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SCIENTIFIC NOTE

NEW COUNTY RECORDS OF *AEDES AEGYPTI* AND *AEDES EPACTIUS* IN COLORADO

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ABSTRACT. In August and September 2017, we conducted mosquito surveillance in southeastern Colorado by using ovitraps and larval sampling. The aim was to determine if there were established populations of *Aedes aegypti* and *Ae. albopictus* in the region. A single female *Ae. aegypti* was reared from eggs collected in La Junta, CO, but *Ae. albopictus* was not detected. Three other species were reared from eggs and/or larvae: *Ae. epactius*, *Culex restuans*, and *Cx. pipiens*. *Aedes aegypti* and *Ae. epactius* were detected for the 1st time in Otero and Baca counties, respectively, and these became new county records for Colorado. Both species were detected in very low numbers, suggesting extremely low population density or sporadic introductions into southeastern Colorado.

KEY WORDS *Aedes aegypti, Aedes epactius,* Colorado

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The yellow fever mosquito, *Aedes aegypti* (L.), is an invasive species that has been in the USA for centuries (Nelson 1986). *Aedes aegypti* is a vector of several arboviruses of public health importance, including yellow fever virus (Reed et al. 1900), dengue viruses (Rudnick and Chan 1965), chikungunya virus (Moore et al. 1974), and Zika virus (Marchette et al. 1969). It is believed to have originated in Africa (Tabachnick 1991), and currently has a worldwide distribution in the tropics and subtropics. Occasionally, *Ae. aegypti* populations have been detected in temperate zones in North America. In the USA *Ae. aegypti* has been found throughout the southern states north to New Jersey, Illinois, Kansas, and the Southwest, including California, Arizona, and southern New Mexico (Darsie and Ward 2005; Hahn et al. 2016, 2017). The Asian tiger mosquito, *Aedes albopictus* (Skuse), is also an invasive species first detected in the USA in 1985 (Sprenger and Wuithiranyagool 1986). It is a vector of dengue virus (Rudnick and Chan 1965), chikungunya virus (Paupy et al. 2012), Zika virus (Grard et al. 2014), and many other pathogens of public health importance (Gratz 2004). *Aedes albopictus* is native to Asia (Benedict et al. 2007); it is believed to have been introduced to the USA from Japan through the used-tire trade (Hawley et al. 1987). In the USA, *Ae. albopictus* populations have been detected throughout the southern states, the eastern seaboard, and some parts of the Midwest (Hahn et al. 2016, 2017).

Aedes aegypti is not native to the state of Colorado and prior to this study it had only been detected once in this state: in specimens collected from Pueblo County in southern Colorado in 2010 (Rose et al. 2015) (Fig. 1). Established populations of *Ae. aegypti* are not known to exist in Colorado; the detection in Pueblo County in 2010 (Rose et al. 2015) was thought to be an accidental introduction and an isolated incident. However, *Ae. aegypti* is capable of causing dengue outbreaks at relative abundance levels of 0.5–1.5 pupae/person (Focks et al. 2000); therefore, even modest introductions or low population levels may pose a public health threat. Similarly, *Ae. albopictus* is not native to Colorado; however, for several years, a population has been detected in Larimer County in northern Colorado (Hahn et al. 2016, 2017), suggesting that Colorado can support populations of *Ae. albopictus*. Surveillance for *Ae. aegypti* and *Ae. albopictus* is not routinely conducted in Colorado, and therefore there is the possibility of undetected introductions or even established low-level populations in the southern part of the state. Currently, routine mosquito surveillance in Colorado primarily focuses on *Culex* vectors of West Nile virus, especially *Cx. pipiens* (L.) and *Cx. tarsalis* Coq. (Fauver et al. 2016). There is therefore the need to find out if there are established populations of *Ae. aegypti* and *Ae. albopictus* in Colorado, especially in the southern part of the state, to evaluate the risk of transmission of *Ae. aegypti*– and *Ae. albopictus*–borne arboviruses in the state.

We placed ovitraps in 7 cities in southeastern Colorado (Pueblo, Walsenburg, Trinidad, La Junta, Las Animas, Lamar, and Springfield). Sampling efforts varied from city to city depending on availability of suitable sampling sites. In each city, we selected sites that were more likely to support populations of *Ae. aegypti* and *Ae. albopictus*: junkyards, tire shops, abandoned houses, railroad yards, and container-cluttered homes. We placed 6 ovitraps in the proximity of each sampling site. In Pueblo we sampled 2 sites (12 ovitraps), in Walsenburg 1 site (6 ovitraps), in Trinidad 1 site (6

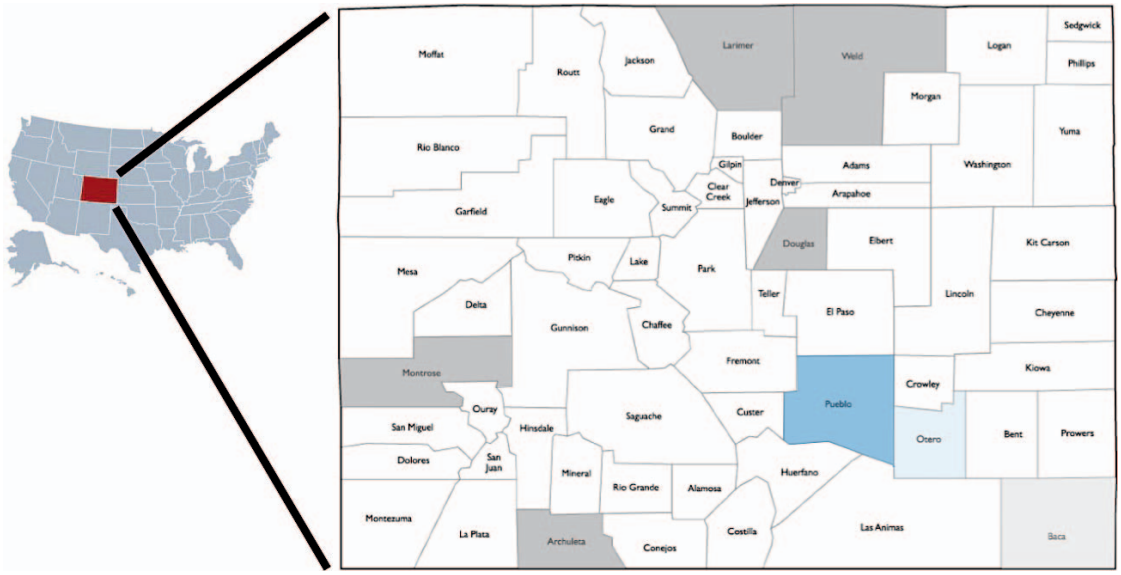

Fig. 1. The distribution of *Aedes aegypti* and *Ae. epactius* in Colorado after adding in our new records from the fall of 2017. The previous *Ae. aegypti* detection is represented by a darker blue shade (Pueblo County) and the new detection by a lighter blue shade (Otero County). The previous *Ae. epactius* detections are represented by the darker gray shade (Larimer, Weld, Douglas, and Montrose counties) and new detection by the lighter gray shade (Baca County).

ovitraps), in La Junta 3 sites (18 ovitraps), in Las Animas 1 site (6 ovitraps), in Lamar 2 sites (12 ovitraps), and in Springfield 1 site (6 ovitraps). Overall, we used 66 ovitraps and the same sites were sampled in both August and September 2017. Ovitraps were water-filled 22-oz black plastic cups (Giacona Container Corporation, Jefferson, LA) with seed germination paper (Anchor Paper Company, St. Paul, MN) as the oviposition substrates. Ovitraps were placed in the field on August 11 and September 8, and collected from the field on August 18 and September 15, respectively. In addition, we collected larvae from discarded containers and from the ovitraps by using turkey basters and white plastic trays. The larvae were placed in plastic 4-oz Whirl-Pak bags (Cole-Palmer, Vernon Hills, IL) and transported to the Centers for Disease Control and Prevention laboratory in Fort Collins, CO, where they were reared to adult stage and identified to species using the taxonomic keys of Darsie and Ward (2005). Larvae were reared in the water in which they were collected (tire water) and not fed in the laboratory. The eggs were hatched and reared in tap water and fed on liver powder (MP Biomedicals, LLC, Solon, OH). Mosquito rearing was done in Forma Environmental Chambers (Thermo Electron Corporation, Marietta, OH) at 28°C, 80% RH, and photoperiod of 16 h light and 8 h dark. Pupae were placed in tap water, in 100-ml plastic cups in mosquito breeders (BioQuip, Rancho Dominguez, CA). The adult mosquitoes were identified soon after emerging.

We conducted 462 trap-nights, but we only were able to count 470 eggs on substrates from Springfield, CO. There were large amounts of debris on the substrates and that made it difficult to detect and count the mosquito eggs accurately. Despite that, we flooded all substrates without detectable mosquito eggs and 1 larva emerged from the substrates from La Junta collected in September 2017. We reared the larva to the adult stage and identified it as an *Ae. aegypti* female. We also flooded the substrates from Springfield, but very few of the eggs hatched; we were able to rear 2 larvae to the adult stage and both were female *Ae. epactius* Dyar and Knab. In addition, we identified 4 *Cx. restuans* Theobald and 42 *Cx. pipiens* L. from larvae collected in Springfield. Overall, we collected 4 species: 1 *Ae. aegypti* from La Junta, and 2 *Ae. epactius*, 4 *Cx. restuans*, and 42 *Cx. pipiens* from Springfield. The single female *Ae. aegypti* was reared from an egg collected in La Junta, is the 2nd detection of *Ae. aegypti* in southern Colorado in 7 years, and the 1st detection of this species in Otero County (Rose et al. 2015; Hahn et al. 2016, 2017). We do not know if there are established populations of *Ae. aegypti* in southern Colorado, especially since there is no routine mosquito surveillance in southeastern Colorado, outside of the city of Pueblo; more investigations are needed to address this issue.

We did not detect *Ae. albopictus* in southeastern Colorado, and the reason why is not clear at this time. There is the possibility that this species does not exist in southeastern Colorado, and the maps published by

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Hahn et al. (2016, 2017) support this assumption. However, our studies took place over a short period, only 14 days, and there is the possibility that we did not detect *Ae. albopictus* because of this short study duration.

We report the 1st record of *Ae. epactius* in Baca County and in southeastern Colorado. The specimens were collected as eggs at a tire shop in Springfield, CO. This increases the number of counties in Colorado in which *Ae. epactius* has been detected to 6 and suggests a much broader distribution of this species in Colorado. The public health importance of *Ae. epactius* is currently not well understood. *Aedes epactius* is a competent vector for Jamestown Canyon Virus (JCV) (Heard et al. 1991), but it has not yet been associated with JCV outbreaks despite the fact that the distribution ranges of JCV and *Ae. epactius* overlap (Calisher 1983, Darsie and Ward 2005). Furthermore, JCV has not yet been detected in field-collected *Ae. epactius*. However, *Ae. epactius* is an aggressive human biter and in some areas it is a nuisance species (Carpenter and LaCasse 1955). *Aedes epactius* is widely distributed in North and Central America (Carpenter and LaCasse 1955, Weissmann 2016), but it is not a common species in the state of Colorado. It was previously collected in 2 western counties, Archuleta and Montrose (Harmston and Lawson 1967), and 3 northern counties, Larimer, Weld, and Douglas (Rose et al. 2017) (Fig. 1).

Our results strongly suggest that routine surveillance for mosquito vectors is needed in southeastern Colorado to understand the true range of *Ae. aegypti*, and to enhance our understanding of the dynamics and seasonality of *Ae. aegypti* and other vector species in this region. In addition, more surveillance is needed to establish if *Ae. albopictus* exists in this part of the state.

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