The Downfall of the Blue Bloods

The horseshoe crab is a 450 million-year-old prehistoric creature, older than the frightening Tyrannosaurus Rex; however, unlike the dinosaur, the horseshoe crab is a modern-day hero, helping the battle against the newest COVID-19 outbreak (Pavid). This luminary being has survived five mass extinctions because of its unique characteristics, but the chances of surviving human exploitation for the lucrative biomedical research are slim. To mitigate the horseshoe crab crisis, steps must be taken now before it is too late. Conservationists and scientists must work together to protect our health and ecosystem by saving the horseshoe crabs from the barbaric practice of bleeding.

Horseshoe crabs are a remarkably ancient group and are often known as “living fossils” since their fossil relatives are observed as far back as in the Ordovician Period, around 488 to 443 million years ago. Despite their name, these animals are not crabs but are closely related to scorpions, spiders, and extinct arthropods (“Horseshoe Crab”). Currently, there are four different species of horseshoe crabs: the Japanese or tri-spine horseshoe crab, the Indo-Pacific horseshoe crab, the mangrove or the round-tailed horseshoe crab, and the well-known American horseshoe crab (Pavid). They have a primitive appearance with a rounded, rigid, brownish-green exoskeleton and a long spiketail. However, their appearance isn’t what makes them so spectacular; it’s their blue blood.

Yes, blue blood. Humans have red blood due to hemoglobin, a protein that carries oxygen to the body’s cells. Hemoglobin has iron contained in it, which gives off that reddish hue. Contrary to humans, horseshoe crabs are devoid of hemoglobin and instead carry the copper-based molecule, hemocyanin, for oxygen distribution, and it is this copper that turns the blood of the horseshoe crab bright blue (Kurlwich). According to the National Oceanic and

Atmospheric Administration, just a gallon of horseshoe crab blood can cost up to an estimated $60,000 (Frank et al.). So why is the blood of a horseshoe crab that exorbitant? The answer lies within the granular amebocytes found in the blood. Dave Grant, a naturalist at Brookdale Community College's Ocean Institute, reports that amebocytes "work similarly to white blood cells and are believed to be a precursor to them" (Law). When they come in contact with endotoxins, bacterial substances that cause fevers and death, amebocytes release a cascade of defense mechanisms, neutralizing the threat of invasive pathogens (Gannon et al.). If a horseshoe crab is lacerated, its insides are exposed to dangerous microorganisms found in the ocean. The moment bacteria is sensed, a clump of blood-clotting granular amebocytes is instantly unleashed, sealing out the invaders, thereby, preventing infection (Kurlwich). This fascinating superpower has led to the exploitation of hundreds of thousands of these horseshoe crabs each year.

This unique ability of amebocytes has allowed technicians and biomedical companies to confirm the safety of drugs, vaccines, and medical devices using the Limulus Amebocyte Lysate (LAL) test, named after the American horseshoe crab, Limulus *polyphemus.* The lysate is produced by extracting blood from the crab. Suppose the drug or medical device product contains pathogens. In that case, the Limulus lysate will form a jelly-like clot around it, providing blood technicians with the conclusion that this device is contaminated and is unsafe to use (Law). Pharmaceutical and medical device companies employ the LAL test all around the world to ensure that their products are uninfected and safe to use. The LAL test can detect microscopic amounts of pathogens or endotoxins, as low as 0.1 parts per trillion. Minuscule amounts of toxins in the human body can cause dangerously high fevers leading to death and using the ability of a horseshoe crab's blood to verify the safety of the supplies before human use has saved thousands of lives (Law). This technique has been used since the 1970s, and it's

fantastic for humans. Vaccines save us from all sorts of diseases; unfortunately, it's not as wonderful for horseshoe crabs. Hundreds of thousands of them are rounded up and bled every year, leading to their decline (Pavid). Additionally, horseshoe crabs have a central nervous system and are found to be disoriented after the bleeding procedure. This illustrates that the brutal practice is unethical and needs to stop.

These magnificent creatures also play an important role in our ecosystem. The International Union for the Conservation of Nature asserts that the horseshoe crab is a vital link to coastal biodiversity as one of their ecological functions is to lay millions of eggs on beaches for different kinds of wildlife. Migratory shorebirds rely on the fatty eggs of the Atlantic horseshoe crabs for nourishment on their migration journey, and sea turtles prey on the horseshoe crabs' eggs and larvae (Fenton et al.). Additionally, its large stiff shell operates as a habitat for sponges, mussels, and snails, and they keep the sediment around the coastline healthy which benefits fishermen and beachgoers as well (Pavid). As these horseshoe crabs go around helping the environment, the medical industry captures and takes them to a laboratory where they are forcefully bled. According to *Business Insider,* each year, the medical industry catches around 600,000 horseshoe crabs and drains 30% of their blood. Approximately 50,000 horseshoe crabs in the United States die every year as a result of being captured and bled (Toliver). Because biomedical companies are greedy and collect horseshoe crabs extensively from one specific area, it hinders their ability to repopulate sustainably. As the horseshoe crabs are carelessly thrown back into the ocean like unwanted garbage after narrowly escaping the inhumane vampire-like process, they must struggle to survive after losing a third of their blood. Hundreds of thousands of horseshoe crabs share this heinous experience each year. In fact, in 2016, the International

Union for the Conservation of Nature moved the American horseshoe crab up to “vulnerable”, one stage beneath endangered (Frank et al.).

I wanted to determine how many people understood the severity of this issue, so I created a survey and sent it to high schoolers, college students, teachers, and adults to see how many people knew the “importance of horseshoe crabs.” Out of the twenty-one participants, fourteen of them selected the option of “I don't know anything about the importance of horseshoe crabs“, five chose “I somewhat know about the importance of horseshoe crabs”, and two chose “I am completely aware of the importance of horseshoe crabs.” From this small experiment, I concluded that many people do not know the crucial role horseshoe crabs play in our ecosystem and the development of biomedical companies. This leaves us with the ethical dilemma- How do we address the worldwide downfall of horseshoe crabs while still keeping vaccines and other medical equipment safe?

The most effective solution is the use of a LAL alternative test that would replace the need for the precious horseshoe crab blood. In 1997, Singaporean scientists Ling Ding Jeak and Bo How discovered a synthetic-based reagent known as Recombinant Factor C (rFC) (Maloney et al.). This was integral for the development of an “animal-free endotoxin detection” device. This rFC utilizes a protein cloned from a horseshoe crab as its active ingredient and when an endotoxin binds to the reagent, the rFC molecule catalyzes a reaction, resulting in the production of a fluorogenic compound. The fluorescence is measured once before and once after the endotoxin is introduced. The difference in fluorescence is proportional to the concentration of endotoxin within a sample and is used to calculate the final endotoxin result (Maloney et al.). Unfortunately, biomedical companies had little incentive to spend their time and money on a newer and less familiar alternative. Both the FDA and U.S. Pharmacopeia refused to license and

regulate it. Despite the struggle, Ding's work fascinated other scientists from around the globe, especially the Lonza Group, a Swiss multinational manufacturing company that commercialized this endotoxin test. Compared to the LAL test, this alternative does not require horseshoe crab blood and yields fewer false positives. Additionally, it's less wasteful and thus less expensive (Cramer). More importantly, approximately 100,000 horseshoe crabs annually would be preserved in just North America (Maloney et al.).

On a personal scale, we can support organizations such as The Ecological Research & Development Group (ERDG) and the IUCN SSC Horseshoe Crab Specialist Group, which advocate for the conservation of horseshoe crabs. In our local communities, we could start projects educating people and raising awareness about this issue. We could also urge legislators to establish regulations protecting the exploitation of these crabs by organizing petitions.

Although horseshoe crabs may seem insignificant and ugly at first glance, they are vital to our health and environment. This ancient creature has provided nutrition for migrating shorebirds for centuries and is now being used to confirm the safety of vaccines and medical equipment. Their blue blood is saving our lives but costing them their own. By encouraging companies to use an alternative man-made test called rFC, we can save thousands of horseshoe crabs across the globe. Join me in the conquest to protect the noble horseshoe crabs; sign one or more of the petitions currently online urging legislators to require the biomedical industry to use synthetic blood.

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