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Detecting ticks on light versus dark clothing

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Running head: Ticks on light versus dark clothing

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It is common belief that ticks are more visible and easier to detect on light clothing in comparison with dark clothing.

We studied which of the clothing, light or dark, had the least attractive effect on *Ixodes ricinus*, thus minimizing exposure and thereby in theory help to prevent tick borne diseases in humans.

Ten participants, exposed by walking in tick endemic areas, wore alternately light and dark clothing before every new exposure. Nymphal and adult ticks on the clothing were collected and counted.

In total, 886 nymphal ticks were collected. The overall mean in found ticks between the both groups differed significantly, with 20.8 more ticks per person on light clothing.

All participants had more ticks on light clothing in all periods of exposure. Dark clothing seems to attract fewer ticks.

Key words: tick, prevention, dark, light, clothing

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INTRODUCTION

The incidence and the variety of diseases associated with tick-bites have continued to grow worldwide (1). Second only to mosquitoes, ticks are currently considered as the most important vectors of human infectious diseases in the world. Diseases such as Lyme borreliosis (LB), erlichiosis, relapsing fever, tularemia, tick-borne encephalitis (TBE) and babesiosis are caused by a variety of infectious pathogens, including different types of bacteria as well as viruses and protozoa (2-4). In Sweden more than 10.000 individuals are affected each year (5).

Recommended methods to prevent tick-bites and tick-borne diseases are e.g control of the vector by achieving a less favourable environment for the tick and its reservoirs, the use of pesticides and/or insecticides, exclusion of reservoir animals such as deer from residential areas by fencing, or to recommend avoidance of tick-infested areas. Personal protection is another option in high-risk areas; for example, rapid removal of attached ticks by daily inspections, the use of repellents, wearing protective clothing that cover major parts of the body including tucking trouser turn-ups into socks to keep ticks from gaining access to exposed skin (6, 7) .

In Europe, *Ixodes ricinus* (*I. ricinus*) is the main vector of tick-borne diseases (8). These eyeless ticks are considered to sense the presence of a host by ground vibration. They seek their hosts by climbing vegetation and orientating towards them mainly by sensory receptors responding to odours, humidity, temperature, ammonia and carbon dioxide (2, 9).

However all studied ticks, even eyeless species, have been found to have some type of photosensilla and optic ganglia in their synganglion. Little is known about the physiology of vision in ticks and it is doubtful whether they are capable of detailed form perception. Tick species are thought to respond to shadows, variations in light intensity and perhaps are capable of vague discrimination of shapes. Some tick species have been shown to recognize differences between light and dark areas and accordingly modify their behaviour (9-11).

Usage of light clothing is generally recommended by public health authorities as a practice in personal precautions to reduce the risk for disease transmission, owing to the belief that ticks are more visible and easier to detect in comparison with dark clothing (12, 13). However, studies confirming the advantages of wearing light clothing or whether brightness of clothing influences the tick when selecting its hosts have not been found.

The aim of the present study was to determine whether light or dark clothing had the least attractive effect on *I. ricinus* and thus in theory potentially prevent diseases in humans by decreasing exposure to ticks and consequently to tick bites.

MATERIAL AND METHODS

Study Area and Population

The study was a randomized cohort study with cross-over design. Two geographical places (with a total of 6 exposure squares), situated in an extremely highly tick endemic area on the archipelago of the Baltic Sea in the southeastern part of Sweden, were chosen for exposure. The study was carried out at the end of September (2 d) during daylight h between 10 a.m. and 6 p.m. The weather conditions were 10-12 °C, cloudy, with a wind speed of 5 to 9 m per s and air humidity between 50% and 79%. The vegetation consisted predominantly of deciduous forest and the undergrowth was covered with leaf litter, grass and rocks.

Healthy adult males and females (non pregnant), aged 18-65 y were invited to participate in this experimental study. The first 10 to volunteer (five male and five female) were included and agreed to expose themselves to ticks. Signed forms of consent were obtained. All participants involved were given information about ticks and signs of symptoms of tick transmitted diseases. The participants were observed for any symptoms of tick-borne diseases occurring during a period after the exposure up to the next tick season.

Exposure

The 10 participants were randomized into two standardized exposure-groups. In the first group 2/5 wore light clothing and 3/5 wore dark clothing and vice versa in the second group. The exposure squares, sited side-by-side, measured 25x25 m. The participants were exposed twice in each square; once wearing light clothing and once wearing dark clothing. The participants alternated wearing light (white) and dark (black) clothing each time they entered a new exposure square for the first time. In total they exposed themselves 6 times wearing light clothing and 6 times wearing dark clothing. Every exposure was of a 3,5 m duration. Electronic pedometers were used to standardise the distance walked for each participant during exposure. After each exposure the participants placed themselves upright on a stool standing in a tub with the base covered with water. Each participant had 2 persons

searching and collecting ticks on the clothing and placing them into cryo tubes. The search proceeded until no ticks could be found. Those ticks, which had dropped into the water in the tub, were also collected. All the nymphal and adult ticks were counted.

Clothing

Clothing consisted of 1 light and 1 dark set, exactly alike. Each set consisted of a T-shirt (100% cotton), a fleece-jacket (100% polyester), trousers (100% cotton) and soft leather shoes.

Tick density

Before the participants entered the 6 study areas, the ticks in the areas were sampled to obtain the number of actively host-seeking *I. ricinus*. The sampling method used was blanket-dragging, i.e. a blanket (1x1.5 m) of white, woolen flannel, dragged over the ground vegetation measuring 20 m in each area. The blanket was then inspected and any ticks found were collected and placed into cryo tubes.

Tick detection

To test whether the detection of ticks differed between the scrutiny of dark versus light clothing, blind experimental attachment was conducted. When wearing the different clothing (dark and light), a randomized number of ticks were placed on the participant. Randomization was carried out by drawing lots, numbered 1-20 prior to each application. The ticks were searched for, collected and counted. The number of ticks placed on clothing was unknown for both the exposed participant and the searchers.

Statistics

A paired sample *t*-test and Wilcoxon signed rank test was used to detect differences in attractive effect on *I. ricinus* between the groups wearing light or dark clothing. *p*-values below 0.05 were considered to indicate significance and

95% confidence intervals (CI) were based on the normal distribution. To test if the difference between the groups wearing light and dark clothing was normally distributed, expected normal quintiles were calculated using Blom's proportional estimation formula.

A chi-square test (4-fold table) was used to test significant differences between detecting ticks on light versus dark clothing.

The statistical software package, SPSS, (Version 11.5) computer program was used for the statistical analysis (14).

Ethics

The study was approved by the research ethics committee of the University of Lund.

RESULTS

Tick density

By blanket-dragging, 2 adult ticks and 46 nymphal ticks per 20 m and 1 adult tick and 55 nymphal ticks per 20 m were found respectively in area 1 and 2.

Differences in number of ticks on clothing

The 10 participants fulfilled all their 12 exposures, i.e. altogether they exposed themselves for 3,5 h with light clothing and dark clothing respectively.

In total, 892 adult and nymphal ticks were collected, 5 of 6 adult ticks were found on

light clothing. Of the 886 nymphs, 547 (62%) were found on light clothing and 339 (38%) on dark clothing, i.e. the participants in each group had mean numbers of ticks of 54.7 (SD 18.1) and 33.9 (SD 9.2) in light and dark groups respectively. The overall mean in found ticks between both groups differed significantly with a difference of 20.8 (SD 16.5) more ticks per person on light clothing (Paired sample *t*-test, $t=3.982$; $df=9$; $p=0.003$; 95% CI 8.98-32.62, Wilcoxon signed rank test, $p=0.005$).

In 11/12 occasions of exposure the total number of ticks were more on light (median 43.5 range 18-81) compared to dark (median 26 range 19-44) clothing. Taking into account the total number of ticks per participant when wearing light (median 53.5 range 31-88) versus dark (median 31 range 25-50) clothing, all participants had more ticks on light clothing on all exposure occasions.

Differences in tick detection

In total, a blind number of ticks were applied 26 times to the participants wearing light and dark clothing respectively. No significant difference between detection of ticks on light versus dark clothing was seen. Of applied ticks 91% were found on light clothing versus 93% on dark clothing.

Symptoms of tick-borne diseases

During the observation period up to the next tick season, none of the participants developed symptoms of tick-borne diseases.

DISCUSSION

Recent studies show evidence of a widespread increased risk to develop tick-borne diseases (1-5). Despite an increase in our knowledge about the appearance of such diseases, as well as the behaviour of the tick, we are still limited in our ability to reduce the number of affected individuals. The need for appropriate preventive methods in public health protection is obvious. Several methods are proposed, but for some of these, the advantages do not seem to have been evaluated. One of the personal precautions is the general recommendation to use light clothing in order to easier detect and remove ticks and thus prevent tick-borne diseases (6, 7).

However, our results show more ticks on light clothing in comparison with dark clothing. Although only 10 participants were included, however all participants had more observed ticks on light clothing on all occasions of exposure.

A natural assumption is that ticks are easier to detect on light clothing than dark clothing. We therefore attempted to minimize the risk of a lower standard of tick detection on both dark clothing and light clothing after every exposure in tick endemic areas. Two persons examined every participant, who by being placed in an elevated position permitted a thorough detection of falling ticks, until no ticks were detected.

In addition, to minimize possible bias due to failure of detecting ticks on dark clothing in comparison with light clothing we tested this hypothesis. The results showed no significant difference in detection of ticks. In fact, the participants found fewer ticks on light clothing.

Another possible shortcoming in our study could be different behavior by the participants in the different exposure areas when wearing light versus dark clothing. However, by using pedometers we standardized the distance walked in each area. Also, by measuring equal time for exposure we standardized the conditions affecting the participant's similar behaviour in the different exposure squares.

Thus, our findings would appear to be due to the fact that *I. ricinus* seems less attracted to dark clothing compared to light clothing and not due to above mentioned shortcomings in the study design. Also, another study found that the

wearing of light clothing did not appear to be protective against Lyme borreliosis (15).

If the question of brightness (light and dark) solely, or chemical components used in clothing dyes has an influence on tick attachment, it might possibly be answered by an experimental setting in a laboratory. Other ticks of the family Ixodidae are known to be positively attracted to light. Even if *I. ricinus* does not have eyes with corneas, they are described to exhibit positive phototaxis towards horizontal light and also to change direction towards the light accordingly to whether the source of light changes (10, 16-18). Perret et al. found photosensitive cells located dorsolaterally in all 3 life stages of *I. ricinus*. They described that the nerve from each light receptor is directly connected to the neural centre, the synganglion (11).

All ticks collected by blanket-dragging and exposure are considered as host-seeking. However, in this study we focused on nymphal ticks since they are believed to be the major transmitter causing human tick-borne diseases when taking into consideration the different stages of *I. ricinus* development (8, 19).

During the exposure time, in total 7 h (420 min), 886 nymphal ticks were collected from the clothing. To minimize the risk of tick transmitted diseases after the

study, the length of time of exposure was determined when the majority of the participants reported several ticks on their clothing in a pilot exposure.

To conclude, this study gains even greater credence by being performed under field conditions that authenticates the behavior and strategy of ticks when searching for hosts in their natural settings. Clearly, other factors than brightness of clothing influence the ticks' choice of human host. However, we have endeavored to eliminate possible confounding factors by standardised exposure areas, clothing and behaviour of the participants, thus minimizing differences of personal factors.

We found that all participants had more ticks on light clothing on all occasions of exposure. Dark clothing would appear to attract fewer ticks. However, to

understand the underlying mechanism of the ticks' host-seeking behavior,
further studies are needed.

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