains used for infection

	Strain						
Parameter	1	5	13	31	32	s.e.	Prob
Sick days/total observed	0.34^{b}	0.21^{a}	0.16^{a}	0.12^{a}	0.40^{b}	0.04	<0.001
Fever days/total observed	0.17^{a}	0.26^{a}	0.27^{a}	0.32^{ab}	0.41^{b}	0.04	<0.01
Rabbits with fever occurence	0.88^{a}	1^{b}	0.67^{a}	1^{b}	0.63^{a}	0.09	<0.05
Rectal temperature	39.0^{a}	39.4^{b}	39.7^{c}	39.9^{d}	39.6^{c}	0.08	<0.001
P.m. reisolation rate	0.21^{a}	0.34^{a}	0.38^{a}	0.60^{b}	0.41^{a}	0.08	<0.01

Means with different letters on the same row differ significantly

Table 4: Proportion of observed animals and days with occurrence of clinical signs of sickness and with fever, *P. multocida* reisolation rate by inoculation route applied

	Inoculation route					
Parameter	aero.	conj.	id.	im.	s.e.	Prob
Sick days/total observed	0.08^{a}	0.07^{a}	0.50^{b}	0.34^{b}	0.04	<0.001
Fever days/total observed	0.08^{a}	0.11^{a}	0.50^{b}	0.45^{b}	0.04	<0.001
Rabbits with fever occurence	0.67^{a}	0.67^{a}	1^{b}	1^{b}	0.08	<0.01
Rectal temperature	39.1^{a}	39.3^{b}	40.0^{d}	39.6^{c}	0.08	<0.001
P.m. reisolation rate	0.29	0.41	0.41	0.40	0.08	n.s.

Means with different letters on the same row differ significantly

The mean rectal temperature was above 39.5°C in groups infected id. and im., while rabbits in groups infected aero. or conj. were not feverish (P<0.001). All rabbits had fever at least once in groups infected id. or im., but only 67% in groups infected aero. or conj. (P<0.01). The number of days with prevalence of illness and fever was low in groups infected aero. or conj. compared to the doubled (P<0.001) frequency at groups infected id. or im. *P.m.* reisolation rate however did not differ by the way of inoculation. Observed parameters of sickness were influenced by the infective strain too. Rabbits infected with strain 31 had the highest *P.m.* reisolation rate (P<0.01) and average rectal temperature (P<0.001), all were feverish and had high proportion of days with fever. Contrary to these, signs of illness were observed with the lowest frequency (<0.001) at this group, what could be explained by the absence of sneezing or forced breathing. The depression as the only sign caused by abnormally high body temperature is often difficult to observe on rabbits kept in confinement.

Feed intake on the first week p.i. (Figure 1) was significantly different in the experimental groups by the way of inoculation (P<0.005). Uninfected control rabbits and rabbits infected by aero. or conj. route consumed more feed (999, 1067 and 893 g, respectively) compared to those infected id. or im. (527 and 673 g, respectively). Strain effect was not important. The decreased feed intake and weight gain could be partly explained by the higher body temperature caused by the infection. On the second week after inoculation feed intake recovered to the normal level (1184±104 g, P<0.001). The only exception was the group infected with the strain 31. In line with the decreased feed intake on the first week the rabbits gained mere 34.4±23.2 g in average independently from the inoculation strain. The apparently high variability of the results was explained again by the route of the infection (P<0.001) with negative (i.e. weight loss) values at groups infected id. or im. (-80.6 and -21.7 g, s.e. 56.5 and 50.2, respectively) and higher values at rabbits infected aero. or conj. (201.0±63.6 and 80.9±48.4 g, respectively). Control rabbits gained 232±69 g with the same amount of feed consumed.

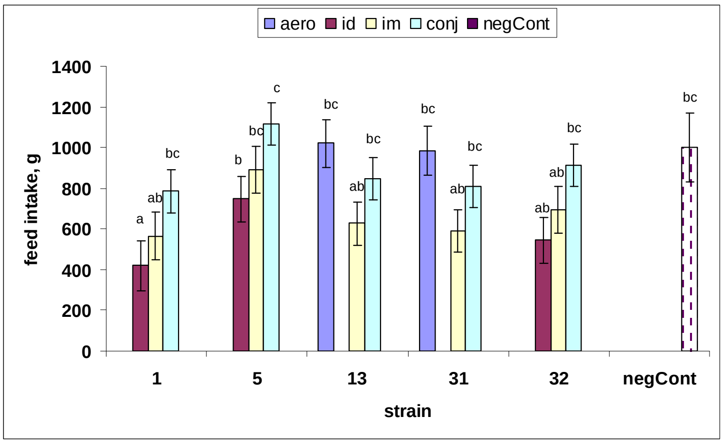

Figure 1: Average feed intake of rabbits experimentally infected with five different *P. multocida* strains and four different routes until day 7th post inoculation

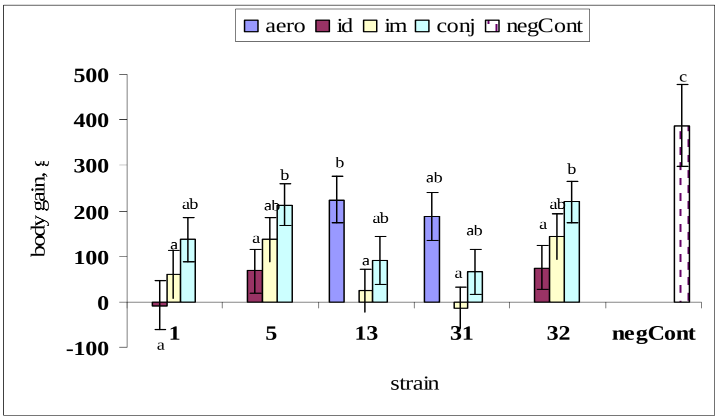

Figure 2: Body weight change of the rabbits experimentally infected with 5 strains of *P. multocida* by aerogen, conjunctival, intradermal and intramuscular route until day 14 p.i.

On the second week the rabbits gained better ($P<0.001$) 156±24 g in average but the differences caused by the way of inoculation remained the same. Weight gain summarized for the two weeks is demonstrating the same tendency (Figure 2) and the highest value was found again ($P<0.05$) at the non-infected control group. As shown on Figure 3, serum antibody level of rabbits infected by im. route increased significantly ($P<0.05$) reaching 200-350% of the level before infection independently from the strain used for inoculation. Inoculation with strain 1 resulted elevated serum antibody level ($P=0.1$) independently from the inoculation route. Elevated serum antibody level was not found in case of further strains with any other inoculation way and nor in negative control rabbits.

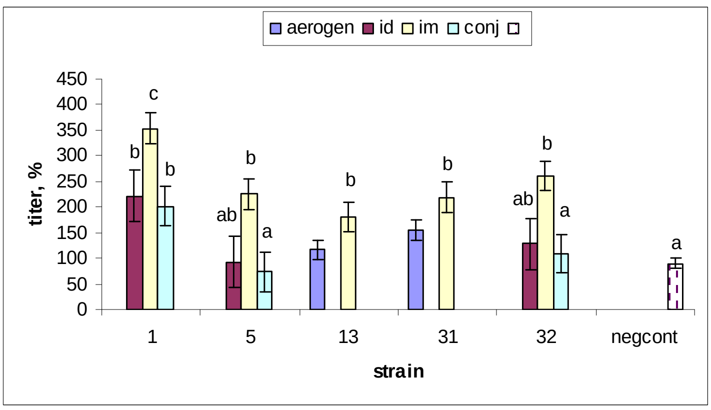

Figure 3: Change of serum antibody titers for rabbits experimentally infected with 5 strains of *P. multocida* by aerogen, conjuntival, intradermal and intramuscular route

CONCLUSIONS

The rabbit virulence of *P.m*. strains collected from chronic forms of disease on farms with enzootic *pasteurellosis* could be best evaluated by im. or id. inoculation, and observation of rectal temperature, apparent clinical signs of illness, *P.m.* reisolation rate and feed intake. Forceful statistical models can facilitate challenge experiments designed with small number of experimental rabbits.

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