The complement system plays a key role in opsonization and immune clearance of engineered nanoparticles. Understanding the efficiency, inter-subject, and inter-strain differences of complement opsonization in preclinical species can help with translational nanomedicine development and improve our ability to model complement response in humans. Dextran-coated superparamagnetic iron oxide (SPIO) nanoparticles and a wide range of non-magnetic iron oxide nanoparticle formulations are widely used in magnetic resonance imaging and as clinically approved iron supplements. Previously we found that opsonization of SPIO nanoworms (NW) with the third complement protein (C3) proceeds mostly via the alternative pathway in humans, and via the lectin pathway in mice. Here, we studied the pathway and efficiency of opsonization of 106 nm SPIO NW with C3 in different preclinical species and commonly used laboratory strains. In sera of healthy human donors (n = 6), C3 opsonization proceeded exclusively through the alternative pathway. On the other hand, the C3 opsonization in dogs (6 breeds), rats (4 strains) and mice (5 strains) sera was either partially or completely dependent on the complement Ca^{2+}-sensitive pathways (lectin and/or classical). Specifically, C3 opsonization in sera of Long Evans rat strain, and mouse strains widely used in nanomedicine research (BALB/c, C57BL/6 J, and A/J) was only through the Ca^{2+}-dependent pathways. Dogs and humans had the highest between-subject variability in C3 opsonization levels, while rat and mouse sera showed the lowest between-strain variability. Furthermore, using a panel of SPIO nanoparticles of different sizes and dextran coatings, we found that the level of C3 opsonization (C3 molecules per milligram Fe) in human sera was lower than in animal sera. At the same time, there was a strong predictive value of complement opsonization in dog and rat sera; nanoparticles with higher C3 deposition in animals showed higher deposition in humans, and vice versa. Notably, the opsonization decreased with decreasing size in all sera. The studies highlight the importance of the consideration of species and strains for predicting human complement responses (opsonization) towards nanomedicines.