INQUIRY & INVESTIGATION

The Scene of the Crime: Classroom Integration of Biosafety, Microscopy & Forensics

NABT BioClub RECOMMENDATION

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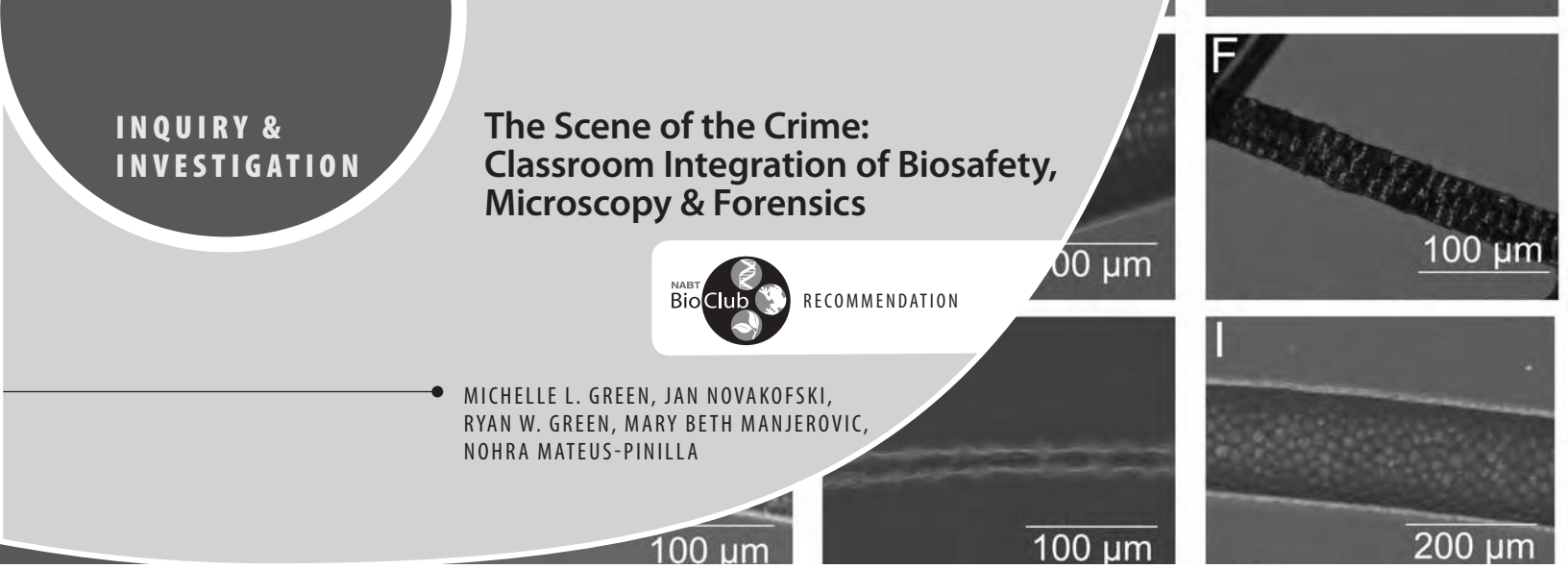

Abstract

Providing both introductory information and biosecurity protocols in laboratory, farm, and field settings is central to student learning and safety. However, even when clear protocols are provided, students do not fully understand the consequences of their actions. We present a crime scene that requires evidence investigation to improve basic skills and inquiry to identify biosecurity breaches. The crime-scene format engages students and encourages critical thinking about the negative effects of actions when working in various environments. This approach not only improves student skills through forensic microscopy but advances student retention of biosecurity requirements.

Key Words: *Forensics; teaching; biosecurity; microscopy; toxoplasmosis; zoonoses.*

Working in labs, farms, or other protocol-structured settings requires frequent, deliberate, and systematic evaluation of procedures and breaches therein. Even within classroom laboratory settings, students must learn the value of following research procedures and safety protocols. Students must be purposeful with their activities and application of scientific technologies to demonstrate their understanding of methods used to reduce negative human impacts that could potentially cause damage to environments and their living components.

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Human activities can clearly affect the surrounding environment, whether that environment is a natural ecosystem, a farm, or a research facility. Negative effects are possible even in educational settings, but biosecurity protocols are designed to minimize harmful impacts that students might have on their surroundings during laboratory, research, and learning activities. At the outset of each university laboratory course, instructors introduce students to such safety protocols, as well as basic skills. While these exercises are critical to the success of the course, it is challenging to present the information in a manner that fosters student engagement, understanding, and retention. Introductory material can be repetitive for biological science majors because they are presented with the material at the beginning of each laboratory course they enroll in over their career. Because students hear the same information in each class, they find first labs to be repetitive and dull, which results in poor student engagement. Unfortunately, poor engagement reduces student understanding and information retention (Carini et al., 2006), which can, in turn, negatively affect student learning throughout the semester. Furthermore, nonretention of safety information can cause students to threaten their own safety and the safety of individuals around them, as well as negatively affect the animals used either in the lab or in future research fieldwork. Given the potential for injury and university liability, it is imperative to develop methods to improve student understanding and retention of basic skills and safety procedures.

The goal of this module was to provide students with detailed knowledge of biosafety and animal-welfare protocols that must be followed when working with live animals. Throughout the semester, the students enrolled in our course complete activities with swine and dairy cattle at university-operated farm facilities. All laboratory exercises involving animals are overseen and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee. Because animal health is critical, students must comply with biosafety training exercises, including the Basic Training Program for Animal Users (IACUC, online module); the Occupational Health and Safety (OHS) Training (online module) required by federal regulations for animal use at research institutions; and a health-screening questionnaire (privately reviewed by OHS to alert students to personal health concerns). As instructors, we require students to pass an online quiz that covers introductory safety information. Although training completion is a good step, we find that many students do not understand practical biosafety concepts and, most importantly, do not understand the impacts of their actions on the farms.

The laboratory module presented here is designed specifically to promote student understanding of biosafety protocols and the

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consequences of protocol breaches. We used the popularity of crime-scene television programs to fuel student interest. The crime-scene format provided a novel way to combine biosafety information with basic microscopy skills.

At the conclusion of the laboratory exercises, students were expected to demonstrate understanding of their potential impact on systems and animals during course activities by (1) evaluating and applying biosafety information, including knowledge of common zoonotic diseases and biosecurity procedures; (2) differentiating between samples, based on an understanding of cell and hair structure; (3) reviewing and applying microscopy techniques to examine trace evidence found at a crime scene; and (4) developing recommendations to prevent costly disease outbreaks, based on collected evidence and observations from biosecurity breaches.

○ Background & Planning

To address our aim of helping students gain practical knowledge of biosecurity protocols, we used hair microscopy in the context of a hypothetical scenario in which biosecurity violations occurred at a university farm. Students were required to study background information prior to class and complete a prelaboratory assessment. The following sections outline the background topics.

Importance of Biosecurity Rules

Our students conduct laboratory sessions at the Imported Swine Research Laboratory (ISRL), an Animal Biosafety Level 2 facility that requires strict compliance with safety protocols. Strict protocols are in place because students may be exposed to zoonotic diseases (infectious diseases that can be transmitted between humans and animals) or bring diseases to the farm. We discuss general biosecurity strategies designed to reduce the risk of disease transmission, including personal cleanliness, limited-access policies, avoidance periods after visits to other farms, and humane pest abatement. Specifically, we discuss fomites (inanimate objects contaminated with infectious organisms), because they are the most frequent carriers of disease. Common fomites include skin cells and hair often found on dirty boots, on clothing, in dirt under fingernails, on vehicle tires, and especially on cell phones. The background information segues into a discussion of the importance of student compliance and the consequences for noncompliance.

Zoonotic Diseases

We open the discussion by asking students what diseases they can contract from wildlife, to which most students respond with "rabies" and "malaria." We further the discussion using a handout on important zoonotic diseases related to farm biosecurity (Table 1). Students specifically discuss *Toxoplasma gondii* and critically consider general characteristics, symptoms, and consequences of infection. The major discussion points include the following:

1. *Toxoplasma gondii* is one of the most common parasites found worldwide (Dubey, 2010).
2. Nearly any warm-blooded animal can be an intermediate host, but the sexual stage occurs only in cats (Dubey, 2010).
3. Cats become infected by eating infected prey.
4. The parasite is passed in the cat's feces in an oocyst form that causes infection in intermediate hosts.
5. Most human infections occur from ingesting undercooked meat containing tissue cysts, but infections also occur after consuming oocysts in contaminated food or water (Dubey, 2004).
6. The Centers for Disease Control and Prevention (CDC) considers *T. gondii* a leading cause of deaths attributed to foodborne illness (CDC, 2013).
7. Human infection does not cause serious illness in most adults, but severe disease can occur in immune-suppressed individuals and congenitally infected children (Dubey, 2010). Schizophrenia and suicidal behavior have been linked to acute toxoplasmosis in humans (Torrey & Yolken, 2003; Brown et al., 2005), and primary infection of pregnant women can lead to birth defects or fetal loss (Jones et al., 2003).
8. Severe toxoplasmosis in pigs is considered rare, but outbreaks are associated with feed contamination from cats on the farm premises.
9. Clinical signs in adult pigs include anorexia, fever, limb weakness, and death; congenital toxoplasmosis in piglets resulted in stillborn young and birth defects (for a review, see Dubey, 2010).

Microscopy

Students are provided a handout with microscopy information. Any detailed explanation of light microscopy will suffice. Because microscope details are widely available, we do not include them here.

Hair Morphology

Students review information on the structure and development of hair. We discuss keratinocytes, dead cells that previously accumulated the protein keratin but lost cytoplasmic organelles. Hair fibers are composed of keratinized epithelial cells arranged in layers (outer cuticle, middle cortex, inner medulla), and growth differences in the layers result in distinctive patterns (Hausman, 1920). We also discuss the regression (telogen) phase of hair follicles because telogen hairs shed easily and are often found at crime scenes.

○ Materials

- One microscope per student group
- Microscope supplies: lens paper, lens cleaner, immersion oil
- Clear rulers or grid slides to measure field of view

Table 1. Examples of diseases presented in the module.

Disease	Common name	Cause	Affected organisms
Avian influenza	Bird flu	Virus	Pigs, birds, humans
H1N1 influenza	Swine flu	Virus	Pigs, humans
Human influenza	Flu	Virus	Pigs, humans
Coronavirus	Common cold	Virus	Humans
Salmonella	Salmonellosis	Bacteria	Birds, humans
Toxoplasma gondii	Toxoplasmosis	Parasite	Warm-blooded animals

Figure 1. Map of the Imported Swine Research Laboratory facility, indicating access points, buildings, and sample collection locations.

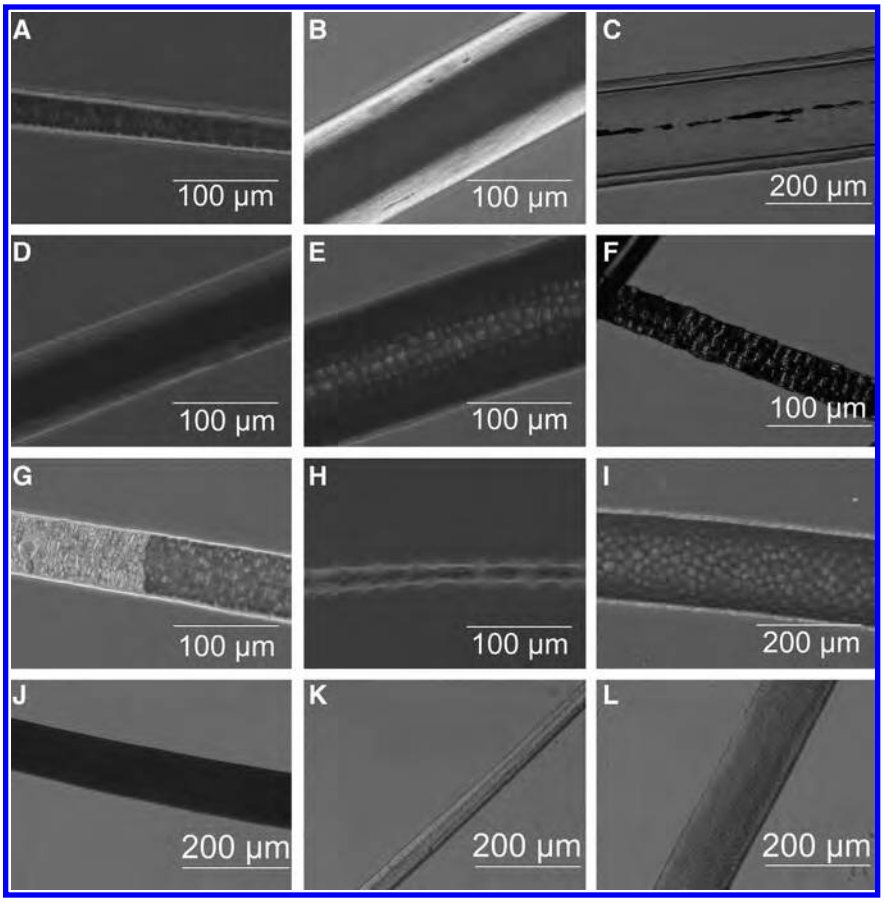

Figure 2. Example atlas of hair specimens that might be found at hypothetical crime scenes, providing example photographs as standards by which to measure prepared slides. (A) Cat, 40×; (B) dog, 40×; (C) pig, 20×; (D) horse, 40×; (E) rabbit, 40×; (F) mouse, 40×; (G) squirrel, 40×; (H) raccoon, 40×; (I) deer, 20×; (J) human (black pigment), 20×; (K) human (gray pigment), 20×; and (L) human (brown pigment), 20×. Specimens can be collected from pet owners, hunters, farmers, veterinarians, hair salons, zoos, or museums.

- Map of the facility (Figure 1)
- Diagram of hair-scale pattern-logic tree (for examples, see Petraco & Kubic, 2004)
- Example atlas of mammalian hair (Figure 2)
- Evidence and practice slides (e.g., letter "e", crossed threads, etc.)

○ The Case

The Scene of the Crime

The following premise is provided to students and discussed as a group before evidence investigation begins.

Dr. Knowsalot is investigating the spread of toxoplasmosis using a PCR test. Toxoplasmosis is a zoonotic disease that infects humans, pigs, and other species. Historically negative tests for toxoplasma at ISRL abruptly changed to a positive reaction last week. Worried, Dr. Knowsalot rushed out to collect samples, hoping to find the source of the problem. At the farm, Dr. Knowsalot collected a variety of fibers and a smudge that resembles blood. She noted new tire tracks on access roads but the tread patterns were smeared. When the Farm Manager heard of the positive result, she was worried about toxoplasmosis in her herd, biosecurity procedures, and potential losses of tens of thousands of dollars from a serious disease outbreak. The Manager asked us to help determine if there was a biosecurity breach and make specific recommendations to prevent future problems. She provided us with current biosecurity procedures, a facility map, the samples, and information on farm personnel.

The Suspects

We present four suspects to account for potential biosecurity breaches:

1. *Farm Manager: Medium height, dark hair. Manages several farms. Conscientious and knowledgeable. Teaches students about farm management and best practices for handling livestock. Most recent management concern was hiring the cheapest possible pest control company to control mice on the farm.*
2. *Farm Worker: Short, black hair, has three children, involved in activities that keep him busy. A recent hire, works some days at ISRL, some days at the poultry farm. He is responsible for treating any minor injuries on the farm. Although it is against the rules, he goes from the poultry farm to ISRL on some days because he is committed to giving first aid to injured birds and piglets.*
3. *Graduate Student 1: Medium blonde hair. Underpaid like most students, drives an old pickup that has never seen a carwash. Tries to save money on food and laundry. Her research compares a lactation gene between cows and pigs. Working at two farms and a lab, she is busy and takes shortcuts between locations to save time. Has a gray cat that likes to sleep in her clothes basket and play in her boots.*

4. *Graduate Student 2: Tall, light brown hair, easygoing. Allergies have flared up, leaving him sneezing and coughing. Wears insulated coveralls, even though they are difficult to wash, and beat-up boots. His research comparing the health of domestic pigs and feral hogs provides plenty of time on site at the farm. A confirmed outdoor type, he supplements his meager salary by hunting deer and ducks. His constant companion is his hunting dog.*

The Investigation

Students carry out a standard microscopy activity using practice slides to learn about magnification, total magnification, parfocal attributes, contrast, and determining the size of the field of view. Students receive six samples (Table 2) and estimate diameters of hair or cells; then they sketch and describe key characteristics such as the cuticle, cortex, medulla, and pigmentation. We provide an atlas of hair samples for comparison (Figure 2) and a key to the patterns of cuticle and medullary epithelium of animal hairs. Once students determine the sample source, they generate a hypothesis to explain why the specimen was at the crime scene and complete the assessment (for examples of student explanations, see Table 2).

○ Student Assessment

Students complete a lab report using evidence to identify violations and guilty suspects. While completing the lab report, they are encouraged to discuss the case with lab partners. As part of the report, students are asked the following questions and required to support their claims with evidence:

1. *What biosecurity breaches took place, and who was responsible?*
2. *Which biosecurity breach and person were likely responsible for introducing toxoplasmosis?*
3. *What other pathogen(s) may have been introduced by biosecurity breaches you identified?*
4. *What recommendations would you make to prevent future problems?*

Students often return favorable ideas, such as installing a washer and dryer at the farm for washing coveralls. Interestingly, some students suggest firing individuals that committed violations. While the recommendations are hypothetical, it is noteworthy that students recognize the severity of the violations and voluntarily suggest harsh consequences. This highlights the importance of student compliance at the farms, reinforcing the importance of learning good biosecurity practices, and understanding consequences that are in place for student biosecurity violations.

○ Expected Outcomes & Discussion

Instructors provide discussion questions after lab activities are completed, allowing for formative assessment by monitoring group discussions in the classroom. The following questions help students connect biosecurity violations to practical consequences beyond the classroom:

1. *Given the presence of toxoplasma on the farm, what are the impacts to both humans and animals?*
2. *Based on what you know about biosecurity at a small research facility, what is the potential human impact on large production animal facilities?*
3. *Based on what you know about zoonotic diseases, how might students negatively affect a natural ecosystem? Are you at risk of toxoplasmosis or other infections when working with wildlife?*

Based on the material presented, students should connect human impact to health outcomes for the animals as well as to financial effects on large farms where veterinary services and loss of animals could occur. Students also connect human impact to natural ecosystems where zoonotic diseases are a concern. Retention of information has improved with this laboratory, providing confidence that students will use the information in future laboratory, farm, and fieldwork settings. The retention of the information is tied to critical-thinking skills necessary to complete the lab activity in a format applicable to

Table 2. Information provided to students for laboratory activity and examples of student explanations of the evidence.

Slide ID	Location	Evidence	Suspect	Sample Source & Example Explanations
I	A	Dog hair	4	No biosecurity breach – Either didn't wash coveralls and brought dog hair to farm or allowed dog to run around at the farm; dogs are intermediate hosts of *T. gondii* but an unlikely source of *T. gondii* infection
II	B	Deer hair	4	No biosecurity breach – Washed boots and removed deer hair prior to entering sensitive areas; no risk of *T. gondii* infection
III	C	Dark human hair	1; 2	No biosecurity breach – Either suspect could have dropped telogen hairs in this location; no risk of *T. gondii* infection
IV	C	Chicken blood	2	Biosecurity breach – Left chicken blood smears, didn't wash hands or clothes between farm visits, not a source of *T. gondii*
V	E	Mouse hair	1	Biosecurity breach – Suspect 1 needs to improve pest control; mice are intermediate hosts of *T. gondii*, farm cats eating infected mice could be a source of *T. gondii*
VI	F	Cat hair	3	Biosecurity breach – Didn't wash coveralls and carried cat hair into the facility; didn't wash hands after cleaning litter box at home and brought *T. gondii* oocysts to farm; possible source of *T. gondii*

future fieldwork as well as the creative format that links the lab to popular media.

Module Modification

Although we implemented this learning module in a course for Animal Science majors, the module can be applied to a variety of courses and formatted to engage nonmajors, biology majors, and high school science students. The module structure allows instructors to tailor the activity to their needs. We suggest identifying the most damaging student error(s) that may occur in a course, followed by developing a case based on that specific error. For example, if noxious chemicals must be used in safety hoods, generate a case in which staff mysteriously become ill and in which evidence (e.g., hair and fiber samples, timelines of lab courses and staff work hours, diagrams of ventilation systems) leads students to discover chemicals left on the bench top. In light of the recent scares involving anthrax and smallpox at the CDC (CDC, 2014a) and the National Institutes of Health (CDC, 2014b), modules identifying high-level biosecurity breaches may be particularly intriguing to students. The module structure can otherwise be used as a final exam activity, in courses such as parasitology or microbiology, in which students identify unknown samples selected from those they have previously studied. Whether instructors choose to reinforce safe behavior or challenge students to a unique final exam, the module format provides opportunity for student engagement.

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