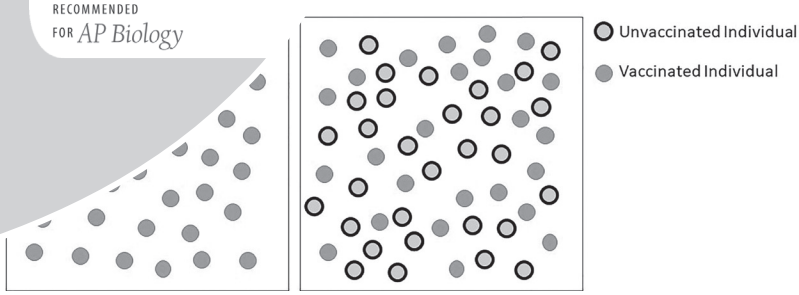
INQUIRY & INVESTIGATION

Modeling Herd Immunity: An Introductory Course Activity

RECOMMENDED FOR *AP Biology*



AMBER M. EADE, CORTNEY DEAN, STACY L. HRIZO

Abstract

Teaching epidemiology, particularly more advanced concepts such as vaccine efficacy and herd immunity, can be very challenging in science classes geared for non-science majors. Nevertheless, as became quite evident during the recent COVID-19 pandemic, a thorough understanding of such topics is highly important to all individuals due to its role in informing future decision-making related to public health. One method for easing comprehension of difficult science content is to employ hands-on activities that engage students in critical thinking while visually demonstrating difficult concepts. While introductory activities illustrating the spread of disease through unprotected populations already exist, those that model the more advanced topics of vaccination and herd immunity are limited. In addressing this gap, the present manuscript describes an activity that expands on prior exercises through the addition of common biological buffers (MOPS and HEPES) mimicking vaccination status. Further, detailed exploration of buffer concentrations and interaction conditions during the development of this activity underscores potential avenues for class discussion of real-world outcomes. This includes the concept that exposure to a pathogen does not invariably result in illness, and vaccination does not always guarantee immunity against infection. Overall, the resultant activity creates avenues to enrich student comprehension of epidemiology, providing valuable insights into disease transmission, effectiveness of vaccination, and the dynamics of herd immunity.

Key Words: *Epidemiology; herd immunity; vaccination; infection model.*

○ Introduction

Pathogens require the availability of susceptible hosts to successfully reproduce and spread. Interestingly, there is an inherent dichotomy present when pathogens infect their host targets. When infecting a host, pathogens enhance their own transmission potential by spreading to additional new hosts. Simultaneously, the host organisms immune response triggers the development of a natural immunity against the pathogen. The resulting immune response ultimately diminishes the pathogens ability to reinfect the original host and limits its future transmission to other individuals. As the overall immunity against a pathogen subsequently increases within a population, the occurrence of the pathogen tends to decline. When a significant portion of the population becomes immune to a pathogen, its propagation becomes restricted and, in some instances, diseases can become eradicated. This fundamental concept, known as herd immunity, is vitally important in protecting vulnerable populations, such as those with compromised immune systems, from diseases that may produce life-threatening outcomes (Metcalf et al., 2015). The medical community has taken advantage of the concept of herd immunity using vaccinations. Instead of relying on natural immunity acquisition, which carries risks of the potential negative effects of illness due to infection with the pathogen, individuals can receive a vaccination that will prompt their immune systems to develop resistance against a specific pathogen without the untoward consequences. If a substantial portion of the population receives vaccinations, the transmission of the targeted pathogen will slow and may cease altogether, just as would occur with natural immunity.

Given the role of vaccination in the management of many modern-day diseases, the ability to demonstrate the impact of this procedure has become of interest. Moreover, discussions regarding why some individuals still experience unexpected outcomes, such as becoming ill even following vaccination, are important lessons in understanding the limitations of vaccines. Teaching epidemiology of this nature in the undergraduate student setting holds significant importance due to its role in informing future decision-making related to both public health concerns and personal healthcare choices. This became increasingly apparent during the COVID-19 pandemic, where the spread of pathogens and use of vaccines became regular discussion points throughout the world. As students may find the biological concepts underlying pathogen transmission and vaccine effectiveness difficult, employing engaging activities that visually demonstrate concepts can add contextual cues that make material easier to understand. This is especially advantageous in classes attended by non-majors, who may possess limited familiarity or interest in the subject matter. Many introductory activities showcasing how pathogens propagate within a population are available for use in non-majors' biology courses. However, activities that effectively model more advanced topics such as vaccine efficacy and herd immunity are limited.

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○ Activity Overview

Previously utilized laboratory activities demonstrate the spread of viral disease through an unprotected population, effectively conveying fundamental principles of epidemiology. Such activities utilize water-filled vials to simulate "uninfected" individuals while one "infected" vial contains sodium hydroxide (NaOH). Through a series of manual fluid exchanges, representing physical interaction between members of a community, NaOH is transmitted through the vials, thereby "infecting" other members of the population. Toward the conclusion of the activity, the addition of a phenolphthalein indicator solution to each vial exposes any changes in the fluid pH caused by the movement of NaOH during fluid swaps. Phenolphthalein is a weak acid that will change its color from clear to pink as it detects increases in pH in the range of approximately 8.2–9.8. The resulting color can range from light pink for slight increases in pH to hot pink for larger increases in pH. When added to each vial at the end of the class activity, phenolphthalein causes the vials exposed to the NaOH turn a bright pink color, while the unexposed vials remain clear. This procedure effectively identifies participants whose vials became "infected" with NaOH during the activity. The color change caused by the pH indicator creates excitement in the room as students reveal the outcome of their interactions with classmates.

Activities of this nature are both enjoyable and instructive, effectively illustrating how infections can disseminate through community interactions in an engaging manner. However, these activities do not address the concept of herd immunity and broader impacts of vaccination on pathogen transmission. A more recently developed activity employed the use of a Tris buffer to mimic the effect of vaccination, effectively expanding on commonly used laboratory activities that modeled disease transmission (Schwingel, 2018). This builds on the initial demonstration by introducing "protected" members to the population and adds in a discussion of the overall effects of herd immunity. As described below, we have since expanded on this activity further, exploring different concentrations of additional buffer options and exchange conditions in a way that provides additional discussion of real-world outcomes. Specifically, recognition of the potential outcomes resulting from different combinations of fluid interactions during this activity prompts student discussion of why some members of a community do not become ill following exposure to a pathogen whereas others who hope to be protected via vaccination can still become infected. This is an important discussion due to the variable nature of herd immunity following vaccination for various pathogens (Fine, P.E., 1993). The level of immunization required to achieve herd immunity can differ greatly based on the pathogen at hand. Herd immunity against measles, for example, requires 95% of the population to be vaccinated, whereas the vaccination threshold for polio herd immunity is 80% of the population (Plans-Rubió, 2021; Lai et al., 2022). Thus, through this exercise students may not only gain an understanding of the concept of heard immunity, but also develop more in-depth appreciation of the potential for vaccine limitations and breakthrough infections.

○ Updated Protocol Development and Testing

Development of the present activity involved evaluating the outcomes (i.e., infected vs uninfected) created through a variety of possible combinations of fluid exchanges that could be encountered during an in-class activity. Lab activities modeling pathogen transmission have students exchange fluids with either 3 or 4 partners. Due to the high number of samples that could be achieved through larger numbers of fluid exchanges, development of the present protocol started with only 3 exchanges. By adding the use of a buffer to mimic effects of vaccination, the types of fluid included in the exchanges increased from 2 in the original activity to 3 in the updated version (NaOH – infected, H20 – uninfected/unvaccinated, and Buffer – vaccinated).

Several considerations were made in determining which of all possible fluid exchange combinations required examination. In designing the fluid swaps for our study, several combinations of fluid did not require testing due to having no potential for an effect or being duplicative of another swap combination. For example, as the activity is designed to begin with only 1 vial of NaOH (*infected*) and all fluid exchanges are novel, there was no potential for any exchange combination to include more than 1 instance of full-strength NaOH. Similarly, as the testing outcome for a vial containing only a buffer or water alone would already be known (i.e., negative), there was no need to test outcomes of exchanges that contained all the same substance or any combination of only water and buffer. Combinations of water and NaOH are already known to produce a positive outcome from the traditional lab activity, thus also do not require testing. Finally, since the protocol involves moving ½ of the vials' contents during each exchange, it was not necessary to test combinations in which the initial content and fluid 1 content would be reversed. Thus, if the initial vial contained NaOH and the first exchange was water, it was not necessary to test an initial vial of water with a first exchange of NaOH. These considerations drastically limited the number of fluid combinations that required examination. The remaining fluid swap combinations that were utilized for this study are illustrated in Table 1 and further described below.

The determination of which combinations to use in the activity was based on allowing validation of expected real-world outcomes for end-of-activity discussions regarding how various interactions can prevent vs promote infection status. Specifically, three outcomes were important to maintain viability of the activity: (1) the original "infected" NaOH vial could not become "uninfected" following dilution with water and/or buffer, (2) the full-strength buffer vials should convey some level of protection to the population, and (3) the water vials must illustrate potential effects of exposure to uninfected and infected members of the population. The 12 fluid exchange combinations presented in Table 1 allowed for the testing of these outcomes.

To examine the effects of commonly used biological buffers acting as vaccines, the first attempt at introducing buffers included 0.1 M MOPS (4-(3-sulfonatopropyl)morpholin-4-ium) and 0.1 M HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid). As described below, these concentrations were adjusted following initial outcomes that were inconsistent with the goals of the activity. Overall, it is imperative to realize that the goal of this activity is to provide meaningful population-based outcomes related to initial infection, potential of infection transmission, and potential for protection of the population due to vaccination. As real-world scenarios do allow some vaccinated individuals to become infected as well as unvaccinated individuals to avoid infection, it is not critical to analyze the outcome of every individual interaction. Instead, end results related to the goals of the activity were most important to examine. Thus, it was more imperative to ensure that initially

Table 1. Fluid Exchange Combinations Tested.

Exchange Combinations					
Vial #	Initial Contents	Fluid 1	Fluid 2	Fluid 3	Interaction Representation
1	NaOH	Water	Water	Buffer	Infected individual interacting with 2 uninfected and 1 vaccinated individual
2	NaOH	Water	Buffer	Buffer	Infected individual interacting with 1 uninfected and 2 vaccinated individuals
3	NaOH	Buffer	Buffer	Buffer	Infected individual interacting with 3 vaccinated individuals
4	NaOH	Water	Water	Water	Infected individual interacting with 3 uninfected individuals
5	Water	Water	Buffer	NaOH-D	Uninfected individual interacting with 1 uninfected, 1 vaccinated, and one carrier individual
6	Water	Buffer	Buffer	NaOH-D	Uninfected individual interacting with 2 vaccinated, and one carrier individuals
7	Water	Water	Buffer	NaOH-S	Uninfected individual interacting with 1 uninfected, 1 vaccinated, and 1exposed individual
8	Water	Buffer	Buffer	NaOH-S	Uninfected individual interacting with 2 vaccinated, and 1exposed individual
9	Water	Buffer	NaOH-D	NaOH-S	Uninfected individual interacting with, 1 vaccinated, 1 carrier, and 1exposed individual
10	Buffer	Water	Water	NaOH-D	Vaccinated individual interacting with 2 uninfected and 1 carrier individual
11	Buffer	Water	NaOH-D	NaOH-S	Vaccinated individual interacting with 1 uninfected, 1 carrier, and 1 exposed individual
12	Buffer	NaOH	NaOH-D	NaOH-S	Vaccinated individual interacting with 1 infected, 1 carrier, and 1 exposed individual

Note. NaOH-D = Carriers. Partial infection (1/2 strength due to 1 dilution) that may still transmit infection. NaOH-S = Exposed. Low-level of exposure (1/4 strength due to two dilutions) that may be less likely to transmit infection status.

infected vials remain infected, as the transmission of vaccination from one individual to another is unrealistic, than it was to prevent vaccinated vials from becoming infected, which is a possible real-world outcome. As such, the expected outcomes of the resulting 12 potential fluid exchange combinations that were evaluated are presented in Table 2 alongside the observed outcomes following testing with different buffer concentrations. Although not all expected outcomes were observed, the last-attempt outcomes are consistent with the goals of the study, provide realistic examples of what can occur in real-world scenarios, and prompt discussions regarding expectations vs reality with respect to vaccination efficacy.

With respect to the first testing requirement noted above, the maintenance of infection following dilution was examined using combinations of NaOH with water and/or buffer (Table 1, Vials 1–4, and 12). The positive result at all concentrations of MOPS and HEPES in Vial 4 (Table 2) illustrate the effect of NaOH cannot be simply diluted out with 3 water exchanges. However, once buffer was added, it was determined that the initial concentration of MOPS (0.1 M) was too strong and negated the effects of initial infection in each instance (Table 2, vials 1–3). As the goal of the activity was not to allow the effects of vaccination to be transferred to an initially infected individual, these results illustrated the need to decrease the concentration if using MOPS as a buffer option. Thus, our last-attempt trials occurred using 0.085M MOPS.

Notably, adding water to NaOH during this activity does result in dilution of the initial infected vial. Resulting effects of "partial" transmission of infection in later exchanges required consideration. Therefore, exchanges in later vials are noted in Table 1 as full strength (NaOH), partial dilution due to 1 prior water exchange (NaOH-D), and a second dilution due to 2 prior water exchanges (NaOH-S). As exchanges use half of the fluid from the original vial, a single dilution cuts the NaOH concentration by half, whereas a second swap then decreases it to approximately one quarter of its original concentration. For discussion purposes, it is possible to consider these different levels of "exposure" in real-world terms of potential for an infection to be transmitted. For example, transmission can occur not only with those who are infected and symptomatic, but also through exposure from carriers who are asymptomatic or early in the stages of infection but still producing infectious particles. Given the exercise starts with only 1 infected vial and the first fluid exchange immediately dilutes that concentration by half, full-strength NaOH can exist only in the initial contents of the vial or as the first fluid swap. Similarly, as it takes 2 water swaps to reach the NaOH-S status, this exchange can occur only as the third exchange (i.e., Fluid 3 of Table 1). It was unnecessary to test the effects of diluting the infection with buffer, as the effect of initial infection is anticipated to outweigh buffer in all instances. Thus, if the outcome of NaOH is not negated by three full-strength buffer exchanges,

Table 2. Infection Outcome following 3 Fluid Exchanges.

Vial #	Expected Outcomes	First-Attempt Outcomes		Last-Attempt Outcomes	
		0.1 M MOPS	0.1 M HEPES	0.085 M MOPS	0.2 M HEPES
1	Initial Infection Status Persists (+)	–[1]	+	+	+
2	Initial Infection Status Persists (+)	–[1]	+	+	+
3	Initial Infection Status Persists (+)	–[1]	+	+	+
4	Initial Infection Status Persists (+)	+	+	+	+
5	Acquisition of Infection (+)	–	+	+	+
6	Possible Acquisition of Exposure (+) or Protection via Herd Immunity (–)	–	–	–	–
7	No Transmission of Infection (–)	–	–	–	–
8	No Transmission of Infection (–)	–	–	–	–
9	Acquisition of Infection (+)	–	+	+	+
10	No Transmission of Infection (–)	–	+[2]	–	–
11	No Transmission of Infection (–)	–	+[3]	+[3]	+[3]
12	No Transmission of Infection (–)	+[3]	+[3]	+[3]	+[3]

+ = color change = *infected*

– = no color change = *uninfected*

[1] = Buffer inappropriately transmitted protection

[2] = Buffer ineffective against secondary transmissions

[3] = Buffer ineffective against multiple exposures

such as that outlined in Vial 3 (Table 2), then there is no reason to believe that it would be altered by diluted levels of buffer.

To test the second requirement of initially vaccinated (i.e., buffer) vials conveying protection when exposed to the pathogen in most instances, it was important to test the range at which the pathogen could be experienced (i.e., partial exposure through diluted levels of NaOH vs combinatorial exposure with full and diluted NaOH). Additionally, as vials 6, 8, and 9 started with water and experienced a first swap with a buffer, the outcome for these vials would be the same as for vials that started with buffer and initially swapped with water. This is confirmed by the fact that vials 9 and 11, which started with opposite contents, have identical outcomes (see Table 2). The outcomes of these vials can be simultaneously discussed as relates to individuals who started vaccinated and encounter multiple exposures to infected individuals vs. those who were not vaccinated but encounter a mixture of vaccinated and infected individuals.

Looking at Table 2 outcomes for Vials 6 and 8–11, it is evident that, even with a dilution from a first exchange with water, the 0.1 M MOPS was successful against effects of NaOH-D and NaOH-S either presented independently or combined. However, this concentration was ineffective with repetitive transmission including both full and partial infection (Vial 12). Initial concentration of 0.1 M HEPES, on the other hand, was too weak and allowed transmission of infection status following a single dose of NaOH-D (Vial 10) unless additional buffer exposure occurred, preventing dilution of the initial buffer concentration (Vials 6 & 8). As it is possible for some individuals who are vaccinated to still end up positive for the pathogen, the results of the vaccine not preventing transmission at the most extreme levels of what could be modeled with this activity, such as what was observed in Vial 12, were not concerning. However, it was important for vaccination to provide some level of protection to the population. Thus, the 0.1 M HEPES not protecting against even partial infection transmission was problematic. This led to the recognition that the HEPES concentration needed to be adjusted for future use. Increasing the HEPES concentration to 0.2 M effectively returned the buffer to being effective against partial transmission (Vial 10) while still allowing those with multiple exposures to become infected (Vials 11 & 12). While these results do not ensure that all "vaccinated" vials maintain protection against infection, they do allow an appropriate combination of infection versus protection for discussion purposes.

Finally, for those vials that started with water and maintained a first swap with water (vials 5 & 7), appropriate results for the level of infection exposure were desired. Thus, it was deemed acceptable for a vial that swapped with buffer and the lowest level of infection (i.e., NaOH-S) to maintain an uninfected status (Table 2, Vial 7), while a larger level of infection (i.e., NaOH-D) should produce infection (Table 2, Vial 5). The initial level of MOPS used was too strong to allow the appropriate level of infection status to be observed, but decreasing to the 0.085 M level resolved this issue.

As can be seen in the "Last-Attempt Outcomes" columns of Table 2, the concentrations of 0.085 M MOPS and 0.2 M HEPES met all testing requirements by (1) allowing initial infection to remain present even after multiple interactions with vaccinated vials, (2) allowing vaccination to convey a partial level of protection, and (3) illustrating appropriate combinations of interactions in the unvaccinated population. Identical outcomes were achieved in three separate lab trials.

Following determination of the appropriate buffer concentrations, a prior lab activity that illustrated infection without the use of vaccines was modified to include the new vaccination component. Protocols were designed to allow for groups of 24 and 40, modeling the sizes of typical undergraduate non-majors biology classes. The resultant activity includes 3 'rounds' each consisting of a different rate of vaccination (0%, 25%, and 75%). The protocol was first tested in the lab and subsequently in student groups consisting of either biology majors or non-majors. In the lab setting, protocols were tested and revealed infection rates of 20–33% when unvaccinated, 7.5–17% with 25% vaccination, and 0% when 75% of the vials were vaccinated. Thus, an appropriate decrease in infection rate was clearly demonstrated as vaccination rate increased. While formal student feedback data following the activity was not collected, students were reported to appear receptive to the new protocol and remained engaged during the activity.

As expected, due to the fact that students get to choose who to exchange fluid with during the activity (*see Lesson Guide below*), each attempt at this activity with different student populations produced slightly different results. However, the overall ability to illustrate the concepts of pathogen transmission and vaccine effects are consistently evident. When testing the protocol with student groups, the percent of individuals infected at the end of the exercise appropriately decreased as a result of vaccination. Unvaccinated rounds produced a 28–40% infection rate, whereas the infection rate decreased to 10–25% when 25% of the population was vaccinated and even further to only 3–5% infected when 75% of the population had been vaccinated. These results are very similar to what was achieved in the lab setting and illustrate that no matter what order the students opt to exchange fluids, the overall outcome appropriately models the intended scenario of herd immunity.

Following determination of the appropriate buffers and resulting protocol for a set of 3 exchanges, increasing to 4 fluid exchanges using the same overall protocol was also attempted. The increase in fluid exchanges resulted in too many opportunities for initially infected NaOH vials to encounter buffer solutions at the 75% vaccination rate. This resulted in the NaOH vials testing negative when exposed to the phenolphthalein indicator. As such, this violated the first goal of the activity, which was to be able to maintain a positive outcome in vials that began as "infected." With the increased buffer exchanges, the result of 4 swaps effectively led to the transmission of vaccination status, which is unrealistic in the human population. As such, we opted to limit the activity to only 3 rounds of fluid swaps.

○ Lesson Guide

What follows is a brief description of the resulting lab activity, including overall lesson plan for introducing the activity and discussing results. The ultimate objective of this activity is to demonstrate the concept of herd immunity using a hands-on simulation that showcases how disease transmission can be affected by varying vaccination rates within a population. To accomplish this task, students must first be introduced to the basic concepts of epidemiology and investigate disease transmission in unvaccinated individuals. Therefore, this is a three-part lesson that includes an introduction in addition to a multi-step activity (see Figure 1). Prior to engaging students in the hands-on activity, a brief lecture and discussion occur focusing on factors contributing to disease transmission, terminology pertinent to transmission rates, the role of organizations in tracking diseases, and how vaccinations assist in limiting transmission opportunities. Subsequently, the hands-on activity is introduced and faculty lead students step-by-step through the process, ensuring uniform progression and preventing any student from falling behind or moving too far ahead of the group. To pique interest and keep students engaged with the subject matter, the initial explanation of the activity includes informing students that the exercise they are about to complete simulates the transmission of a hypothetical sexually transmitted infection.

To ensure recognition of the distinct differences in transmission rates in vaccinated versus unvaccinated populations, this activity consists of two distinct observation rounds (see Figure 1): the first models general disease transmission with an unvaccinated population and the second demonstrates transmission within populations with an approximately 25% and then 75% vaccination rate. Optionally, if time allows, this protocol can be further adapted to include additional rounds showcasing transmission rates for other levels of vaccination (i.e., 50%). In each round, students engage in simulated transmission by exchanging fluid from vials containing water (representing healthy fluid), sodium hydroxide (representing the infection), or a buffer solution (mimicking vaccination). Students complete 3 rounds of fluid exchange with different partners, after which their infection status is revealed via use of a Phenolphthalein pH indicator. Throughout the activity, students document each of their own transmission exchanges. Once all exchanges have occurred, students collectively create a data spreadsheet that contains all information on the order of fluid exchanges as well as the results provided by the pH indicator. This information allows students to calculate the transmission rate and engage in discussions

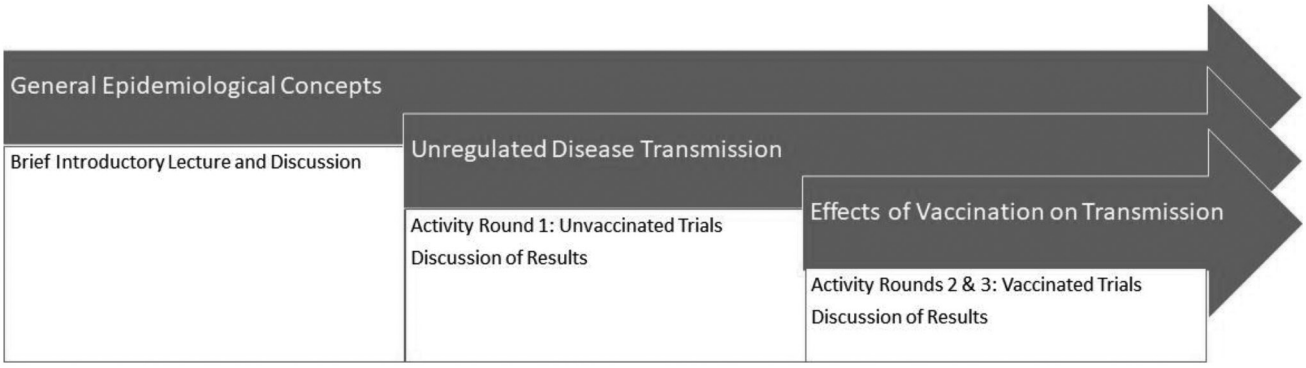

Figure 1. Activity Layout.

The suggested activity plan includes progression through a three-part progression lesson in which the hands on activities build on knowledge gained in the prior steps.

regarding disease propagation across human populations experiencing varying vaccination levels.

Through completion of this modified lab activity, students can explore the concept of herd immunity and relate it to the transmission of diseases throughout the human population. Students gain experience working with pairs during fluid exchange and working in large groups during the compilation of final data sheets and tabulation of results. Additionally, students are prompted to discuss not only the overall effects of different vaccination rates on the end result of population infection, but the potential outcomes for individuals who are vaccinated vs unvaccinated when they come in contact with an infected individual. Advanced concepts such as active infection, secondary transmission, and breakthrough infections may also be discussed in context of possible fluid exchange outcomes. Overall, it is expected that this activity will enhance opportunities for exploration of epidemiology-based topics in a classroom setting.

○ Supplemental Materials

The following appendices contain step-by-step instructions for instructors and students and are available as Supplemental Material with the online version of this article:

- Appendix 1: Instructor Activity Guide
- Appendix 2: Student Worksheet

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AMBER M. EADE (amber.eade@sru.edu) is an Associate Professor in the Department of Biology at Slippery Rock University. CORTNEY DEAN was an undergraduate student in the Department of Biology at Slippery Rock University. STACY HRIZO (stacy.hrizo@sru.edu) is a Professor in the Department of Biology at Slippery Rock University. Correspondence regarding this article should be addressed to Dr. Stacy Hrizo, 1 Morrow Way, Slippery Rock, PA 16057.