Background:
Leishmaniasis is an important zoonotic disease and a continuing public health challenge in the Kingdom of Saudi Arabia (KSA). Cutaneous leishmaniasis is particularly prevalent in the southwestern regions, including Asir and Al-Bahah Provinces. Ongoing research is essential to clarify the transmission cycle, reservoir hosts, and ecological factors influencing the spread of Leishmania spp. This study aims to examine the distribution and infection dynamics of Leishmania species among rodent hosts across diverse ecosystems in KSA, specifically in Asir and Al-Bahah Provinces.

Methods:
Wild rodents were trapped using live cage traps in October 2020 in Asir and in August 2021 in Al-Bahah. Identification of the captured small mammals was based on morphological characteristics and molecular confirmation using the mammalian mitochondrial cytochrome *b* (CYTB) gene—Leishmania spp. Infections of the captured rodents were determined by amplification and sequencing of the *Leishmania* spp—specific internal transcribed spacer 1 (ITS1) region.

Results:
In Asir, all captured rodents were identified as *Acomys dimidiatus* (Eastern spiny mouse); whereas *A. dimidiatus*, *Dipodillus dasyurus* (Wagner’s gerbil) and *Mus musculus* (house mouse) were captured in Al-Bahah (Al-Katheri).

Molecular analysis targeting the ITS1 region confirmed the presence of *Leishmania* DNA in 13 rodent samples, namely seven samples from the Asir Province (n = 27) and six samples from the Al-Bahah Province (n = 55), which were all collected from rodents identified as *A. dimidiatus*. Both *Leishmania tropica* and *Leishmania major* were detected in the Asir study areas, while only *Leishmania major* was identified in the Al-Bahah area. Parasite DNA was detected in the liver, spleen, and lung tissues, but was not found in the kidney tissues.

These findings contribute to a better understanding of the epidemiology and transmission dynamics of leishmaniasis in the region