Swine dysentery is classically associated with infection by Brachyspira hyodysenteriae; however, the proposed novel species ‘Brachyspira hampsonii’ has recently been isolated from clinical cases of dysentery in North America. Selective anaerobic culture is a highly sensitive method for detecting Brachyspira in clinical samples but requires several days for completion often followed by molecular testing for speciation. Alternatively, in situ hybridization applied to sections of formalin-fixed tissue can provide rapid, culture-independent identification of pathogens observed histologically. In this study, a fluorescent in situ hybridization assay was developed for confirmation of a clinical diagnosis of swine dysentery associated with ‘B. hampsonii’ infection. An oligonucleotide probe (Hamp1210) targeting 23S rRNA of ‘B. hampsonii’ was developed after sequence analysis and comparison of numerous Brachyspira spp. clinical isolates with reference sequences available in GenBank. Application of Hamp1210 and a previously published probe for B. hyodysenteriae (Hyo1210) to diseased colonic tissues from pigs successfully detected the target species in both experimentally infected pigs and naturally infected pigs, and the Hamp1210 probe consistently detected both clade I and clade II isolates of ‘B. hampsonii’. In situ hybridization incorporating these probes can reduce the delay from sample submission to pathogen identification in cases of swine dysentery where formalin-fixed tissues are available.