# A rapid diagnosis and treatment of Ornithonyssus bacoti infection

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# Abstract

Mites serve as pathogens, allergens, or microbial containers, which can seriously damage the health of humans and animals. The substantial amount of mite species and their similar morphology make it complicated to identify and classify. Our mouse breeder incidentally noticed papular-type erythema with itching and peeling of the skin in several places, and an investigation revealed that this symptom was caused by an uncommon parasite that appeared on the skin and around the nest of the mice. By morphological observation, DNA extraction, PCR amplification, and DNA sequencing, we roughly identified the category of the parasite as a mite. We then designed a specific primer Cox1, amplified and sequenced the mitochondrial cox1 gene fragment of the mite, calculated the intra and interspecific differences, and reconstructed the phylogenetic tree for comparative analysis. We named this species Ornithonyssus bacoti - KF. According to the ivermectin gradient test, we found that 0.1 mg/ml concentration of ivermectin solution was the most effective for mite removal in the bath, with no recurrence after 6 months of treatment. This study provides a new specific primer sequence for the detection of Ornithonyssus bacoti, which provides a new method for the rapid identification of Ornithonyssus bacoti. This study provides accurate diagnosis and precise treatment for the feeding and breeding of rodents to prevent mite infection.


# Introduction

Mites are tiny arachnids with diverse species, with 500,000 to 1 million species of mites estimated to be present on Earth. Mites inhabit a diverse assemblage of ecological niches and lifestyles, ranging from free-living, predatory or phytophagous, to exclusively parasitic (Fischer and Walton 2014). A variety of mites, including the Sarcoptes scabiei, Notoedres cati, Cheyletiella spp, Dermanyssus gallinae, Ornithonyssus bacoti, Ophionyssus natricis and Neotrombicula autumnalis can attack the surface of human skin and cause discomfort (Beck and Pfister 2006). Mites can parasitize vertebrates, including domestic animals, birds, rodents, and other wildlife. As a highly efficient vector for the transmission of pathogens, transmitting various important pathogens in medicine and veterinary medicine (Wu et al. 2019). Studies in recent years have shown that many infectious diseases caused by the bite or mechanical transmission of mite vectors, such as scrub typhus, viral hemorrhagic fever renal syndrome (HFRS), and plague, have posed a serious threat to human and animal health (Jiang et al. 2017).

The accurate identification of mite species is of great importance for scientific research and clinical disease treatment. The complex taxonomy of mites is largely based on morphology, which is often suspect. For microarthropods, it is more difficult to obtain data dependent on ecological, behavioral, or internal characteristics, and therefore species delimitation and identification are mainly based on the identification of external morphology. However, species formation and morphological differences are not always accompanied by a relationship (Bickford et al. 2007), and therefore, determining and delimiting species boundaries based on morphological criteria alone is not sufficiently accurate and reliable. In addition, the morphological identification of mites is challenging. This is due to the wide variety of mite species, their large numbers, diverse phenotypes, morphological similarities, and the scarcity of molecular

data as well as the small number of specialized researchers, and the fact that mites in the worm or larval stages often lack distinct morphological features (Zhao et al. 2020). Molecular identification is based on the complement and validation of morphological identification, and an accurate molecular diagnostic tool for species identification can be of great value. With the continued development of molecular biology techniques, we can identify and determine the phylogenetic relationships of a range of mite species simply and rapidly using DNA sequence analysis (Ernieenor et al. 2018). Polymerase chain reaction (PCR) is a polymerase chain reaction technique that has the advantages of speed, high sample volume, and low cost. And it has been found to be useful for the identification of a variety of important mite species. The most commonly used molecular markers in mite identification and phylogenetic studies are the internal transcribed space 2 (ITS2) of the rDNA region and the partial sequence of mitochondrial cytochrome oxidase subunit I (cox1, commonly referred to as COI in barcoding studies) (de Rojas et al. 2002; Ben-David et al. 2007; De Rojas et al. 2001).

In this study, morphological identification of the collected unknown insect species was firstly performed, and it was tentatively inferred to be mites. Subsequently, DNA extraction was then performed and the sequences were compared by PCR amplification with known mite universal primer sequences. On this basis, primers specific for the O-cox1 mtDNA region and the O-ITS rDNA region were designed, amplified, and then sequenced again for sequence comparison, and identified as the Ornithonyssus bacoti. According to the ivermectin gradient test, we found that 0.1 mg/ml concentration of ivermectin solution was the most effective for mite removal in a pharmacological bath. This study provides accurate diagnosis and precise treatment for rodent feeding and breeding to prevent mite infection.

## Materials And Methods

# Collection of mite samples

Mite samples were collected from mice skin and around the cage using the transparent tape application method and placed into vessels containing PBS buffer. Thirty mites were collected per dish, 10 dishes were collected, one dish was used to observe the morphology, and the rest were frozen and stored for subsequent identification experiments. The mites in the vessels were placed in the middle of the slides with sterile forceps in advance by adding a small drop of PBS buffer, and the morphology of the mites was observed under the light microscope to identify the species and photographed for retention.

## Dna Extraction

The collected mite microsomes were put into sterile EP tubes, and each tube was added with the appropriate amount of completely prepared Lysis buffer (containing proteinase K, 25 μl/ml), and the tissue was fully digested in a warm bath at 55°C for 3h; the supernatant was centrifuged, and 1/2 volume of saturated NaCl was added, mixed by turning up and down, and centrifuged; the supernatant was transferred, and 2 times the volume of 100% ethanol was added, and placed in a -20℃ Centrifuge,

discard the supernatant, add the appropriate amount of pre-cooled 70% ethanol (95% ethanol for the third time) and wash the DNA, repeat the operation three times; centrifuge, discard the supernatant, place the supernatant on an ultra-clean table and dry for 20 min, add 15 μl of 1*TE buffer, and dissolve the DNA at room temperature for 1 h. The DNA was fully dissolved using a Thermo Fisher NanoDrop-2000 spectrometer. The purity and concentration of DNA were determined using a NanoDrop-2000 spectrometer, and the amount of DNA extracted from each tube was calculated.

## Pcr Amplification

PCR was performed on a BioRAD PCR amplifier. DNA template 6 μL, 10×Buffer 3 μL, dNTP 3 μL, F1/R1 (10 μmol/L) 0.36 μL each, Taqase 0.36 μL, ddH2O 16.92 μL. PCR reaction conditions: 94 ℃ for 3 min; 94 ℃ for 30 s, 52 ℃ for 60 s, 72 ℃ for 60 s, 40 cycles; final extension at 72 ℃ for 2 min. The amplified products were electrophoresed on 1.8% agarose gel, stained with ethidium bromide, observed by a UV transmission detector, and photographed for retention.

## Dna Sequencing

All PCR products were sent to Biotech Bioengineering Co., Ltd (Shanghai, China) for non-purified DNA sequencing. All specimens were sequenced in both directions and the primer combinations for this step had the same combinations as those used in PCR amplification.

## Gene Sequence Analysis

Species were identified by BLAST analysis of amplicon sequences based on their coverage and homology to the corresponding sequences in the GenBank database. The most relevant sequences were searched in GenBank on the NCBI website for comparative analysis, and the phylogenetic tree was reconstructed using the maximum likelihood method (ML), homology analysis, and evolutionary tree construction using MEGA 11.0 software. The ML tree was reconstructed using the Kimura 2-parameter (K2P) model in MEGA 5.0, and the reliability of the nodes was tested by bootstrap analysis using 1,000 replicates. The phylogenetic tree was visualized with tree diagram 2.

## Ivermectin Gradient Test

Ivermectin injection (10 mg/ml), produced by Henan Province Huazhou Biotechnology Co. 0.1mg/ml, 0.05mg/ml, and 0.02mg/ml concentrations of ivermectin solution, distilled water was used as the control group, and three replicate tests were done using 6cm diameter vessels with 30 Ornithonyssus bacoti per vessel. One live mite examination was performed respectively at 1st, 2nd, 4th, 6th, and 8th after drug administration. The mites were observed under a low magnification microscope and those seen to be

active were judged to be positive, otherwise, they were negative. The average number of dead mites each time was recorded.

## Results

# Morphological identification results

Under the optical microscope, the adult Ornithonyssus bacoti was found to have four pairs of legs with pincer-like chelicerae, a relatively wide posterior part of the body, and dense epidermal setae on the back. The crawling speed was fast, and the mite was dark gray in the starved state and dark red after sucking full blood (Fig. 1).

## Electrophoretic Analysis Of Pcr Products

The PCR amplification was carried out with the extracted genomic DNA of the worm as the template and F1/R1 as the primer, and the amplified fragments obtained were consistent with the expected results. The success rate of amplification was 99%.

## Dna Sequencing And Sequence Analysis Results

The mitochondrial DNA of the mite was PCR amplified by designing primer Cox1. The primer sequences are shown in Fig. 2. Nucleotide BLAST of the generated sequences in the NCBI GenBank database confirmed the identity of the species, as all the highest hits were Cox sequences of the Ornithonyssus bacoti. Among them, two sequences (GenBank: FM179677.2, MH553336.1) were more than 98% similar to this species, one sequence (GenBank: MH553338.1) was more than 97% similar to this species, and the remaining sequences were more than 80% similar. Therefore, the first three sequences were compared with this species by individual sequence comparison, and the sequence identity was extremely high as seen in Fig. 3. The coverage scores of all sequences mostly exceeded 90%. From the homology results, the homology rate was > 97% with Ornithonyssus bacoti and > 85% with Ornithonyssus sylviarum. The maximum likelihood (ML) method was used to reconstruct the phylogenetic tree as shown in Fig. 4.

## Results Of The Ivermectin Gradient Test

As can be seen from Fig. 5, the mortality rate of all three different concentrations of treated Ornithonyssus bacoti exceeded 50% two hours after dosing. After 8 hours of dosing, all of the 0.1 mg/ml concentration group died; the 0.05 mg/ml concentration group had a 90% kill rate; the 0.02 mg/ml group had an 87.5% kill rate, and all of the control group survived. The mortality rate of live mites in the experimental group gradually increased with the increase of the dosing time. It can be seen that 0.10 mg/ml ivermectin solution had the best insecticidal effect. In addition, the rate of drug lethality decreased significantly in the latter 4 hours. Finally, we selected a concentration of 0.1 mg/ml of ivermectin solution

to exterminate the Ornithonyssus bacoti in the environment for more than eight hours of treatment. The positive rate of Ornithonyssus bacoti in the environment was reviewed after 1 month, 2 months, 3 months, and 6 months, respectively, and the results were negative. It indicates that ivermectin at this concentration has a good mite removal effect.

# Discussion

The Ornithonyssus bacoti, also known as the tropical rat mite. It is a specialized hematophagous mite with a high reproductive capacity and resistance to starvation, as well as a long life span. It is a zoonotic ectoparasite that often parasitizes rodents and other small mammals that feed on blood, such as rats, mice and voles, and tree shrews, and is often found on the surface of the body and in the nests of the host, causing abnormal symptoms such as weight loss, wounds, and loss of hair on the back. Under certain conditions, Ornithonyssus bacoti can spread from rodents to their temporary hosts, humans, and bite the skin, causing papular erythema, itching, and peeling, and affecting people's daily life (Beck 2008). Previous studies have reported the detection of Bartonella, Coxella beccinalis, and Rickettsia Riccifera in Ornithonyssus bacoti (Reeves et al. 2007). Therefore, effective identification of medically important mites is essential to control the development of mite-borne diseases.

The use of DNA sequences for specimen identification began in the 1980s (Kloos and Wolfshohl 1982; Rollinson, Walker, and Simpson 1986). Exponential advances in molecular biology have so far solved countless organism identification problems. DNA barcoding is a diagnostic technique for species identification using sequential diversity sequences of short, standardized genetic regions. DNA barcoding is an important tool in taxonomy and phylogenetic research, and it has become a practical method to identify specimens and measure species diversity. In recent years, there has been an increasing number of research using DNA barcoding to solve different biological problems in different disciplines, such as species discovery, specimen identification, species definition, etc (DeSalle and Goldstein 2019). The main advantages of DNA barcoding are the following: (i) providing standardized and high-tech identification tools to help identify species at any life stage, such as parasites, endangered species, pests, etc., enabling more taxonomists to easily and accurately obtain systematic analyses; (ii) better delineation of taxa and discovery and description of new species through cluster analysis based on gene sequences; (iii) facilitating the development of DNA sequencing technology in the field of biodiversity (Savolainen et al. 2005). In conclusion, DNA sequencing technologies can enable us to better understand biodiversity. However, for unknown species, DNA barcoding has some limitations. In other words, DNA barcoding can only be classified by matching the DNA sequence of known species. In this case, DNA barcoding serves only as a tool (Hebert et al. 2003).

Many earlier studies in the literature have shown that rDNA ITS2, mtDNA 16S, and cox1 have been widely used for molecular classification and identification of many lower taxonomic species (Marcilla et al. 2001; Burger, Shao, and Barker 2014; Matzen da Silva et al. 2011). The most widely used noncoding region in mites is the partial sequence of mitochondrial cell COI in the second internal transcriptional septal region of the ribosomal gene cluster (ITS2). ITS2 consists of three genes, 18S rDNA, 5.8S rDNA,

and 28S rDNA, which are transcribed into RNA but do not undergo the translation process. Since these three rDNA genes are not overly versatile, the likelihood of these sequences being selected is very low, allowing for rapid accumulation of replacement (CRUICKSHANK 2002). This is very effective for the identification of low taxonomic species. The 28S rDNA structural domains jD5, jD6, and jD8 were identified as universal DNA barcodes for molecular classification and identification of mites. Thus, these three structural domains have their advantages and disadvantages. Their simultaneous use can effectively improve recognition efficiency. Among the different DNA regions used in molecular systematics, the mt DNA gene is a suitable tool for intraspecific variation and species identification due to its relatively high evolutionary rate and strict matrilineal inheritance. In addition, because the mitochondrial genome has a high copy number in most cells, molecular diagnostic tools targeting mitochondrial sequences may have higher detection sensitivity than those targeting nuclear genes (CRUICKSHANK 2002). For animals, the major DNA barcodes are the mitochondrial. The 658 base pair (bp) region of the gene cytochrome, cox1. mitochondrial cytochrome oxidase subunit 1 is considered a good candidate for barcoding animal species (Hebert et al. 2003).

In this study, we first designed primers for the 28S rDNA structural domains D5, D6, and D8, and the PCR amplified products were stained with ethidium bromide after agarose gel electrophoresis and observed in a UV transmission detector, and the species was tentatively identified as a mite based on the positive bands shown. Subsequently, we amplified, cloned, and sequenced the mitochondrial cox1 gene fragment of the mite, calculated the intra- and interspecific differences, and reconstructed the phylogenetic tree for comparative analysis before identifying the species as Ornithonyssus bacoti.

As we know, ivermectin is a new type of antiparasitic drug that can kill a variety of parasites and vector organisms in vivo and in vitro, with the characteristics of broad spectrum, high efficiency and low toxic side effects. Therefore, in response to the sudden appearance of Ornithonyssus bacoti, we selected ivermectin solution at a concentration of 0.1 mg/ml to treat the infected mice with a drug bath, and used the same concentration solution for the complete disinfection of the surrounding environment. The positive rate of cypress avian spiny mite in the environment was reviewed after 1 month, 2 months, 3 months and 6 months, respectively, and the results were negative. The good mite removal effect of ivermectin was further confirmed.

# Conclusion

This study provides a new specific primer sequence for the detection of the Ornithonyssus bacoti, which provides a new method for the rapid identification of the cypress avian spider mite. It also provides accurate diagnosis and precise treatment for rodent feeding and breeding to prevent mite infection. Due to their high mobility, high fecundity, and high viability, Ornithonyssus bacotis are highly susceptible to become potential invaders in rodent feeding facilities. Therefore, we believe that this study will provide some value for similar mite invasion cases in the future.

# Declarations

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### Conflicts of interest

The authors declare that there is no conflict of interest.

### Availability of data and material

All the data generated or analyzed in this study are contained in this article.

### Authors' contributions

Huimin Xu drafted the initial article;Ying Wang and Jiaqi Fang reviewed and revised the article; Jiaxin Wang conducted the figures. Yun Zhou and Weiguo Li guided the writing.

### Ethics approval

The protocol was approved by Committee of Medical Ethics and Welfare for Experimental Animals, Henan University School of Medicine, in line with the "Guidelines for the Care and Use of Laboratory Animals" published by NIH.

### Consent to participate

The authors consented to participate.

### Consent for publication

The authors agreed to publish this paper.

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# Figures

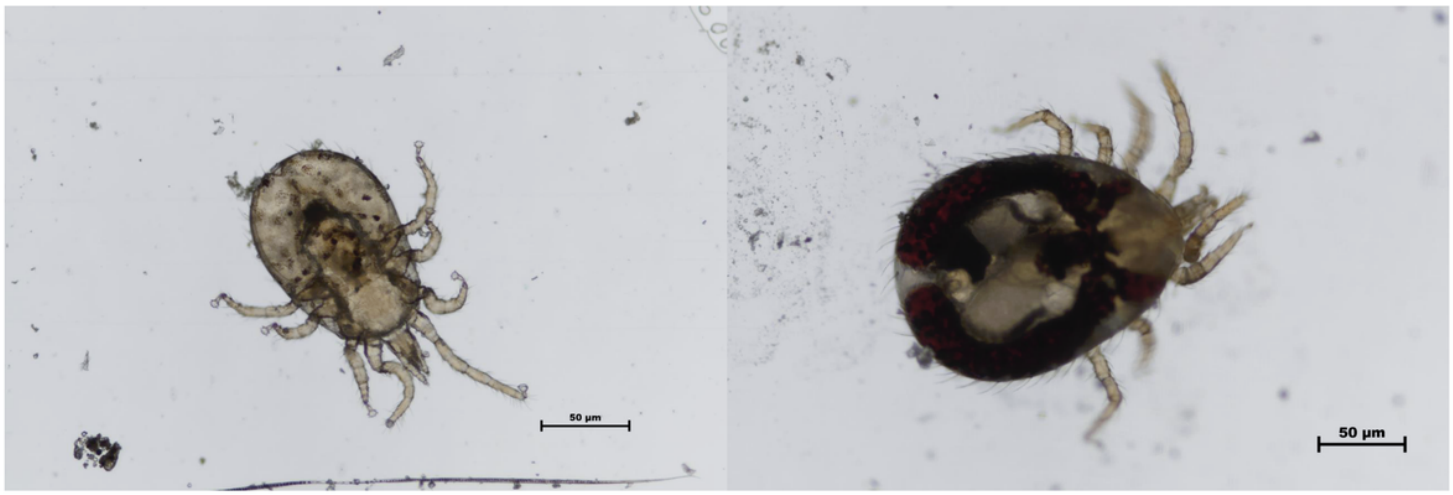


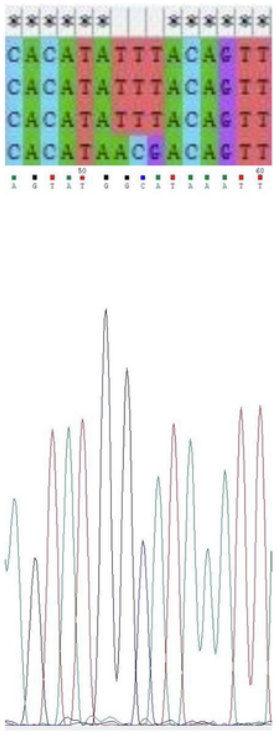

**Figure 1**

a. Microscopic form of Ornithonyssus Bacoti before satiety (10×10); b. Microscopic form of Ornithonyssus Bacoti after satiety (10×10).

| Gene Fragment | Length(bp) | Primers(5'-3') | Tm(℃) |
|---|---|---|---|
| jD-5 | 419 | F: TTC TGA CGT GCA AAT CGA T | 55.25 |
| | | R: GGC AGG TGA GTT GTT ACA CA | 58.04 |
| jD-7 | 465 | F: CAG ATC TTG GTG GTA GTA GCA | 56.83 |
| | | R: CCT TGG AGA CCT GCTGC | 57.19 |
| jD-8 | 314 | F: GCA KCAG GTC TCC AAG G | 54.00 |
| | | R: GTT TTA ATT AGA CAG TCG GATTC | 53.59 |
| COX1 | 410 | F: ACAATCTGTAACATACGCCCA | 57.38 |
| | | R: GCTCGTGTATCAATATCTAGTCCA | 57.74 |

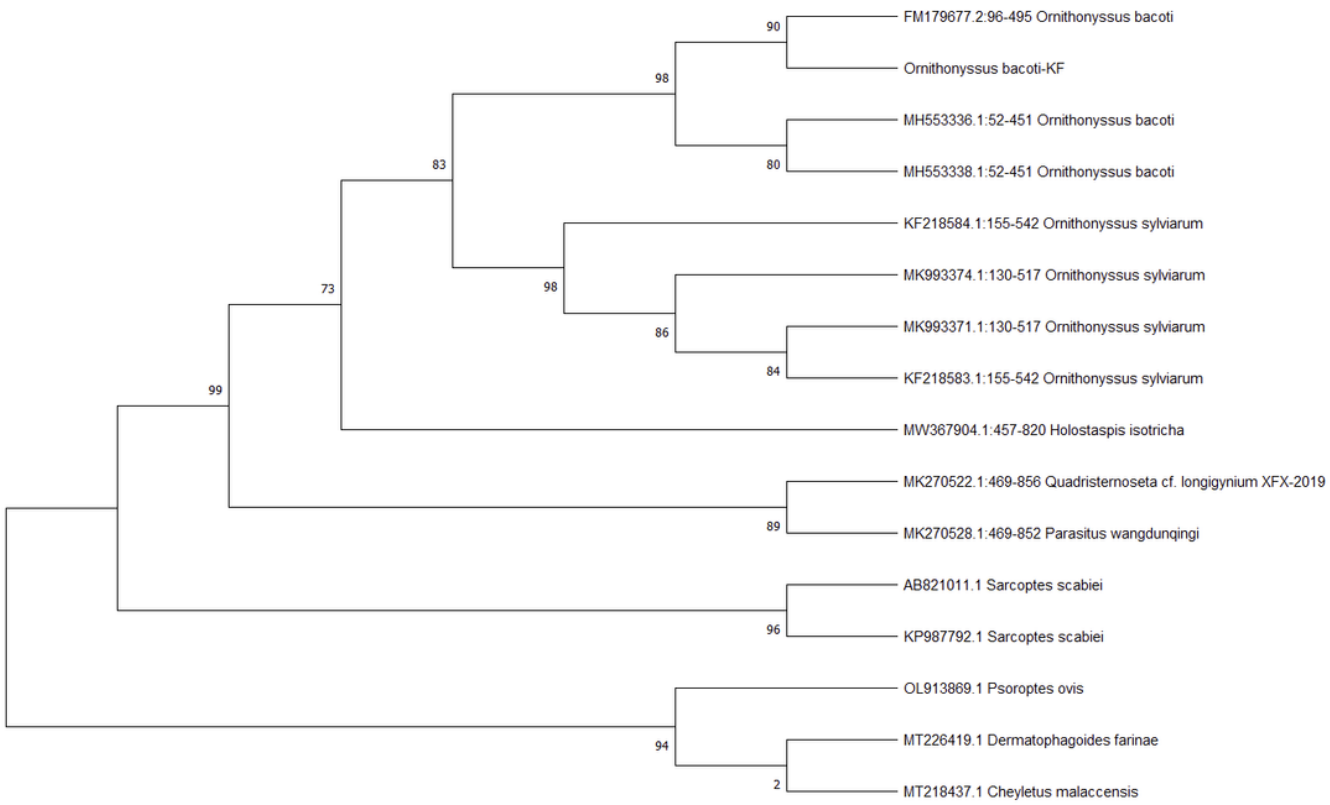
## Figure 2

Universal or degenerate primers for divergent regions of 28S rDNA for mite species and specific primers for COX1 mtDNA for Ornithonyssus Bacoti.




## Figure 3

Sequence comparison of FM179677.2, MH553336.1, and MH553338.1 with the sequence of Ornithonyssus bacoti in Genebank.




## Figure 4

Phylogenetic tree reconstruction using maximum likelihood (ML) method for similar species.

| Groups (mg/ml) \ Fatality rate(%) | 1h | 2h | 4h | 6h | 8h |
|---|---|---|---|---|---|
| 0.00 | 0 | 0 | 0 | 0 | 0 |
| 0.02 | 17.5 | 50 | 60 | 75 | 90 |
| 0.05 | 17.5 | 57.5 | 72.5 | 85 | 87.5 |
| 0.10 | 27.5 | 60 | 87.5 | 90 | 100 |

## Figure 5

The insecticidal rate of ivermectin at different gradient concentrations was calculated at 1 h, 2 h, 4 h, 6 h and 8 h after administration, respectively.