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ORIGINAL PAPER

The effect of temperature on the viability of *Demodex folliculorum* and *Demodex brevis*

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Abstract *Demodex folliculorum* and *Demodex brevis* are obligatory parasites of the pilosebaceous unit in humans and are cosmopolitan in terms of their distribution. This study was conducted to explore the effect of temperature on the viability of *D. folliculorum* and *D. brevis*. Both types of parasites were collected with the cellophane tape method, then randomly grouped and placed into separate moist cabinets. They were divided into 15 groups and exposed to experimental temperatures ranging from −15°C to 60°C. Curve diagrams and scatter plots on the relationship between temperature and the corresponding survival time were drawn and analyzed. It is demonstrated that temperature has a tremendous influence on the viability of *D. folliculorum* and *D. brevis*. Survival time and temperature are inversely correlated in the temperature range of 5–37°C. Both *D. folliculorum* and *D. brevis* can survive better at low temperatures than at high temperatures. The most suitable maintenance temperature is 5°C, and the optimal temperature for *D. folliculorum* and *D. brevis* to develop in vitro is 16–20°C. Temperatures below 0°C and above 37°C are harmful to the mites. The lethal temperature is 54°C, and the effective temperature that kills *Demodex* mites is 58°C.

Introduction

Demodex folliculorum and *Demodex brevis*, two types of parasitic *Demodex* mites in humans, have been found in almost all age and racial and geographical groups. They live inside the sebaceous glands and hair follicles, sucking nutrients from the hair roots and damaging the cell walls. Previous research has indicated that *D. folliculorum* and *D. brevis* are conditional-pathogenic parasites. Severe infestation of *D. folliculorum* and *D. brevis* in the skin and long-time infection lead to disorders combined with a weakened immune system. In the past decades, interest in *D. folliculorum* and *D. brevis* has grown considerably, especially in their pathogenicity. Progressively, more epidemiological investigations demonstrate a very close link between *Demodex* infestation and facial diseases such as rosacea (Erbagci and Ozgoztasi 1998; Powell 2004; Moravvej et al. 2007), eruptions resembling rosacea (Georgala et al. 2001), pityriasis folliculorum (Garcia-Vargas et al. 2007), and blepharitis (Anane et al. 2007). However, there are still few studies on the ecology of *D. folliculorum* and *D. brevis*, and maintenance in vitro has not been successfully achieved. Empirical studies lack large numbers of standard *D. folliculorum* and *D. brevis*, which critically restricts further study of their pathogenicity. For a long time, the only means to obtain *D. folliculorum* and *D. brevis* samples was to conduct a census using the cellophane tape method, which is time-consuming and labor-intensive. In addition, the *D. folliculorum* and *D. brevis* obtained in this manner may not meet the requirements for standard experiments and cannot be kept for long due to their aptness to die.

For the past few years, we have been engaged in studying the tolerance of the mites to various environmental conditions so as to find a proper maintenance method. In this study, we report the effects of temperature on the motility and the survival time of both *D. folliculorum* and *D. brevis* in vitro. The aim of this study is to establish the conditions needed for longer preservation and maintenance of *D. folliculorum* and *D. brevis* in vitro so as to permit extended periods of investigation of viable specimens potentially leading to prevention and control of the parasites.

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Materials and methods

Mites

The mites were collected from volunteers of undergraduates between the ages of 18 and 23, using the cellophane tape method (Wu 2005). In *Demodex* studies, it is a simple and straight-forward test and widely accepted in the mainland of China. In this procedure, the subjects' face is washed with warm water, after which, 2×7 cm pieces of cellophane tape are applied to the forehead, cheeks, nose, and chin just prior to night sleep. In the morning, the tape is removed and pressed onto slides for observation and counting by light microscopy. The mites were randomly divided into groups.

Informed consent was obtained from the volunteer human subjects used in this study. This study was approved by the Ethics Committee of Xi'an Jiaotong University School of Medicine.

Groups

The experimental temperatures ranging from −15°C to 60°C were classified into three groups—low temperature (−15–0°C), moderate temperature (5–37°C), and high temperature (45–60°C)—which were further divided into 15 subgroups. Preliminary experiments were conducted first, and a suitable observation interval time was fixed according to the maximum and the minimum survival time of *D. folliculorum* and *D. brevis* There were at least ten mites in each group, and each group was observed at least three times.

There were three low temperature subgroups, of −15°C, −5°C, and 0°C. The mites were kept in an ice box in the freezer compartment of a refrigerator. Observation was carried out every hour for the first two groups and every 2 h for the 0°C group. Before each observation, the mites were placed at 16–20°C to reach anabiosis, which was critical for the observation.

The moderate temperature group contained seven subgroups, with temperatures at 5°C, 8–10°C, 16–20°C, 25–26°C, 29–30°C, 32–33°C, and 36–37°C. The mites in the first two subgroups were placed in the moist cabinet in the fridge and were observed every 4–6 h before anabiosis. The 16–20°C subgroup was placed directly on the bench (the natural temperature difference of the laboratory was detected with thermometer every day during the study), and the other mites were all placed in wet boxes and then put into incubators. The regular interval time between observations was 6–8 h.

The high temperature category contained subgroups at temperatures of 45°C, 54°C, 56°C, 58°C, and 60°C. The mites were placed in wet boxes first and then in thermostatic water bath boxes under different conditions. Observations were performed every 30, 3, 2, 1, and 1 min, respectively, at room temperature. For immobile mites, a second observation was needed after anabiosis.

Motility assessment criteria

The mites' motility was categorized into five categories. It was necessary to reach anabiosis before the motility assessment. Anabiosis consisted of putting the mites at 16–20°C for 30 min, especially for those mites that were in the low and high temperature groups.

"–": If the mite's chelicera or legs remained motionless for 1 min, a second observation was performed after 30 min. If the mite was still motionless, it was considered to be dead;
"±": The mite was motionless after being placed at low temperature for 4 h but began moving after anabiosis;
"+": Moved weakly; chelicera or one to two legs moved one to two times per minute;
"++": Moved obviously; chelicera or three to five legs moved three to five times per minute;
"+++": Moved actively; chelicera or six to eight legs moved more than six times per minute.

Curve diagram and scatter plot

The curve diagram of survival time (median) for both *D. folliculorum* and *D. brevis* was drawn to show the influence of temperature on the two types of mites. The scatter plot of survival time (median) at temperatures between 5°C and 37°C was plotted to analyze the association between temperature and survival time, and the correlation coefficient and regression equation were obtained.

Data analysis

Statistical Package for the Social Sciences 11.5 statistical software was used to carry out the H test on independent multi-samples with correlated data.

Results

The effect of temperature on *D. folliculorum*

Survival time of D. folliculorum at different temperatures

The survival time of *D. folliculorum* differed significantly depending on the various temperature treatments (Table 1). Under the low temperature conditions (ranging from −15°C to 0°C), the mites' survival time decreased as the temperature decreased. They could only survive 5.5 h at −15°C, which was significantly shorter than the survival

times for the −5°C and 0°C subgroups (χ^2=75.807, P< 0.01). There were significant differences in survival time between the seven moderate temperature subgroups at 5–37°C (χ^2=12.667, P<0.01). The higher the temperature, the longer the mites survived. The optimum survival temperature for *D. folliculorum* was 5°C, with a duration of 110 h. However, the median survival time at 5°C was not significantly different from that at 8–10°C or 16–20°C (χ^2=5.627, P>0.05). Survival time was significantly longer at 16–20°C than at 25–26°C and 29–30°C (χ^2=23.937, P< 0.05). As the temperature rose to 36–37°C, the survival time decreased substantially to 16.5 h. There were also significant differences between the high temperature subgroups (χ^2=172.489, P<0.01). The mites could survive for 90 min at 45°C, but survival time decreased sharply to 5 min when exposed to 54°C. The difference between the two groups was significant (χ^2=38.278, P< 0.01). When the temperature rose to 56°C, 58°C, and 60°C, the survival time of *D. folliculorum* was just 3, 1, and 1 min, respectively. There was no significant difference between the 58°C subgroup and the 60°C subgroup (χ^2=0.024, P>0.05).

Motility of D. folliculorum after being kept at −15–37°C for 4 h

D. folliculorum kept at different temperatures ranging from −15°C to 37°C exhibited differences in motility after 4 h in a moist chamber (Table 2). At low temperatures, ranging from −15°C to 10°C, the mites hardly moved but did not die immediately. When returned to a suitable temperature, motility was restored. The motility increased as the temperature increased. *D. folliculorum* performed well at 16–20°C and above. Motility was mainly + to ++ in the 16–20°C subgroup, ++ to +++ in the 25–26°C subgroup, and +++ in the 29–30°C subgroup. When the temperature was increased to 37°C, the mites were extraordinarily active, with a motility index of +++, and many of them even crept.

The effect of temperature on *D. brevis*

Survival time of D. brevis at different temperatures

Temperature obviously influenced the survival time of *D. brevis* (Table 3). In the three low temperature subgroups, the shortest survival time (5 h) occurred at −15°C, where this survival time was remarkably shorter than the survival time of the −5°C and 0°C subgroups (χ^2=61.473, P<0.01). Differences were also found between the seven moderate temperature subgroups (χ^2=165.145, P<0.01). *D. brevis* survived the longest, 145 h, at 5°C. There was no remarkable difference between subgroups 8–10°C and 16–20°C (χ^2=0.752, P>0.05). However, significant differences were found between the 16–20°C, 25–26°C, and 29–30°C subgroups (χ^2=80.961, P<0.01). The survival time decreased as the temperature increased. When the temperature rose to 36–37°C, the survival time decreased rapidly to 17 h. There were also notable differences between the five high temperature subgroups (χ^2=235.589, P<0.01). The differences between any two of the subgroups were significant, except for the difference between the 58°C subgroup and the 60°C subgroup (χ^2=0.024, P>0.05).

Table 1 Survival time of *Demodex folliculorum* at different temperatures

Group	Temperature (°C)	Number of mites	Survival time		
			Longest	Shortest	Median
Low temperature	−15	41	11.0	2.0	5.5
	−5	71	32.5	3.0	21.5
	0	59	39.0	3.0	23.0
Moderate temperature	5	42	270.0	7.0	110.0
	8–10	37	274.0	6.0	81.0
	16–20	53	184.0	6.0	68.0
	25–26	67	151.0	4.0	49.5
	29–30	36	84.0	4.0	34.0
	32–33	50	65.0	4.0	20.0
	36–37	39	54.0	2.0	16.5
High temperature	45	33	215	30	90
	54	107	27	3	5
	56	30	6	2	3
	58	30	2	1	1
	60	30	1	0	1

The survival time unit of low and moderate temperature is hour, and the unit of High temperature is minute

Table 2 Motility of *Demodex folliculorum* after being kept in −15–37°C for 4 h

Group	Temperature (°C)	Number of mites	Activity of mites				
			−	±	+	++	+++
Low temperature	−15	41	5	36	0	0	0
	−5	71	1	70	0	0	0
	−3~0	59	6	53	0	0	0
Moderate temperature	5	42	0	42	0	0	0
	8–10	37	0	37	0	0	0
	16–20	53	0	0	11	38	4
	25–26	67	7	0	0	10	50
	29–30	36	2	0	0	6	28
	32–33	50	3	0	2	10	35
	36–37	39	5	0	9	15	10

Motility of D. brevis after being kept at −15–37°C for 4 h

The motility of *D. brevis* also changed depending on the temperature (Table 4). At 8–10°C, the mites became motionless but regained motility after anabiosis. At 16–20°C, *D. brevis* moved with a motility index of + to ++. At 25–26°C, motility of *D. brevis* increased gradually. When the temperature was increased to 29–30°C, *D. brevis* were active, with a motility index of +++. The mites were extraordinarily active at 37°C and displayed plentiful creeping and crawling.

Comparison of survival time between D. folliculorum and D. brevis at different environmental temperatures

As shown in Fig. 1, it is evident that the survival time of both *D. folliculorum* and *D. brevis* had a similar tendency of varying with temperature. Both *D. folliculorum* and *D. brevis* had their longest survival time at 5°C. However, *D. brevis* survived longer than *D. folliculorum* at temperatures of −15°C, −5°C, 0°C, and 5°C (χ^2=10.320, χ^2=5.069, χ^2=50.842, χ^2=4.329, all P<0.05). This suggested that *D. brevis* can tolerate hypothermia better than *D. folliculorum* can. For the six *D. brevis* subgroups and the six *D. folliculorum* subgroups exposed to temperatures ranging from 8–10°C to 36–37°C, there was no significant difference between each pair of corresponding subgroups of *D. brevis* and *D. folliculorum*. In the 45°C, 54°C, and 60°C subgroups, *D. folliculorum* survived longer than *D. brevis* (χ^2=44.662, χ^2=15.206, χ^2=34.186, all P<0.05). No significant difference was found in the survival times of *D. folliculorum* and *D. brevis* when the temperature reached 58°C and above.

Table 3 Survival time of *Demodex brevis* at different temperatures

Group	Temperature (°C)	Number of mites	Survival time		
			Longest	Shortest	Median
Low temperature	−15	34	11.0	0.5	5.0
	−5	61	57.0	1.0	34.0
	0	52	58.0	6.0	35.0
Moderate temperature	5	71	246.0	12.0	145.0
	8–10	55	204.0	4.0	87.0
	16–20	80	228.0	7.0	88.0
	25–26	90	110.0	5.0	40.0
	29–30	58	94.0	7.0	30.0
	32–33	56	75.0	5.0	27.5
	36–37	54	32.0	3.5	17.0
High temperature	45	58	60	30	30
	54	43	13	2	3
	56	53	5	1	2
	58	55	2	1	1
	60	45	1	0	1

The survival time unit of low and moderate temperature is hour, and the unit of High temperature is minute

Table 4 Motility of *Demodex brevis* after being kept in −15–37°C for 4 h

Group	Temperature (°C)	Number mites	Activity of mites				
			−	±	+	++	+++
Low temperature	−15	34	11	23	0	0	0
	−5	61	4	57	0	0	0
	−3~0	52	0	52	0	0	0
Moderate temperature	5	71	0	71	0	0	0
	8–10	55	9	46	0	0	0
	16–20	80	0	0	32	39	9
	25–26	90	0	0	17	28	35
	29–30	58	0	0	10	19	29
	32–33	56	0	0	17	25	14
	36–37	54	15	0	23	11	5

Association analysis between temperature and survival time

Figure 2 shows a scatter plot of the survival time of *D. folliculorum* and *D. brevis* at different temperatures ranging from 5°C to 37°C; there is an evident inverse correlation between survival time and temperature. The correlation coefficient for *D. folliculorum* was −0.989, with the regression equation $Y=-2.8215X+117.83$; the correlation coefficient for *D. brevis* was −0.955, with the regression equation $Y=-3.6869X+145.29$.

Discussion

This study systematically investigates the association between temperature and the in vitro vitality of *D. folliculorum* and *D. brevis* by observing the survival time and the motility of both *D. folliculorum* and *D. brevis* at different temperatures, ranging from −15°C to 60°C. This temperature range was further divided into 15 ranges. The results demonstrate that the viability of *D. folliculorum* and *D. brevis* in vitro is closely related to the environmental temperature. The survival time of the mites decreases as temperature rises, which suggests an inverse correlation between survival time and temperature.

Generally speaking, the mites can survive longer at lower temperatures than at higher temperatures. Our findings indicate that 5°C is the ideal temperature for keeping the mites in vitro; the mites survive longest (*D. folliculorum* 110 h, *D. brevis* 145 h) at this temperature. However, when temperature drops below 0°C, the mites' survival time decreases significantly (*D. folliculorum* 23 h, *D. brevis* 35 h); this effect is most significant at −15°C, the temperature at which the mites have the shortest survival time (*D. folliculorum* 5.5 h, *D. brevis* 5 h) and even lose motion. Therefore, temperatures below 0°C are considered harmful to the mites. We also found that both *D. folliculorum* and *D. brevis* not only have a relatively longer survival time (*D. folliculorum* 68.0 h, *D. brevis* 88.0 h) but also have better motility at 16–20°C; therefore, this is considered to be the optimal temperature for *D. folliculorum* and *D. brevis* to live and develop. When the temperature rises to 25–26°C, mites become active, with a motility index of ++ to +++, and have a remarkably shorter survival time than those at 16–20°C. When the temperature reaches 37°C or above, the mites do not survive as long and appear extraordinarily restless. Thus,

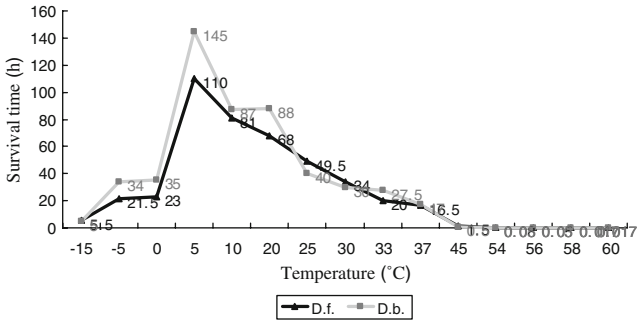

Fig. 1 Curve diagram of the survival time of *Demodex folliculorum* and *Demodex brevis* in different temperatures (median)

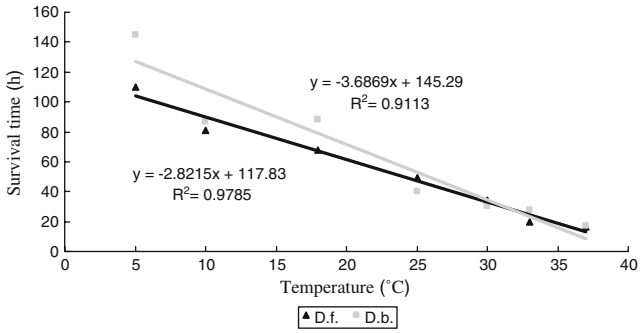

Fig. 2 Scatterplot of survival time of *Demodex folliculorum* and *Demodex brevis* in 5~37°C

temperatures of 37°C and above are harmful to the survival of the mites. The lethal temperature is considered to be 54°C, for the mites can only survive 3–5 min under these conditions; 58°C is regarded as the most effective temperature to kill mites, as they die in 1 min.

A suitable temperature range is essential for the normal physiological function of animals. Temperatures that are too high or too low are harmful to life and may even cause death. *D. folliculorum* and *D. brevis* are no exception. We find that mites can bear low temperatures and survive the longest at 5°C. This coincides with Li's (2004) report that *Demodex* mites can live about 1 week at 5°C. The reason may be that the mites' organisms maintain a hypometabolic state. Their energy consumption falls and their movement stops, but their vital functions remain normal even in a low-temperature environment. If kept at a suitable environmental temperature, they can regain motility and survive for a long time. However, if the temperature drops to 0°C or below, it can cause crystallization in the mites, which, in turn, can cause plasmogen disruption and damage to intracellular and intercellular minute structures. Low temperatures cause solvents to freeze and change electrolyte density, which results in changes in osmotic pressure; this in turn causes irreversible damage to the mites and promotes their death. In contrast, when the environmental temperature is 37°C, it makes the mites feel irritated and they move restlessly to escape. Their metabolism speeds up, their energy consumption increases, and their survival time shortens significantly. If the temperature rises further, the mites are sure to die due to protein coagulation and denaturation, loss of enzymatic activity, insufficient oxygen, functional disorders of the emunctory system, and paralysis of the nervous system.

In this experiment, we found that 16–20°C is the optimal temperature range for the mites to develop, although it does not coincide with the conclusion of domestic specialty books (e.g., Wu 2005) that 37°C (close to the temperature of the human body) is the optimal temperature. The conclusion of the specialty books is based on the studies of Chen (1985) and Wu and Meng (1990). Moreover, there is a notable deviation between our findings in this study and those in our previous report that 25–26°C was the optimal temperature for the maintenance of *D. folliculorum* in vitro (Zhao et al. 2005). The major reason for this deviation might be that we have improved the anabiosis conditions of the mites in this experiment. According to the specialty books mentioned above, the anabiosis conditions of experiments should consist of room temperature for 10 min and then incubation at 37°C for 10 min. It turns out that mites act more intensely in such temperature conditions than they do when they are kept solely at room temperature, which is convenient for us to observe the activity of the mites but shortens their survival time. Moreover, temperature changes in repeated anabiosis also contribute to the death of mites. The anabiosis conditions used in this experiment consisted solely of putting the mites at room temperature (16–20°C) for 30 min. The survival time of the mites in six subgroups (−15–20°C) was prolonged significantly. In the 16–20°C subgroup especially, the survival time of *D. folliculorum* increased from 44.5 to 68 h. Therefore, we come to the conclusion that 16–20°C is the optimal temperature range for *D. folliculorum* and *D. brevis* to survive and develop in vitro.

In conclusion, our study investigates the effect of temperature on the viability of *D. folliculorum* and *D. brevis*. Our findings on the optimal temperature are remarkably different from what is reported in specialty books and past studies. This, we believe, may provide more background for further studies on the maintenance and the control of *D. folliculorum* and *D. brevis* in vitro.

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References

Anane S, Touzri RA, Malouche N, El Aich F, Beltaief O, Zhioua R (2007) Which is the role of parasites and yeasts in the genesis of chronic blepharitis? Pathol Biol 55:323–327

Chen GD (1985) Influence of some environmental factors on the survival of two human follicular mites. Acta Entomol Sin 28:75–78

Erbagci Z, Ozgoztasi O (1998) The significance of *Demodex folliculorum* density in rosacea. Int J Dermatol 37:421

Garcia-Vargas A, Mayorga-Rodriguez JA, Sandoval-Tress C (2007) Scalp demodicidosis mimicking favus in a 30-year-old boy. J Am Acad Dermatol 57:S19–S21

Georgala S, Katoulis AC, Kylafis GD, Koumantaki-Mathioudaki E, Goergala C, Aroni K (2001) Increased density of *Demodex folliculorum* and evidence of delayed hypersensitivity reaction in subjects with papulopustular rosacea. J Eur Acad Dermatol Venereol 15:441–444

Li YL (2004) Human parasitology, 6th edn. People's Medical Publishong House, Beijing

Moravvej H, Dehghan M, Mohammad A, Meshkat-Razavi G (2007) Association of rosacea with demodicosis. Arch Iran Med 10:199–203

Powell FC (2004) Rosacea and the pilosebaceous follicle. Cutis 74:9–12 32–4

Wu GL (2005) Human parasitology, 3rd edn. People's Medical Publishong House, Beijing, pp 1034–1039

Wu JW, Meng YC (1990) Laboratory studies on mobility and lifespan of demodectic mites. Acta Acad Med Suzhou 10:94–97

Zhao YE, Guo N, Zheng X (2005) Observations on morphology and the survival temperature range of *Demodex folliculorum*. Acta Entomol Sin 48:754–757