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 - a. 12 point font, Times New Roman, single-spaced
 - b. Title
 - c. Authors (in order of magnitude of contribution)
 - d. Affiliations
 - e. Keywords (4-6)
 - f. Abstract (consult the [Abstract Guidelines](#) for more guidance)

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Last updated: January 2025

Title: Elevated Ambient Carbon Dioxide Levels Induce Attraction but Not Attachment of Adult Ixodes scapularis in Artificial Membrane Feeding

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Keywords: Artificial Membrane Feeding; Attachment Rate; Attractant; Ixodes scapularis; Membrane Contact

Abstract: Numerous feeding studies on tick species have explored disease transmission, vector interactions, and acaricide testing. Traditionally, these studies used animals for feeding. However, artificial membrane feeding offers several advantages including increased standardization of experiments, decreased costs, and improved animal welfare. In vitro conditions must closely mimic natural environments to promote successful feeding attachment. Kairomones produced by the host are strong stimulants that encourage attachment. An important kairomone detected by ticks is carbon dioxide (CO₂). Previous studies have shown elevated CO₂ levels stimulate host identification and attraction and potentially improve artificial feeding rates in some tick species. The objective of this study was to use an artificial membrane feeding chamber prototype to explore the effects of ambient CO₂ in inducing Ixodes scapularis attachment. Differences in attachment rate were explored at an air-typical ambient CO₂ level of 0.04% and an elevated CO₂ level of 4.0%. Tick attachment was not detected in either ambient CO₂ condition during the incubation period, indicating ambient CO₂ does not impact the attachment rate under the presented condition. However, I. scapularis contact with the artificial membrane occurred at an increased rate of 0.014 female ticks in contact with the membrane per hour in the elevated CO₂ condition (4%) compared with a rate of 0.01 ticks per hour in the air-typical CO₂ condition (0.04%) (p=0.048) suggesting that the ambient CO₂ level affects attraction to the blood but does not directly stimulate attachment of I. scapularis.

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