Retrospective allergy diagnosis of lysteriosis and lysteria carrying in animals

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Abstract. Experimental infections of guinea pigs and rabbits with a sublethal dose of L. monocytogenes pathogen caused an allergic reaction in the form of delayed-type hypersensitivity (DTHS), which was detected by an intradermal allergy test with a Listeriose allergen. The status of DTHS in guinea pigs and rabbits was recorded for a longer time as compared to specific antibodies. A positive allergic reaction correlated with listeria, which was confirmed by the isolation of a Listeria culture 6 months after infection of rabbits with a virulent Listeria strain. The research findings showed that an intradermal allergy test with a developed Listeria allergen allows a retrospective diagnosis of Listeriosis and Listeria carrying. A specific feature of Listeria allergen was established through an intradermal provocative test in animals sensitized by heterogeneous microorganisms (Salmonella and E. coli).

1 Introduction

Listeria monocytogenes infection (*Listeriosis*) is a naturally-anthropurgic sapronotic widespread infectious disease that affects many species of animals and people. It is characterized by clinical polymorphism from asymptomatic carriage and subclinical to severe generalized forms of meningitis and meningo-encephalitis, nervous system disorders, septicemia, abortions, mastitis and high mortality [1–5].

A *Listeria monocytogenes* pathogen is a Gram-positive multiform non-spore-forming bacterium that is very stable in the environment and has multiple transmission routes [6–9]. A variety of *Listeriosis* clinical manifestations, including asymptomatic course and *Listeria* carrying, as well as certain difficulties in isolating a pure culture, make it difficult to diagnose the disease [10, 11].

Listeriosis affects many animal species, causes great damage to animal husbandry [12] and poses a serious threat to human health. However, methods for detecting *Listeria* carriers and retrospective diagnosis of *Listeriosis* are not well developed. In this respect, allergy diagnosis is of considerable interest.

The endeavors to obtain a specific L. allergen and develop an allergy diagnosis of *Listeriosis*, undertaken by individual researchers [13, 14], showed an allergy-induced transformation of the body infected with *Listeria monocytogenes*. More successful studies were conducted by T.I. Egshatyan [15], but to date, a specific allergen and allergic diagnosis of *Listeriosis* have not been used in veterinary science and medicine.

In view of the foregoing, the aim of the study was to evaluate the effectiveness of *L.* allergen obtained for detecting animals and *Listeria* carriers that had *Listeriosis.*

2 Material and methods

L. allergen was obtained from the AUV L. monocytogenes biomass according to our method. A fraction isolated from the *Listeria* biomass was a polypeptide with a molecular weight of 10 kDa. The allergen was standardized against a protein content (100 μg/ml).

The experiments were performed on outbred guinea pigs with a live weight of 280–350 g and white giant rabbits. The guinea pigs and rabbits were sensitized by infection with a sublethal dose (100 million microbial cells) of the virulent *T-71 Listeria* strain. A suspension of 2-day *L. monocytogenes* agar culture was administered subcutaneously in a volume of 1 cm^3. Then these animals were subject to intradermal allergy testing.

To establish a specific feature of *Listeria* allergen, an allergy test was performed that required administering 0.1 ml of allergen strictly into the dermis layer of guinea pigs infected with *Salmonella* and *E. coli*.

In rabbits, blood for research was taken from the marginal ear vein. A suspension of *Listeria* cells with a concentration of billion microbial cells inactivated by autoclaving at 1 atmosphere for 30 minutes was used as an antigen in the formulation of the agglutination reaction (AR).

3 Results

After 1 month following *Listeria* infection through intradermal L. allergen tests, the reaction was positive in

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all animals. A positive allergic reaction began after 16–18 hours in the area of allergen administration in all animals sensitized by *Listeria*. It was characterized by the development of an inflammatory reaction (hyperemia, edema, soreness, and swelling up), the diameter of which reached 15–20 mm 24–48 hours later. A local inflammatory reaction of the delayed hypersensitivity type was accompanied by a greater thickness of the skin fold by 1.7–2.0 times by 24 hours after injection (Table 1, Fig. 1).

Table 1. Allergic reaction indices in guinea pigs (n = 10)

No.	Group of animals	Skinfold Thickness (mm)			Responded +/–
		Normal	24 hours	48 hours	
1	1 month after L. monocytogenes infecting	1.6±0.3	2.8±0.3	3.3±0.2	10/0
2	1 month after Salmonella infecting	1.6±0.2	1.7±0.2	1.6±0.2	0/10
3	1 month after Escherichia coli infecting	1.6±0.2	1.7±0.2	1.7±0.1	0/10
4	2 months after L. monocytogenes infecting	1.7±0.2	2.9±0.2	3.2±0,3	10/0
5	3months after L. monocytogenes infecting	1.7±0.1	2.6±0.2	2.8±0.2	6/4

Following an intradermal allergen injection, the local allergic reaction reached a maximum level 48 hours later and almost completely died out after 72 hours. After 1 month all L. sensitized guinea pigs gave a positive reaction when tested for *Listeriosis*.

A local reaction to allergen injection was negative in guinea pigs infected with *Salmonella* and *Escherichia coli*, which indicates a specific feature of the allergen and allergic reaction.

Following 2 months after *Listeria* infection, all guinea pigs had a positive reaction to the allergen, and the intensity of the local reaction was at least 1 month later.

After 3 months following *Listeria* infection through intradermal L. allergen tests, the reaction was positive in all animals, 4 out of 10 guinea pigs (40 %) gave a negative reaction, which indicates a gradual allergic response decrement.

This is consistent with immune responses, since allergic reactions such as delayed hypersensitivity are one of the body's immune responses to an antigenic excitator. Therefore, the next series of experiments performed on rabbits, along with allergic reaction, addressed antibody response as well.

To deal with the correlation of allergic reaction with *Listeria* carrying, experiments were performed on five rabbits that were infected with a culture of *T-71 Listeria* strain at a dose of 100 million microbial cells. Then they were examined serologically for an agglutination reaction (AR) and an allergic intradermal L. test (monthly from 1 to 6 months).

The test results are summarized in Table 2 and Fig. 2.

Table 2. Results of allergic test and serological studies of rabbits infected with *L. monocytogenes*

Timeline	Allergy test +/–	AR	
		+/–	Average antibody titer
Reference	0/5	0/5	0
1 month	5/0	5/0	120
2 months	5/0	5/0	78
3 months	4/1	3/2	40
4 months	3/2	0/5	0
5 months	1/4	0/5	0
6 months	1/4	0/5	0

*positive response/negative response

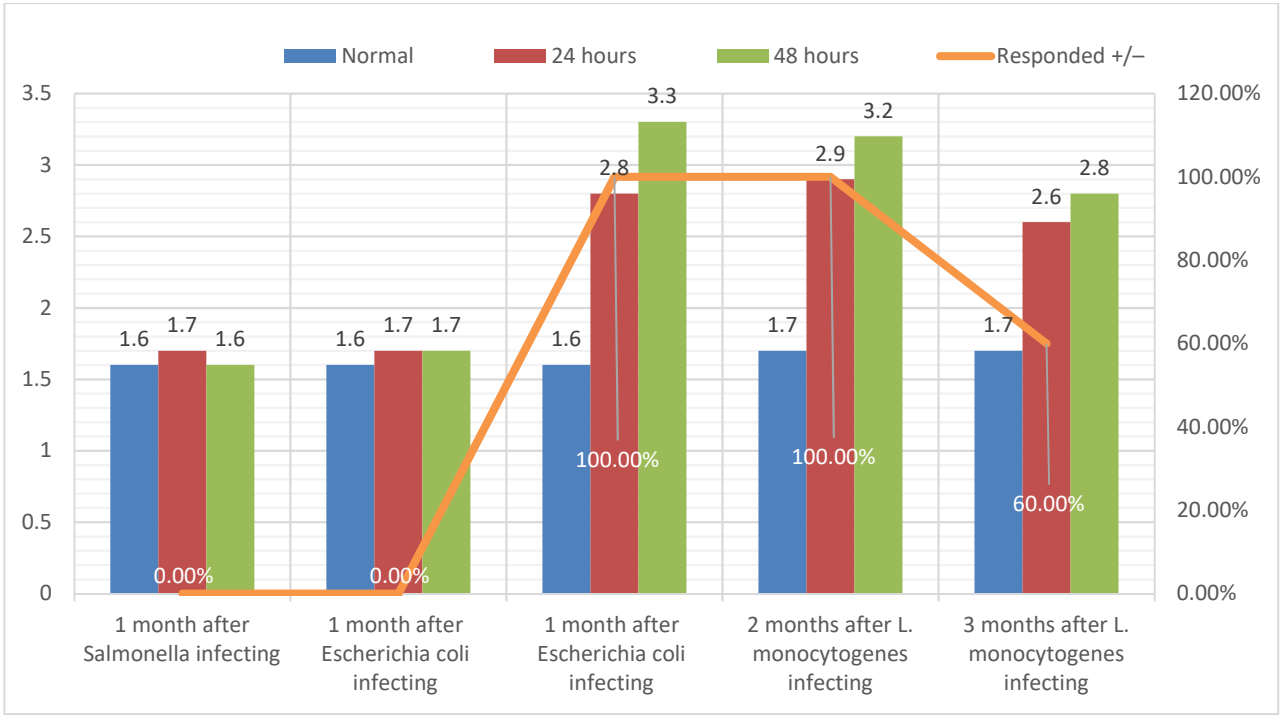

Fig. 1. Indicators of allergic reaction in guinea pigs

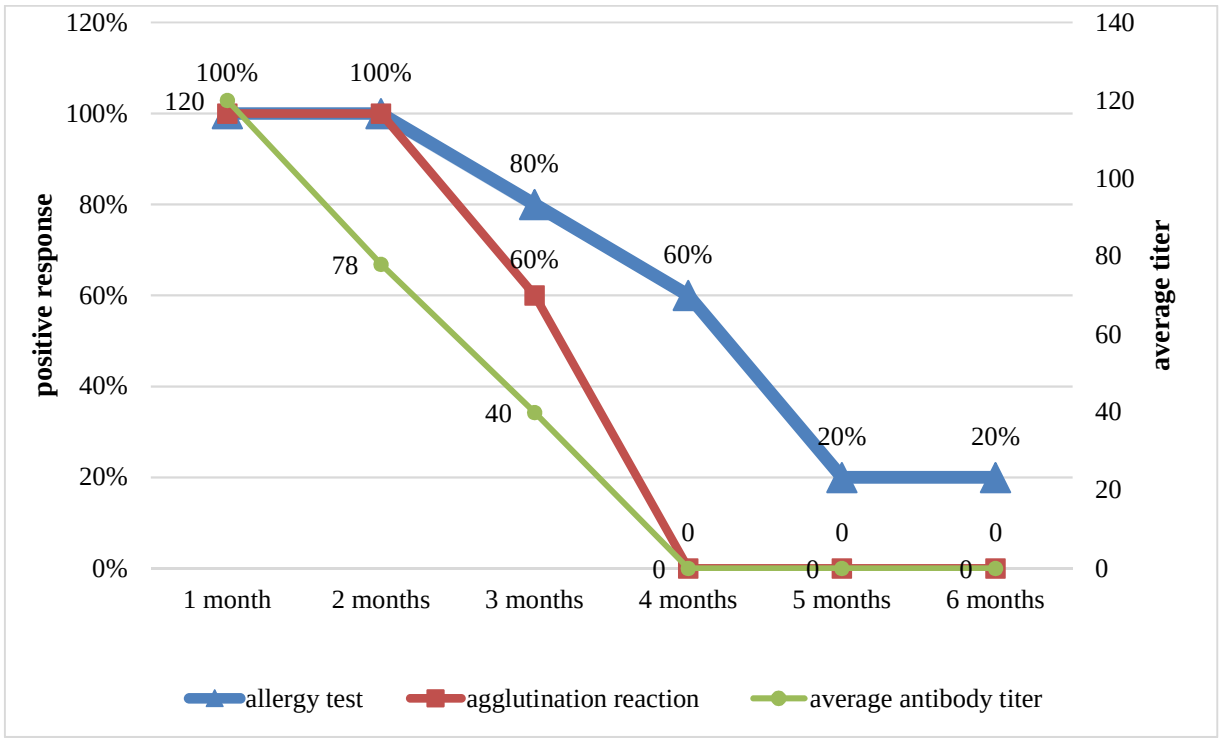

Fig. 2. Results of allergy test and serological studies of rabbits infected with *L. monocytogenes*

Following 1 month after infection, all rabbits responded positively to the administration of allergen, and also had specific antibodies in a titer of 1:120. Antibodies persisted for up to 3 months, albeit in low titers (1:48).

However, after three months, the allergic reaction in four rabbits was positive. After 4 and 5 months, all rabbits in AR responded negatively, whereas the allergic reaction was positive in 60 and 20 %, respectively. 6 months after infection, one of the rabbits alone had an allergic reaction.

During bacteriological studies of pathological material from this rabbit, a *Listeria* culture was isolated from the mesenteric lymph node, which, according to the cultural-morphological properties, corresponded to the initial strain of *L. monocytogenes*.

4 Discussion

When guinea pigs and rabbits were infected with sublethal doses of virulent *L. monocytogenes* strain, no clinical manifestations of *Listeriosis* were observed. However, infecting guinea pigs and rabbits with a *Listeriosis* pathogen was followed by an allergic reaction against delayed-type hypersensitivity, which is one of the forms of immune restructuring.

L. allergen injection was followed by a local reaction after 16-18 hours. It reached a maximum 48 hours later, then faded away almost completely 72 hours later, which is typical of delayed-type allergic reactions. In all guinea pigs, a positive allergic reaction was recorded 1 and 2 months after infection, and after three months, just 60% of sensitized animals responded positively.

An *L.* allergic intradermal test resulted in a negative reaction in guinea pigs infected with Salmonella and E. coli, as well as in intact animals.

Infecting rabbits with a *Listeriosis* pathogen was followed by an allergy-induced transformation of the organism against delayed-type hypersensitivity and the synthesis of specific antibodies. The allergic reaction remained positive in some sensitized rabbits (20%) until 6 months, and antibodies were lost after 3 months.

A *Listeria* culture was isolated from the rabbit that responded positively to an allergen.

The findings indicate that the obtained *Listeriose* allergen and intradermal allergic reaction are highly specific: the intact animals and those infected with *Salmonellosis* and *Colibacteriosis* showed no local reaction after the administration of allergen, as evidenced by the absence of a local positive reaction in animals infected with *Salmonella* and *Colibacteriosis* pathogens.

5 Conclusion

Infecting animals with a *Listeriosis* pathogen causes an allergic reaction against delayed-type hypersensitivity, which is detected by an intradermal test with a *Listeriose* allergen and is highly specific.

The allergic reaction persists for a longer time after infection of animals with *Listeriosis* pathogen in comparison with antibodies and, to a certain extent, correlates with *Listeriosis* carrying.

Thus, the results indicate the possibility of a retrospective diagnosis of *Listeriosis* and *Listeriosis* carrying in animals with an allergy test. The allergen is based on the method developed by the authors.

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