The methods for determining the purity, biochemistry, virulence of *Escherichia coli* strains

Typical *E. coli* strains (Ch-O111-1) and pathogenic *E. coli* strains (LZ06) have been screened for purity in accordance with the current "Chinese Veterinary Pharmacopoeia" purity test method.

Typical *E. coli* strains (Ch-O111-1) and pathogenic *E. coli* strains (LZ06) were subjected to biochemical identification, including oxidase test, sugar fermentation test: glucose, lactose, mannitol, arabinose, maltose, xylose, cottonseed sugar, malonate, citrate, sorbitol, lateral marigold alcohol, methyl red test, phenylalanine dehydrogenase test, hydrogen sulfide test, nitrate reduction reaction, coagulase reaction, DNA enzyme test, contact enzyme reaction, gelatin liquefaction reaction, urease test, V-P test, indole test. The standard strain of *E. coli* CVCC1546 was also selected as the positive strain control. The above tests were carried out using the *E. coli* biochemical identification kit.

The *E. coli* strains (Ch-O111-1) isolated for vaccine study were inoculated with TSB medium and incubated at 37°C for 16 h. The bacterial solution was serially diluted 10-fold, and the appropriate dilution was selected for live bacterial enumeration by surface culture assay, and the enumeration method was performed with reference to the appendix of the current Chinese Veterinary Pharmacopoeia. The bacterial solution was diluted 10 times with sterilized 0.01M PBS, injected from the highest dilution, and 10 SPF grade Balb/c mice (20-22g) were injected intraperitoneally at each dilution, 0.2ml/mice, and the mice were observed continuously for 14 days, and the death of mice was recorded. The LD_{50} was calculated according to the Reed-Muench method.

The virulent *E. coli* strain (LZ06) was inoculated with TSB medium and incubated at 37℃ for 16 hours, sterilized 0.01M PBS 10-fold ratio dilution of the bacterial solution, and the appropriate dilution was selected for viable bacteria counting by surface culture assay, and the counting method was the same as above. The bacteriophage solution was diluted 10-fold, and 10 Balb/c mice (20-22g) were injected intraperitoneally(i.p.) at 0.2ml each from the highest dilution, and observed continuously for 14 days. Mouse deaths were recorded, and the LD_{50} was calculated according to the Reed-Muench method.
In addition, the bacterial solution was diluted with sterilized 0.01M PBS into 2×10^3, 1×10^3, 0.5×10^3 CFU/ml bacterial suspension, and 5 healthy cows (2~3 years old, 10~15 days before parturition, *E. coli* mastitis IgG antibody potency not higher than 1:200) were injected with different dilutions of the bacterial solution intramamammary(i.m.), 1 ml/cow. Cows were observed for 14 consecutive days after injection, and their reactions were closely observed 24 hours after injection, and their body temperature, bacterial count and somatic cell count in milk samples were tested every 3 hours.

Conductivity testing of milk

We measured milk conductivity (a proxy for somatic cell count) in the challenged milk area by DRAMINSKIMA-I somatic cell counter.