רפואה וטרינרית

בטאון הסתדרות הרופאים הוטרינרים בישראל

כרך 80 • חוברת מס׳ 1 • אדר תשפ״ה, 2025

בשיתוף פעולה עם

הארגון הישראלי לרפואה וטרינרית של חיות המחמד

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בין הסתדרות הרופאים הווטרינריים בישראל לבין הארגון הווטרינרי לחיות בית, הושג הסכם לפרסום משותף של מאמרים מדעיים בנושא רפואה וטרינרית בכתב העת רפואה וטרינרית.

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תוכן עניינים

תמונת השער: **זברות** (***Zebras***)

זברות הינן קלוטי פרסה אפריקאים בעלי פרווה עם סידור פסים שחור ולבן אופייני. ישנם שלושה מיני זברה: זברת גרבי (*Equus grevyi*), זברה מצויה (*E. quagga*) וזברת הרים (*E. zebra*). הזברות שייכות לסוג *Equus* יחד עם סוסים וחמורים. פסי הזברות מופיעים בדפוסים שונים, ייחודיים לכל פרט. מספר תיאוריות הועלו עבור תפקידם של דפוסי הפסים, כשמירב ההוכחות מצביעות על תפקיד שלהם בדחיית חרקים עוקצים. זברות נמצאות בטבע במזרח ודרום אפריקה במגוון בתי גידול שונים הכוללים ערבות שיחים, מישורי דשא, אזורים מיוערים ואזורים הרריים. זברות הינן חיות מרעה, ומסוגלות להתקיים מצומח נמוך באיכות נמוכה. האיחוד הבין־לאומי לשמירת טבע (International Union for Conservation of Nature – IUCN) מסווג את מצבן של זברות גרבי כבעלי החיים בסכנת הכחדה, את זברות ההרים כנמצאות במצב פגיע ואת הזברות המצויות כנמצאות במצב סיכון. זברות עשויות לנוע ולנדוד במהלך העונה היבשה לאזורים בהם יותר מקורות מים וצמחיה. זברות מצויות תועדו נודדות למרחקים של 500 ק"מ בין נמיביה ובוטסואנה, הנדידה היבשתית הארוכה ביותר של יונקים באפריקה. בזמן הנדידה, נראה שזברות מסתמכות על זיכרון של מיקומי אזורי מרעה טובים ואף עשויות לחזות את התנאים העתידים הצפויים באזורים אליהן הגיעו. מזונן של הזברות כולל בעיקר דגניים וגמאיים, אבל הן עשויות לאכול גם קליפות עצים, עלים מעצים ושיחים, ניצנים, פירות ושורשים. בהשוואה למעלי גירה, לזברות מערכת עיכול פשוטה יותר ויעילה פחות, ולמרות זאת הן יכולות להתקיים על צמחיה מאיכות נמוכה. תקופת ההיריון בנקבות אורכת כשנה, וכחודש לאחר ההמלטה עשויה הנקבה לחזור למחזורי ייחום. לרוב נולדים סייח או סייחה יחידים, אשר מסוגלים להיעמד, ללכת ואף לרוץ כבר כשעה לאחר הלידה.

קוראי רפואה וטרינרית יקרים,

מ הדורת חודש מרץ של רפואה וטרינרית כוללת מספר מאמרים מרגשים ורלוונטים למספר תחומים במדע הווטרינריה. בטוחני שאתם, קוראינו, תמצאו מידע מעניין ובעל ערך לתחום עיסוקכם.

תודה לך, פרופ׳ **ג׳יובאני די גוארדו** על מכתבך המעניין. אני ממליץ לקוראינו לעבור ולקרוא את ההתייחסות לתפקידה החשוב של התולעת הצנועה *Caenorhabditis elegans* בקידום מדע הרפואה.

ד״ר **רפפורט** מאוניברסיטת תל־אביב מתארת את האתגר שבשמירת חיות מעבדה נקיות מגורמי מחלה, על ידי השוואה בין חולדות ועכברים המוחזקים בתנאים שונים של תברואה ובידוד. כל העוסקים בתחום זה ימצאו בתיאור מידע שימושי ומעניין.

ד״ר **קחטן** וחבריו מציגים מקרה קליני של Benign Pneumatosis Coli בכלב. מצב זה מתאפיין בהצטברות גז בדופן המעי הגס, והוא מתואר לעיתים נדירות גם ברפואת בני אדם, לרוב כממצא אקראי. זהו התיאור הראשון של אבחנה כזו בכלב בישראל, ואחד מהתיאורים הבודדים הקיימים בכלל בספרות.

שני מאמרים מטורקיה מופיעים אף הם בגיליון זה.

פרופ׳ **תורקילמאז** וצוותה ממשיכים ומתארים היבטים מעניינים של עמידות לאנטיביוטיקות. במאמר זה מושם הדגש על *E. coli* בהקשר של בריאות הציבור.

פרופ׳-ד״ר **נורל ארול** מציג מאמר מעניין בתחום הווירולוגיה (חקר הנגיפים) העוסק במחלת ה־Epizootic Hemorrhagic Disease בכבשים, עיזים וגמלים בטורקיה. המאמר מזכיר ומתייחס להתפרצויות של המחלה בישראל ולקווי הדמיון בין האזורים השונים.

ד״ר **שרה וויל פיינשטיין** וחבריה מציגים תיאור מקיף של מקרה הרעלת ניטרט בבקר במרעה אשר נגרמה על ידי אכילת צמחי גדילן מצוי (*Silybum marianum*). הכותבים מדגישים את החשיבות של ניהול שטחי המרעה וקיום ידע על אודות צמחי מרעה העשויים לגרום להרעלות.

ש ל כ ם,

ב ב ר כ ה,

ד״ר טרבור (טוביה) ויינר

עורך ראשי
״רפואה וטרינרית״

חשוב לדאוג לעתיד
כבר היום!

הבנק הבינלאומי בשיתוף הסתדרות הרופאים הווטרינרים, הארגון הישראלי לרפואת חיות המחמד ולרופאים וטרינרים בכלל הסקטורים - מזמינים אתכם לקבוע פגישת ייעוץ פנסיוני ללא עלות.

שירות מיוחד ללקוחות כל הבנקים החברים בהסתדרות הרופאים הווטרינרים, הארגון הישראלי לרפואת חיות המחמד ולרופאים וטרינרים בכלל הסקטורים

בפגישה תקבלו סיוע מקצועי ומקיף, הכולל:

- ✓ ניתוח של התיק הפנסיוני
- ✓ בחינת מרכיבי התיק והתאמתם אליכם
- ✓ המלצה למקסום החיסכון שלכם בגיל הפרישה
- ✓ בדיקה של עלויות התיק

לתיאום פגישה:

03-5196448
052-4473897

ייעוץ פרישה מורחב כולל קיבוע זכויות למס הכנסה

במסגרת ייעוץ הפרישה:

- ✓ נאסוף נתונים על כספים וחסכונות פנסיוניים מכל הגופים הרלבנטיים
- ✓ נטפל בכספי הפיצויים שקיבלת ובהטבות המס המגיעות לך בגיל פרישה
- ✓ נטפל במילוי הטפסים הנדרשים למס הכנסה (טופס קיבוע זכויות / טופס 161 / פריסת מס / רצף קצבה וכו')
- ✓ נתאים את אפשרויות מימוש החיסכון הפנסיוני לצרכיך (הון / קצבה)
- ✓ נסייע לך לנהל את החסכונות הפנסיוניים שלך לאחר הפרישה
- ✓ ייעוץ פרישה יינתן בעלות מיוחדת עבור חברי הסתדרות הרופאים הוטרינרים ובני/ות זוגם.

תקצירים

חקירה סרולוגית רטרוספקטיבית של נגיף מחלה דימומית אפיזואוטית (Epizootic Haemorrhagic Disease) בכבשים, עיזים וגמלים באזור איידין, טורקיה

נ' ארול, נ' ב"ט קוך ו־מ"ת טאן

המחלקה לוירולוגיה, הפקולטה לרפואה וטרינרית, אוניברסיטת איידין אדנאן מנדרס, קמפוס מערבי, איסיקלי, אפלאר, איידין, טורקיה.

נגיף מחלה דימומית אפיזואוטית (Epizootic Hemorrhagic Disease virus – EHDV) גורם לזיהום ויראלי מערכתי הפוגע בעיקר בצבאים אך עשוי גם להביא לנזקים כלכליים משמעותיים בבקר. בטורקיה, גורם מחלה זה תואר לראשונה באזור מוגלה ב־2007, והוא גרם למקרי תמותה גם באזור איידין. המחלה פוגעת במערכת הדם ועשויה לגרום לתמותה. המידע על אודות המאפיינים האפידמיולוגיים, הקליניים והפתולוגיים של המחלה, כמו גם על מידת האלימות של הנגיף בכבשים, עיזים וגמלים מאזור איידין הינו מצומצם. תפקידם של בעלי חיים אלו בהפצת הנגיף אינו ברור דיו. מחקר זה בדק עדויות סרולוגיות לחשיפה לנגיף בדגימות מ־40 עיזים, 55 כבשים ו־68 גמלים. נוכחות נוגדנים סגוליים כנגד EHDV נבדקה באמצעות מבחן ELISA מסחרי. נוגדנים כנגד הנגיף נמצאו ב־5/68 גמלים (7.3%), 7/40 עיזים (17.5%) ו־4/55 כבשים (7.3%). ממצאים אלו מראים שקיימת חשיפה לנגיף בכל מיני החיות הללו באזור המחקר. כיוון שנגיף זה עשוי להביא להדבקה תת קלינית או להתבטא במופעים קליניים לא אופייניים, יתכן ומיני בעלי חיים אלו ממלאים תפקיד באפידמיולוגיה של הנגיף. בנוסף, נגיף זה עשוי לעיתים לגרום להתפרצויות המביאות להפסדים כלכליים באזור. ככל הידוע לנו, זהו הדיווח הראשון על עדות סרולוגית להדבקה טבעית ב־EHDV בגמלים ועיזים בטורקיה. תוצאות סקר ראשוני זה עשויות לסייע בסקרים עתידיים רחבים יותר המכוונים למניעה ושליטה בתחלואה מהנגיף.

אתגרים בשמירת על הסטטוס המיקרוביאלי של בעלי חיים בבית חיות SPF וקונבנציונלי

ד' רפפורט[1]*, מ"ל ארמה[2] ו־מ' הרלב[2]

[1] מעבדת אבחון סנטינלים, מחלקה למיקרוביולוגיה קלינית ואימונולוגיה, הפקולטה למדעי הרפואה והבריאות, אוניברסיטת תל אביב, תל אביב, ישראל

[2] המרכז לשירות וטרינרי, הפקולטה למדעי הרפואה והבריאות, אוניברסיטת תל אביב, תל אביב, ישראל

ניטור מיקרוביולוגי, וירולוגי ופארזיטולוגי מבוצע כשיגרה בבתי חיות. הניטור חיוני להערכת המצב הבריאותי של בעלי חיים המשמשים במחקר. במהלך חמש שנים, בדקנו זיהומים מיקרוביאליים ונוכחות טפילים במכרסמים בבית חיות למחקר השייכים למרכז הווטרינרי של אוניברסיטת תל אביב, הכולל בית חיות SPF (Specific Pathogen Free) וקונבנציונליים. הניטור התבצע בהתאם להמלצות של האיגוד המדעי האירופאי של בעלי חיים (FELASA). כ־955 עכברים וחולדות נבדקו במחקר. החיידקים הנפוצים ביותר היו: *Pasteurella spp., Staphylococcus aureus, Klebsiella pneumoniae, oxytoca), Pseudomonas aeruginosa*).

בנוסף, בודדו חיידקים שלא נמצאים ברשימת FELASA, ביניהם: *Proteus spp., Enterobacter cloacae, Morganella morganii* שהתגלה בשיעור נמוך. תולעי מעיים ואקריות לא נמצאו בבית חיות SPF והראו שכיחות

של 0.5%-8% במתקנים קונבנציונליים. מבחנים סטטיסטים הראו שכיחות נמוכה יותר של פתוגנים ביחידת SPF בהשוואה ליחידות קונבנציונליות. ניתן להגיע למסקנה שלמרות הקירבה הפיזית של שני מתקני בעלי חיים, הסטטוס המיקרוביולוגי נשמר והוא מובהק לטווח ארוך כתוצאה מניטור קפדני סביבתי ובריאותי של החיות, ניהול קפדני של המתקנים ותשתיות מתוכננות היטב.

חקירה של ייצור בטא־לקטמאז רחב טווח, עמידות לאנטיביוטיקה ונוכחות הגן *mcr-1* בבידודי *Escherichia coli* בתרנגולות לפיטום

פ' אוקק[1] ו־ס' תורקילמאז[2]

[1] המחלקה לביולוגיה, מיקרוביולוגיה בסיסית ותעשייתית, אוניברסיטת מניסה סלל ביאר, מניסה, טורקיה.
[2] המחלקה למיקרוביולוגיה, הפקולטה לרפואה וטרינרית, אוניברסיטת איידין אדנן מנדרס, איידין, טורקיה.

זיהוי ייצור בטא לקטמאז רחב טווח (ESBL) ונוכחות גן העמידות לקוליסטין (mobilized colistin resistance gene – *mcr*) בבידודי *Escherichia coli* מעלה את הפוטנציאל להתפתחות עמידות כפולה ודו־כיוונית על ידי שני אלמנטים אלו. הסיכון להעברת חיידקים עמידים שכאלו מבעלי חיים לבני אדם הינו משמעותי בהיבט בריאות הציבור ובריאות בעלי חיים. מחקר זה כיוון לחקור את מופעי העמידות לאנטיביוטיקה של חיידקי *E. coli* המייצרים ESBL אשר בודדו מעופות לפיטום אשר אובחנו עם colibacillosis בחלקה המערבי של טורקיה, ובפרט את הגנוטיפים *bla*TEM, *bla*CTX-M, *bla*OXA, *bla*SHV המקושרים ל־ESBL ו־*mcr-1* המקושר לעמידות לקוליסטין. במהלך המחקר, 247 (75%) בידודי חיידקי *E. coli* בוצעו בשיטות קלאסיות מ־327 מקרים חשודים ל־colibacillosis מכבדי עוף אשר הובאו למעבדה במסגרת אבחון שוטף. זיהוי החיידקים נעשה בשיטות קלאסיות קונבנציונאליות. זיהוי בידודים מייצרי ESBL נעשה פנוטיפית על ידי שימוש ב־CHROMagar ESBL, והגנוטיפים המדוברים זוהו על ידי שימוש ב־PCR. פרופיל העמידויות ל־20 סוגי אנטיביוטיקות מ־9 משפחות שונות נבחן על ידי מערכת מיקרוביולוגית אוטומטית (BD Phoenix, Becton-Dickinson, USA). בידודים שנמצאו עמידים ללפחות אנטיביוטיקה אחת משלוש או יותר משפחות אנטיביוטיקה שונות נחשבו כ־(multidrug resistant (MDR. שכיחות ESBL בקרב כלל הבידודים הנבדקים עמדה על 27%. גן העמידות הנפוץ ביותר היה *bla*TEM (53%), והגנים ל־*bla*CTX-M (27%), blaSHV (8%) ו־*bla*OXA (5%) זוהו גם הם. כל הבידודים מייצרי ESBL נמצאו להיות גם MDR. כל הבידודים נמצאו עמידים ל־tigecycline, 97% מהם עמידים לאמפיצילין ו־91% עמידים לציפרופלוקסצין. הרגישות הגבוהה ביותר זוהתה עבור אמיקצין, ארטפנאם, אימיפנאם ומרופנאם (100%). בנוסף, הגן *mcr*-1 זוהה ב־12% מהבידודים מייצרי ESBL. התוצאות מראות כי ייצור ESBL מצוי בשיעורים גבוהים בבידודי *E.coli* מתרנגולות לפיטום, והגנוטיפ *bla*TEM היה הנפוץ ביותר מבין הגנוטיפים שזוהו. העובדה שכול הבידודים מייצרי ESBL נמצאו להיות גם MDR מדגישה את הקושי האפשרי בטיפול בחיידקים אלו. נוכחות משותפת של ESBL וגנים לעמידות לקוליסטין הנישאים על ידי פלסמיד מלמדת על הסיכון המשמעותי שחיידקים אלו עשויים להוות לבריאות הציבור.

פנאומטוזיס שפיר של המעי הגס בכלבה עם דלף שערי־מערכתי תוך כבדי: דוח מקרה וסקירת הספרות

ע' קחטן, א' שיפוב ו־ד' פארי

בית הספר לרפואה וטרינרית ע"ש קורט, הפקולטה לחקלאות מזון וסביבה ע"ש רוברט סמית, האוניברסיטה העברית, רחובות, ישראל.

פנאומטוזיס במעי הגס הוא תופעה נדירה בה יש נוכחות של אוויר בדופן המעי. באנשים מדובר בתופעה לא שכיחה, אשר עשויה להיות שפירה ואף תתכן כממצא אקראי. ברפואה הווטרינרית מדובר בתופעה נדירה, עם דווחים מועטים מאוד על התופעה כחסרת משמעות קלינית. מתואר מקרה של כלבת ויימרנר מעוקרת בגיל 5 שנים, מאובחנת ומטופלת רפואית עבור דלף שערי־מערכתי (Portosystemic shunt) תוך כבדי, אשר במהלך תהליך האבחון הודגם פנאומטוזיס במעי הגס כממצא אקראי באמצעות מכשיר אולטרסאונד ומכשיר טומוגרפיה ממוחשבת (Computed Tomography; CT). הכלבה טופלה בטיפול שמרני שגרתי עבור דלף הדם, אשר כלל טיפול אנטיביוטי, מגני קיבה ולקטולוז. במעקבים קליניים המשכיים, הכוללים הדמיית הבטן באמצעות אולטרסאונד, לא נראתה עדות לפנאומטוזיס במעי הגס, ולא נראו סימנים קליניים הקשורים למערכת העיכול. האופי השפיר של פנאומטוזיס המעי הגס במקרה זה מעלה תהייה באשר לחשיבות הקלינית של ממצא רדיוגרפי זה, אשר עד היום נחשב כממצא בעל משמעות קלינית רבה בעולם הווטרינרי, לפחות עבור חלק מהמקרים. בנוסף, מקרה זה מדגיש את החשיבות של אפיון וסווג רדיוגרפי קפדני של ממצא הפנאומטוזיס במעי, על מנת לנסות וליצור קריטריונים של שינויים הדמתיים אשר יהיו במתאם עם תחלואה ותמותה, כפי שנעשה ברפואה הומאנית.

הרעלת ניטראטים/ניטריטים אקוטית בעדר בקר: תיאור מקרה

ש' ווייל פינשטיין[1], ב' חלבי[1], י' הדני[1], ש' בראל[2], א' קוניאח[2] ו־מ' בלאיש[2]

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[2] המכון הווטרינרי ע"ש קימרון, בית דגן, ישראל.

בעדר בקר לבשר מסוג סימנטל מעורב, המוחזק במרעה ליד היישוב פסוטה שבגליל המערבי, זוהתה תמותה פתאומית של 44% מהבקר הבוגר במהלך חודש ינואר 2024. חלקת המרעה בה שהה הבקר התאפיינה בגדילה דומיננטית של צמח הגדילן המצוי, הידוע כצמח ניטרופילי המיטיב לגדול בשטחי מרעה המאוכלסים באופן אינטנסיבי. המופע האקוטי שאופיין בשיעור תמותה גבוה, דימום מפתחי האף והעיניים, מט־המו־גלובינמיה, וריכוז גבוה של ניטראטים בנוזל העין הצביע על הרעלת ניטראטים/ניטריטים כתוצאה מאכילה מסיבית של צמחי הגדילן הצעירים: כאשר צמחי מכילי אשלגן ניטראט נאכלים ע"י בקר, הם עוברים פירוק מיקרוביאלי בכרס, במהלכם נוצרים יוני ניטריט שנצמדים להמוגלובין והופכים אותו למט־המוגלובין שאיננו יכול להיקשר לחמצן. בעל החיים במקרה כזה סובל מאי ספיקת חמצן, ומת כתוצאה מחנק.

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מי אנחנו?

המכון למזון לחיות מחמד (PFI) הוא הקול של יצרני מזון לחיות מחמד בארה"ב כבר למעלה מ־60 שנה. החברים שלנו מייצרים את הרוב המכריע של מזון ופינוקים לחיות מחמד המיוצרים בארצות הברית.

באמצעות הסברה והדרכה שלנו ועבודת חברינו, אנו עוזרים ל־186 מיליון חיות המחמד של אמריקה ועוד מיליוני חיות מחמד בעולם, כולל בישראל, לחיות חיים ארוכים ובריאים יותר.

כאן תמצאו את המידע העדכני ביותר הנוגע לתזונה ולמזון של חיות מחמד.

מה חדש הפעם?

מקור חלבון חדש למזון לחיות מחמד: בשר תרבותי

מאת: דויד סטפנוטי
פורסם באינטרנט: 30 בספטמבר 2024
https://doi.org/10.12968/coan.2024.0013

התקציר תורגם מאנגלית

בשר תרבותי הוא מקור חלבון חלופי שפותח כדי לתת מענה לדאגות הקיימות, בריאות הציבור ורווחת בעלי החיים של ייצור בשר קונבנציונלי. מאות חברות סטארט־אפ ומוסדות אקדמיים ברחבי העולם פועלים להפיכת בשר תרבותי למקור חלבון חסכוני עבור בני אדם. עם זאת, בשר תרבותי יכול לשמש גם להאכלת כלבים וחתולים, ולתרום לפתרון בעיות אספקת הבשר איתן מתמודד שוק המזון לבעלי חיים הגדל בשנים האחרונות.

היתרונות של שימוש בבשר תרבותי כמקור חלבון לחיות מחמד יכללו הפחתה של ההשפעה הסביבתית של תזונת חיות המחמד, ירידה בסבל של חיות משק ומספר יתרונות במסגרת Health One, שכן מזון לחיות מחמד מבוסס בשר יפחית משמעותית את הסיכון להפצת פתוגנים, לבטיחות מזון, מחלות זואונוטיות וחיידקים עמידים.

תהליך הייצור ללא אנטיביוטיקה והתנאים האספטיים שהתאים דורשים כדי לגדול בביו־ריאקטורים מובילים ליתרונות בריאות הציבור הללו. עם זאת, בשר תרבותי מעולם לא הופק בקנה מידה למאכל אדם או חיות מחמד.

יש להתגבר על מספר אתגרים טכניים כדי להנגיש לצרכנים את מחירי המזון לחיות מחמד מבוססי בשר תרבותי. כמרכיב חדשני, אין גם עדות להשפעה של האכלת בשר תרבותי לכלבים וחתולים.

באופן עקרוני, בשר מתורבת יכול להיות גם בטוח לצריכה לטווח ארוך וגם מתאים מבחינה תזונתית - וגם עם מספר אפשרויות לשיפור תזונתי פוטנציאלי אפילו עדיף על מקבילו הרגיל.

עם זאת, היצרנים צריכים להוכיח את הבטיחות והתזונתיות של מוצרים מבוססי בשר תרבותיים כדי לקבל אישור רגולטורי ולהעדיף אימוץ צרכנים. וטרינרים, אחיות וטרינריות וטכנאים ימלאו תפקיד קריטי בפיתוח המרכיב החדש הזה בהיבטים רבים, כולל פיתוח מוצר, הערכת בטיחות ותזונה, עריכת מחקר והסברה לצרכנים. סקירה זו מסכמת את היתרונות והאתגרים של שימוש בבשר תרבותי כמרכיב מזון לחיות מחמד.

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Acute nitrite toxicosis, as we had in this case, is manifested by met-hemoblobin formation (which is brown), due to the oxidation of iron atoms in the hemoglobin, from Ferrous iron (Fe+2) to Ferric iron (Fe+3), rendering the hemoglobin unable to carry oxygen. This process can lead to a severe generalized lack of oxygen in organs called methemoglobinemia. In this status, as we have seen in this outbreak, the animal exhibits brown mucous and blood.

Nitrate poisoning in cattle can act very quickly and symptoms may not be seen before the animals are found dead. However, symptoms that can be seen in some cases are weakness, unstable gait, shallow and rapid mouth breathing, rapid and weak pulse, frequent urination, blue or brown discoloration of mucous membranes, tremors, coma and death. Subacute or chronic nitrate poisoning in cattle can result in infertility and abortions.

There are several factors that can affect the quantity of nitrates in the plants.

Some of the most important factors are:

1. The species of the plant. It is known that milk thistle tends to accumulate a high quantity of nitrates.
2. Stalks are usually higher in nitrates concentrations than leaves and grains.
3. Young or immature plants are with higher nitrate contents than mature plants (as we had in this case).
4. Environmental conditions that reduce plants growth such as cloudy or cold weather, can stimulate nitrate accumulation.
5. The presence of nitrate in the plant is higher in night that in the day time.
6. Manure and fertilizers containing nitrogen can lead to excessive storage of nitrates in the plant.
7. **Pathogenesis**
8. The "milk thistle", or in his Latin name *"Silybum marianum"*, belongs taxonomically to the Asteraceae family. It has various common names including milk thistle, blessed milkthistle, Marian thistle, Mary thistle, Saint Mary's thistle, Mediterranean milk thistle, variegated thistle and Scotch thistle. This species is an annual or biennial plant, originally a native of Southern Europe through to Asia, it is now found throughout the world. Typical thistle has red to purple flowers in spring and shiny pale green leaves with white veins (fig. 4). Milk thistle has been used in traditional medicine for centuries (13). Nitrate concentrations in plant dry matter greater than 1.5 NO3 are widely regarded as potentially hazardous to grazing ruminants (14, 15). These guideline values were based on an oral LD50 estimate for cattle of about 0.3 g/kg body weight for nitrate drenched as an aqueous solution (15).
9. Following nitrate-containing plants consumption, nitrate ions are reduced to nitrite ions by the ruminal flora, and are rapidly absorbed and bind to the ruminant's hemoglobin converting it to met-hemoglobin, which inhibits oxygen transport. This process can lead to abortions, fatigue, dyspnea, cyanotic mucous membranes, weakness, and if severe, death due to anoxia. Ruminants are more susceptible because rumen flora can rapidly reduce nitrates to nitrites.

SUMMARY

In this case, 22 of 50 beef cattle succombed due to an acute nitrates poisoning. The herd entered an area with young milk thistle an ingested this plant which was proven by laboratory tests to contain high quantity of nitrates. PM examination revealed a large amount of this plant in the rumen content. The death was quick since it occurred in all the affected animals during one night. Laboratory tests found also nitrates in the occular fluids and blood of several animals . Other laboratory tests to pesticides that can lead to an acute death manifestation like in this case, revealed negative results.

In the field, there was no evidence of other affected animal species such as wildlife or dogs that ate from the carcasses. Furthermore, yound cattle were not affected, due to the lack of rumen and ruminal microorganisms.

This case study demontrates the importance of pasture management and proper knowledge relatting to potential hazardous plants. The natural grazing environment cannot be ignore, rather adressing the cows and the pasture as one holistic eco-system.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Figure 11. Herd's dog that fed from a carcass

visually comparing the reaction zone of the test strip with a provided color scale. Additionally, the presence of nitrite ions is indicated by a color change in the "alert zone" of the strip.

Nitrate Determination in Plant Material (*Silybum marianum*)

Identification and quantification of nitrate was measured based on the method described by Buck (11) and Buxton (12).

Sampling and Sample Preparation

Transport of *Silybum marianum* plant samples to the laboratory and preparation of samples for analysis were conducted following the toxicology lab standard operating procedure (SOP). Initially, 50 grams of plant material were cut and dried at 60°C for 24 hours. Subsequently, 1 gram of chopped dried plant material was placed in a 50 ml test tube for further processing.

Extraction Procedure

Extraction of nitrate from the plant material was achieved by adding 20 ml of diluted acid (1% HCL) to the test tube containing the plant material. A 1 ml aliquot was then taken from the filtered extract for subsequent analysis.

Colorimetric Reaction

To facilitate the colorimetric determination of nitrate, 9 ml of 20% acetic acid was added to the aliquot. Furthermore, 0.4 grams of a color reagent powder were dissolved in the solution to convert any nitrate to nitrite, resulting in the development of a red color indicative of the total nitrate content in the extract. Following vortexing and centrifugation, the intensity of the red color was measured.

Measurement and Calculation

The measurement of nitrate concentration was performed using a colorimetric method employing visible spectrophotometry. Absorbance readings were taken at a wavelength of 520 nm. The concentration of nitrate in the extract was calculated based on the absorbance values obtained. Calibration was performed using reagent blanks and a reference standard solution of 25 ppm KNO3.

LABORATORY RESULTS

Thistle plants were indeed found in the ruminal content. Laboratory analysis revealed 6.3% nitrate content (on DM basis) in the plants found in that pasture. High concentrations of nitrate were found in three samples of intra-ocular fluid from three different cows. The first sample tested contained 100 ppm. In the following day, two additional samples were tested, each containing 80 ppm nitrate. An intra-ocular sample from a fetus found in a dead cow contained 25 ppm. Laboratory findings indeed indicated an acute nitrate intoxication.

DISCUSSION

Nitrate poisoning can arise in animals, especially in ruminants. In cattle, which is the most affected species, nitrate toxicosis can occur by several ways, all of them are by ingestion. Ingestion of excess of nitrates can occur from plants (in the field or with forage given), water containing nitrates (for example whey) or nitrate-containing fertilizers. Due to the rumen flora activity, nitrate ions are converted to nitrite ions, which are ten times more toxic than nitrate ions. The same process can occur in the cecum of horses but in a less extent.

Figure 8. Location of the herd.

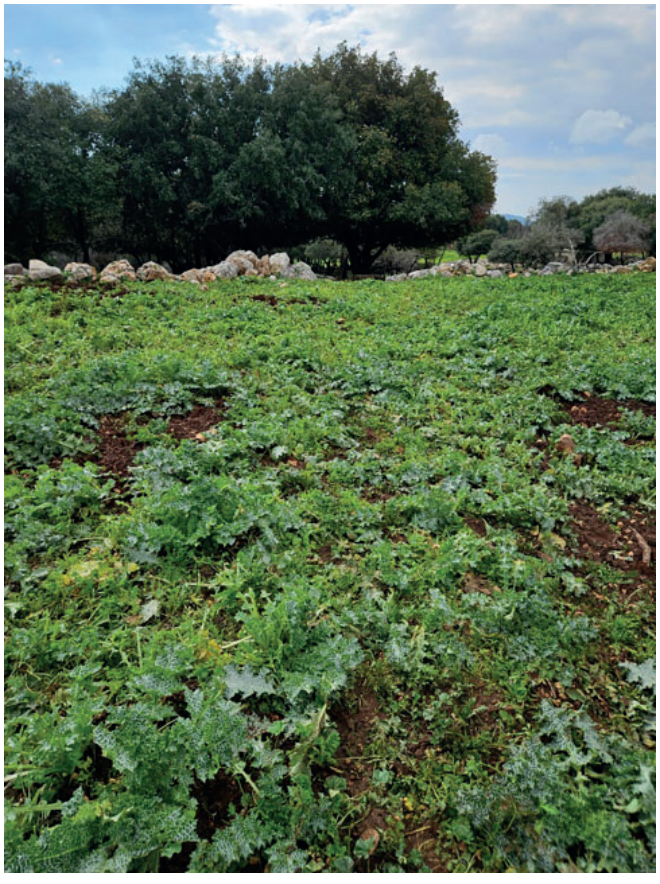

Figure 9. Domination of thistle plants in the pasture.

Figure 10. Flowering thistle.

Testing Method

The MQuant® Nitrate test strips (Merck KGaA, Darmstadt, Germany) were employed for the semi-quantitative determination of nitrate concentrations in ocular fluid. These strips facilitate a quick and easy monitoring process. Nitrate ions in the ocular fluid are reduced to nitrite ions by a reducing agent present on the strip. The resulting nitrite ions react with an aromatic amine to form a diazonium salt, which in turn reacts with N-(1-naphthyl)-ethylene-diamine to form a red-violet azo dye. The concentration of nitrate is estimated by

Figure 6. Living calf near its dam.

Figure 7. Aborted calf a day after the poisoning event.

in Fig. 8) is about 30-50 acre and it was previously used by three different herds. Topographically, the area is rocky and sparsely covered with trees and bushes that are located only around it. In the pasture, young herbs were present and a high quantity of young milk thistle have grown after several days of rain (fig. 9 & 10). Although the herd was accompanied by shepherd dogs that seemed to have eaten or licked the affected cows (fig. 11), there were no clinical symptoms in the dogs. Furthermore, there was no evidence that other animal species such as wild life, have been affected, as generally happens in other acute intoxications such as carbamate or organophosphorus poisoning, due to primary or secondary poisoning. In addition, the breeder reported that already in 2017, the herd suffered from nitrate poisoning.

DIAGNOSIS

Blood, intra-ocular fluid, internal organs and ruminal content were sampled and sent to the toxicology laboratory at Kimron Veterinary Institute.

NITRATE DETERMINATION IN OCULAR FLUID

Sample Collection and Stability

Ocular fluid specimens, collected postmortem, were chosen as the appropriate sample for nitrate determination. These specimens exhibit stability with respect to nitrate concentration for up to 60 hours after death. When refrigerated, stability is maintained for at least 1 week, while storage at -20°C extends stability for up to one month.

Figure 4. The dead bull.

from high mortality rate of 22 out of 50 cows (44%), during one night in January 2024.

The event occurred from the afternoon hours throughout the night, while in the previous day no clinical signs were observed. At morning, the animal carcasses were scattered all around the field (Fig. 1), while they were bloated and a dark blood was excreted from the eyes and nozzles (Fig. 2 & 3). The blood from the nozzles was foamy. During a venous blood sampling it was noticed that the blood didn't coagulates, and its color was indeed red-dark, which indicates met-hemoglobinemia. No signs of diarrhea were found.

This herd has been grazing in the same pasture for the last three months, during this time the cattle received additional poultry manure and potatoes. These cows have been vaccinated against FMD, botulism, rabies, clostridium and babesiosis. Pesticides were not used in the period close to the case. The affected cattle were mature females and the herd's bull (Fig. 4). Young individuals were not affected (Fig. 5 & 6). No clinical signs were observed in the other animals in the herd. Also, no abortions or stillborn were observed during this period. However, a day after the incident, the farmer reported one stillborn (Fig. 7).

The size of the pasture area (which is demonstrated

Figure 5. Living calf among poisoned dead cows.

Figure 1. Group of animals found dead in the field.

Figure 2. Heamorrage from the eyes in an infected cow

Figure 3. Another affected dead cow, lying in a pasture dominated by thistle plants.

Case Study

ACUTE NITRATE / NITRITE INTOXICATION IN BEEF CATTLE

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ABSTRACT

Acute death of 44% of a mixed-Simental adult beef cattle herd occurred in January 2024, in the Western Galilee region, near Fassuta village, in the north of Israel. The grazing area where cattle was held, was characterized by majority of young *Silybum marianum* plants, also known as "milk thistle". This nitrophilic plant tend to thrive in heavily grazed range lands. The acute nature of this event characterized by high mortality, methemoglobinemia and high nitrates concentration in samples, indicated plant-origin intoxication as a result of massive consumption of young thistle plants. Indeed, laboratory analysis revealed 6.3% nitrate content (on DM basis) in the plants found in that pasture, and high concentrations of nitrate were found in three samples of intra-ocular fluid from three different cows: the first sample tested contained 100 ppm, and two additional samples contained 80 ppm nitrate. Additionally, an intra-ocular sample from a fetus found in a dead cow contained 25 ppm. When cattle ingest nitrate-containing plants, nitrite ions are produced by ruminal micro-organisms. Nitrite ions then combine with hemoglobin to produce met-hemoglobin, blocking the transport of oxygen. The outcome is a form of oxygen deprivation, which led to this acute intoxication.

Key words: nitrate-nitrite intoxication, cattle, milk thistle, pasture.

INTRODUCTION

Nitrate intoxication of grazing ruminants, especially cattle, is a well-known phenomenon (1-3). Specific intoxication in cattle caused by grazing *Silybum marianum* plants, also known as milk thistle, was described as early as 1955 (4). This nitrophilic plant encroach rangeland areas where animals gather and defecate, in particular around watering and feeding points (5). Density of thistles is affected by cattle grazing management, such as continuous versus rotational grazing (6), but generally in midEastern rangelands, increased cattle grazing density is associated with a higher frequency of thistles (such as the milk thistle and the syrian thistle) (7). Livestock generally avoid entering dense areas of mature thistle but they will graze young, immature plants. Cattle will not graze thistle beyond the late bud stage and grazing milk thistle is deemed dangerous for cattle because of high and possibly lethal concentrations of nitrates (8, 9).

How often such intoxications occur? In a retrospective study of all suspected bovine intoxications submitted to the California Animal Health and Food Safety Laboratory in the years 2000-2011, Nitrate/nitrite poisoning was the most commonly diagnosed plant-associated intoxication (46% of plant associated intoxications) (10).

CASE DESCRIPTION

A herd of a mixed Simental cattle breed, that was held in a pasture near Fassuta village in the Western Galilee, suffered

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In conclusion, this report describes the first case of pneumatosis coli (PC) in a dog as a potentially incidental and benign finding, with no severe clinical manifestations. While potential predisposing factors, including ICPSS and lactulose therapy, were present, no direct causal relationship could be confirmed. This case underscores the need for increased radiological awareness of PC in veterinary medicine and highlights the importance of distinguishing benign forms of PC from more fulminant types, thus correlating the radiographic findings to morbidity and mortality, as is done in human medicine. Further investigation into the etiology, classification, and clinical significance of PC is warranted to improve diagnostic accuracy and guide clinical management in veterinary patients.

It is important to recognize that gastrointestinal pneumatosis represents a radiographic finding rather than a specific clinical diagnosis. As such, it should be interpreted with the same caution and clinical context applied to any other diagnostic finding. Automatic assumptions of aggressive pathology should be avoided, however, disregarding its potential significance would also be inappropriate. Future advancements in radiographic categorization and pattern classification of gastrointestinal pneumatosis may improve clinical assessments and support the development of more targeted diagnostic and therapeutic approaches for affected patients.

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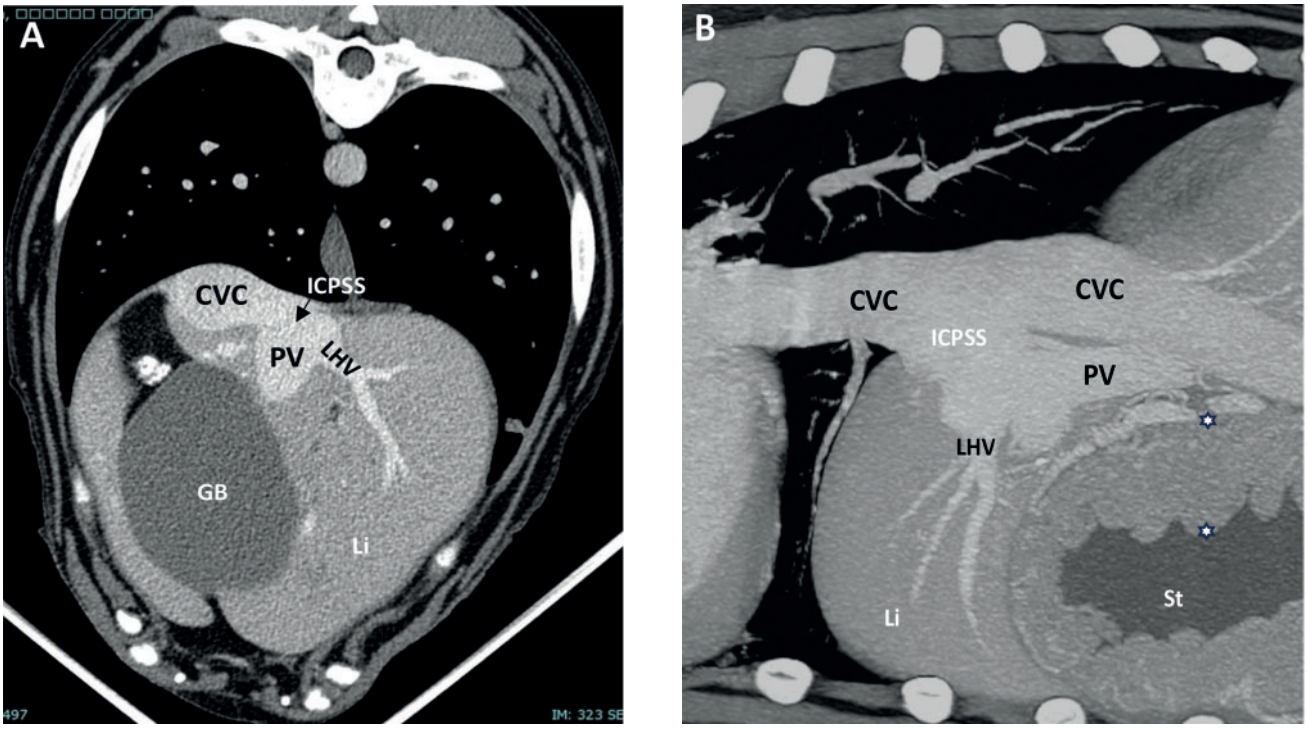


Figure 4: Transverse (A) and sagittal (B) late post contrast abdominal computed tomography images, done with the dog in sternal recumbency. Both images show the presence of a large left divisional intrahepatic congenital portosystemic shunt (ICPSS) between the caudal vena cava (CVC) and the portal vein (PV), at the location where the left hepatic vein (LHV) and the cranial phrenic vein usually enter the CVC. Note the thickening of the stomach wall identified on the sagittal view (between cursors). The sagittal image is of thin-maximum intensity projection (MIP). (Li, Liver; St, Stomach; GB, Gall Bladder).

pneumatosis linearis coli in human medicine. In one case (26), the intramural gas is described as "linear," but no additional characteristics were noted. The accompanying figures depict an extensive circumferential extraluminal air pattern along the large bowel, which appeared non-specific and did not match the linear gas patterns typically reported in human cases (1). Another case (2) includes only a general reference to extraluminal gas tracking along the colon, with no further descriptive detail. The single figure presented also displays extensive and circumferential extraluminal air without a distinctive pattern. The third case (24) lacked any detailed description of the intramural gas and provideed just one figure showing a focal intramural gas pattern with a linear orientation. However, this isolated image may not adequately represent the exact intramural gas distribution pattern which may be better defined on the complete scan. These reports highlight the current limitations in veterinary literature regarding the radiographic characterization of PC and underscore the need for more systematic imaging analysis and standardized descriptions to establish clear diagnostic patterns and improve the clinical interpretation of PC findings in veterinary patients.

The relatively low number of reported PC cases in veterinary medicine, as compared to human medicine, may result from several factors. Computed tomography (CT) imaging, widely used as a screening tool in human medicine due to its availability, lower cost, and ability to be performed without anesthesia, is less commonly employed in veterinary medicine. In addition, technical differences in imaging protocols, such as the lack of oral contrast in veterinary CT studies, may impair the detection of intramural gas, leading to underdiagnosis. Furthermore, radiological awareness of PC may be lower in veterinary medicine, particularly when clinical signs are mild, non-specific, or absent. Subtle patterns of intramural gas may be overlooked, especially when masked by intraluminal gas or confounded by other gastrointestinal findings. The low number of reported PC cases could explain why PC is rarely identified as an incidental finding in veterinary medicine.

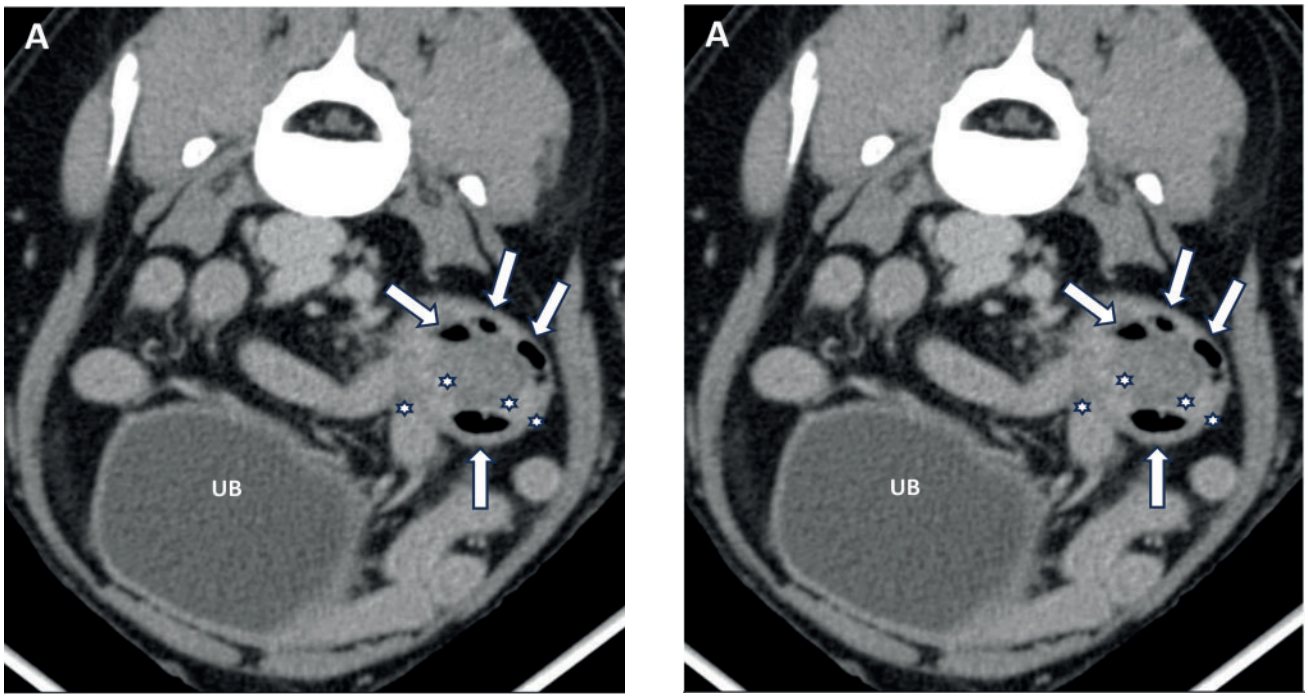


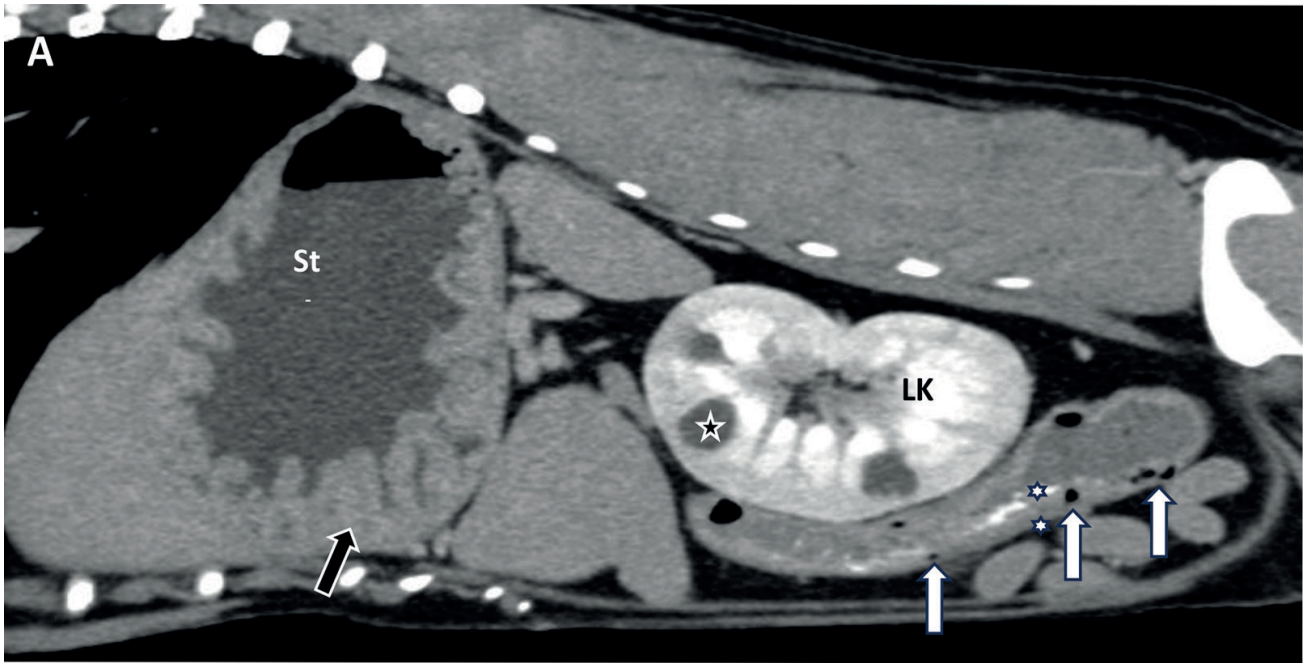
Figure 3: Transverse (A) and dorsal (B) late post contrast abdominal computed tomography images, done with the dog in sternal recumbency. Both images show the presence of irregular to cystic intramural gas collections in the wall of the descending colon (white arrows). Note the irregular thickening of the colonic wall (between the cursors). (Li, Liver; UB, Urinary Bladder).

providing a plausible link between the underlying condition and the development of PC (31). Additionally, Lactulose therapy, commonly used in the management of ICPSS, has been associated with PC in human medicine through the production of carbon dioxide and hydrogen, which may increase intraluminal pressure and allow gas migration through a compromised intestinal wall (6, 10). However, the resolution of PC in this case despite the continued administration of lactulose argues against a direct causal relationship. While antibiotic administration coincided with clinical improvement and imaging resolution, the mild nature of the clinical signs observed makes it unlikely that antibiotics played a definitive role in resolving the PC.

Several cases of PC have been reported in dogs, but only three cases have specifically involved dogs with portosystemic shunts, some of which were treated with lactulose therapy. While both PSS and lactulose are theorized to predispose dogs to pneumatosis intestinalis, no definitive causal association has been established.

The intramural gas pattern observed in this dog's CT scan does not correspond to any of the gas patterns described in human medicine for distinguishing benign pneumatosis intestinalis (PCC) from fulminant pneumatosis intestinalis (PLC) (1, 5). Subjectively, the gas distribution in this case appeared to fall somewhere between the "cystic" or "bubble-like" morphology typically associated with PCC and the "linear" and circumferential morphology characteristic of PLC (Figures 2 and 3). Additional imaging features often reported in PLC, such as bowel dilation, colonic wall thickening, and free intraperitoneal fluid (1), were also present in this case. However, these findings were nonspecific and may have alternative explanations unrelated to PLC.

Radiographic characterization of pneumatosis coli in veterinary medicine remains poorly defined, with limited reports available for comparison. Of the seven veterinary reports describing CT findings of gastrointestinal pneumatosis with imaging of intramural gas (2, 3, 7, 15, 17, 24, 26), only three reports specifically document cases of pneumatosis coli CT imaging (2, 24, 26). The remaining reports either describe pneumatosis in the esophagus and stomach or do not specify the location of the intramural gas. The CT descriptions provided in the three veterinary reports of pneumatosis coli (2, 24, 26) offer limited detail and make it challenging to identify patterns comparable to those described in pneumatosis cystoides coli and



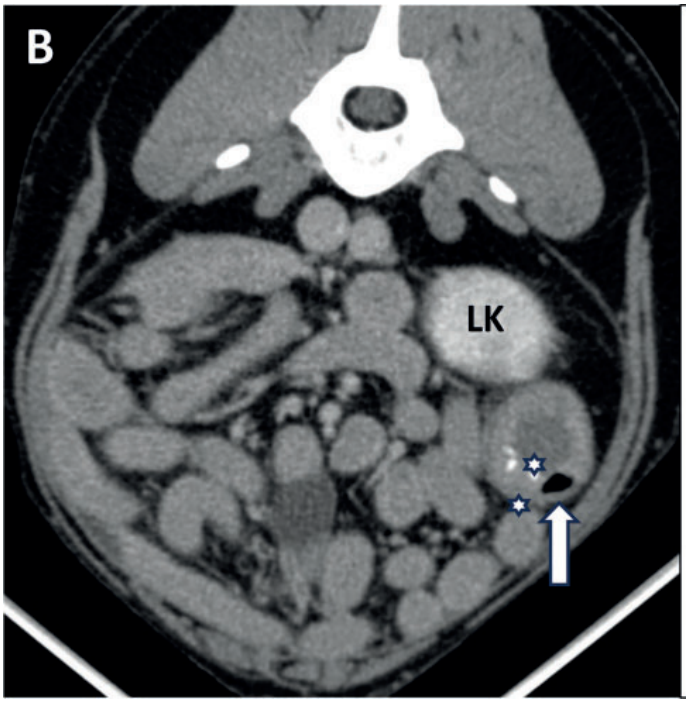


Figure 2: Sagittal (A) and transverse (B) late post contrast abdominal computed tomography images, done with the dog in sternal recumbency.

Foci cystic to linear intramural gas collections are present on the ventral wall of the descending colon (white arrows). Note the irregular thickening of the colonic wall, slightly more evident on the ventral wall (between the cursors).

In the sagittal plane image (A), a mildly enlarged polycystic left kidney (star), and a mild thickening of the stomach wall (black arrow) can be also noted.

(LK, Left Kidney; St, Stomach).

classification of this case as non-aggressive and suggests that PC can occur as a mild or asymptomatic condition in dogs, paralleling benign forms of pneumatosis cystoides coli reported in human medicine.

The precise cause of PC in this case remains unclear, though several potential mechanisms warrant consideration. Gastrointestinal disturbances associated with ICPSS, including altered portal blood flow, intestinal wall hypoxia, and bacterial overgrowth, may predispose affected dogs to the mechanical and infectious mechanisms implicated in the pathogenesis of PI. Approximately 30% of dogs with portosystemic shunts exhibit nonspecific gastrointestinal signs,

IV injection of a non-ionic contrast medium at 2ml/kg as a fast bolus (Omnipaque, GE Healthcare, Cork, Ireland; 300 mg/kg IV). The patient was scanned at 5, 20, 40 and 190 seconds post injection in order to acquire a multi-phase study. Images were viewed with a dedicated viewing software, with 3D capabilities (Fujifilm Synapse, FujiFilm Medical System USA, Stamford, CT), and multi-planar reformatting (MPR) was used whenever necessary for optimal evaluation. The scans were carried out with the patient in sternal recumbency, with a collimation extending from the cranial border of the heart, caudally to the pelvic canal, approximately up until the coxo-femoral joint.

The scans demonstrated multiple focal air collections in the wall of the descending colon, some rounded and others more linear in appearance. The descending colon was mildly dilated, measuring up to 30 mm in diameter, and the wall was thickened (up to 10 mm) and irregular. This change in the colonic wall was confined only to the descending colon, and with a tendency to be more pronounced on its ventral wall, where there were more intramural air collections than in the thinner dorsal wall (Figure 2 and 3). The rest of the small and large intestinal lumen was also mildly dilated, but with no evidence of pneumatosis. Intraluminal gas within the portal vasculature, or pneumoperitoneum were not observed.

The scan also confirmed the presence of a large left divisional ICPSS between the caudal vena cava (CVC) and the portal vein, at the location where the left hepatic vein and the cranial phrenic vein commonly enter the CVC (Figure 4). No obvious intrahepatic portal vasculature was identified. Additional abnormalities observed on the CT scan included a mildly reduced liver size, mild congestion of the extra-hepatic portal vasculature, mildly enlarged polycystic kidneys, mild enlargement of the spleen and pancreas, multiple mineralized foci in the walls of the aorta and proximal cranial mesenteric artery, a diffused thickened stomach wall (up to 26 mm) and a slight amount of free intraperitoneal fluid. In the lung lobes falling inside the field of view, a few small alveolar infiltrates were observed. No other significant abnormalities were observed in the scan's field of view.

At this point, the dog was discharged with a modified supportive treatment plan tailored to address the persistent elevated ammonia levels despite previous therapy. The updated regimen included a dietary change and antibiotic therapy [metronidazole (10 mg/kg, oral, every 12 hours; Flagil, Sanofi, Yakum, Israel) and amoxicillin-clavulanic acid (10 mg/kg, every 12 hours; Augmentin, Glaxosmithkline, Petah-Tikva, Israel)], in addition to the previous medical treatments.

A percutaneous coil embolization of the shunt was recommended as the next step in treatment. Prior to the planned intervention, a blood PCR test for *Ehrlichia canis* was submitted to investigate the cause of thrombocytopenia, and doxycycline therapy (10 mg/kg orally once daily; Doxylin, DEXCEL, Or Akiva, Israel) was initiated as a precautionary measure. The PCR result was negative; however platelet count increased. One month after presentation, an attempt at coil embolization was performed but was unsuccessful. Despite recommendations, the owners declined the option of open surgery for shunt attenuation. The dog's medical treatment remained unchanged throughout this follow-up period.

At the three-month recheck examination following initial presentation, the dog exhibited clinical improvement, with previously soft stools becoming more formed and notable weight gain observed. Ultrasonography at this time revealed no signs of pneumatosis coli (PC) or any other gastrointestinal tract abnormalities. Additionally, portal vein mean velocity (Vmean) measurements were found to be within normal limits. At this point, surgical intervention was recommended again; however, the owners opted to continue with medical management instead. Seven months following the initial presentation, the dog was doing well and remained asymptomatic with regard to the shunt. No gastrointestinal-related signs were present. The dog was receiving lactulose, omeprazole and a hepatic supplement. The owners had discontinued antibiotics for several weeks, with no impact on the dog's well-being. Ammonia levels at this point were 74 μg/dL. The owners were still not willing to pursue surgical intervention.

DISCUSSION

This report describes a unique case of pneumatosis coli in a dog with intrahepatic congenital portosystemic shunt, that presented without specific clinical signs directly attributable to the PC. Unlike most documented cases of pneumatosis intestinalis in veterinary medicine, which often exhibit severe clinical signs or a fulminant disease, this case appeared to be benign and potentially incidental. The absence of severe gastrointestinal or systemic manifestations, supports the

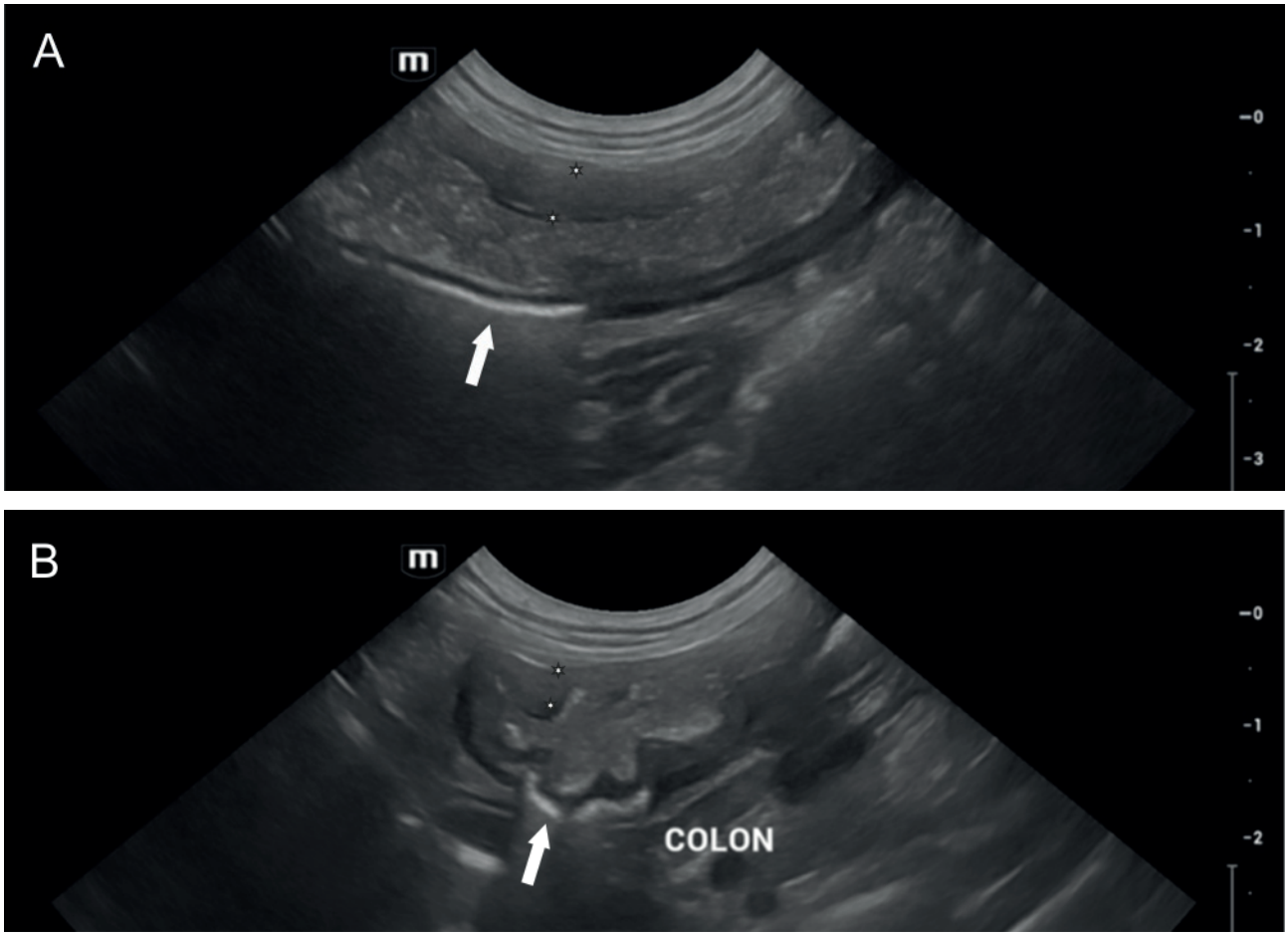


Figure 1: Ultrasonographic images of the colon in a sagittal plane (A), and a transverse plane (B). Mild, irregular thickening of the colonic wall is demonstrated (between cursors). There is an intramural linear gas pattern in the distant colonic wall, showing a reverberation artifact (arrow).

Diagnostic Imaging

Ultrasonography:

Ultrasonography was performed using a micro-convex array transducer in B-mode, at a frequency range of 3–11 MHz (Mindray Vetus 9 and Mindray M-9, Mindray Bio-Medical Electronics, Shenzhen, China). Harmonic imaging was applied in all scans.

Abdominal ultrasonography revealed several abnormalities. The liver was undersized, with no visible intrahepatic portal vasculature. The spleen was mildly heterogeneous and irregular. Both kidneys were mildly enlarged, with multiple cortical anechoic cysts and mildly irregular contours. The gastric wall was diffusely thickened, measuring up to 22 mm, with an indistinct wall layering. The colon appeared slightly distended and contained soft to liquid stool along with some intraluminal gas. In the descending colon, the wall was mildly thickened (up to 5 mm) and irregular, with several intramural gas foci identified as linear submucosal hyperechoic interfaces, producing a reverberation artifact (Figure 1). No additional abdominal abnormalities were identified. There was no evidence of enlarged or abnormal lymph nodes, and no altered echogenicity of intraperitoneal fat or membranes surrounding the colon or elsewhere in the abdominal cavity. Furthermore, intraluminal gas within the portal vasculature, or pneumoperitoneum were not observed.

Computed Tomography (CT):

CT was performed using a 40-slice helical scanner (CT imaging: Philips brilliance 40; 40 slice MDCT; Philips, Cleveland, OH, USA), using a helical scan mode, 1.5 mm thickness contiguous slices, at 120 kvp and 320 mA, and reconstruction algorithms of soft tissue, lung and bone. CT images were acquired before and immediately following manual

intraluminal pressure, allows direct gas migration into the intestinal wall. While the infectious theory proposes that gas-producing bacteria translocate into the intestinal walls. The pulmonary theory attributes the condition to elevated intrathoracic pressure, leading to alveolar rupture and subsequent gas diffusion to the intestinal wall via vascular and lymphatic pathways. All these theories may involve contributing factors such as intestinal mucosal inflammation, physical damage, and immune barrier dysfunction. Additionally, nutritional imbalances, dysbiosis, and gastrointestinal dysmotility are considered potential ancillary causes.

Limited information is available on gastrointestinal pneumatosis in veterinary medicine. Reported cases have been classified based on anatomic location and include the esophagus (7), stomach (Canine – 7, 11, 12; Feline – 13-18; Both – 19), small intestine (Canine – 20- 22; Feline – 8, 23), and colon (Canine – 2, 9, 24-30 ; Feline – 23). A recent retrospective study (3) reviewed cases of gastrointestinal pneumatosis affecting various sites along the gastrointestinal tract, with the stomach and colon reported as the most commonly affected locations. Most cases of gastrointestinal pneumatosis in veterinary patients present with severe clinical signs, requiring intensive care or surgery, developing life-threatening complications and an overall mortality of up to 53% (3). Notably, no significant difference in mortality was observed based on the anatomic location of pneumatosis (3). Unlike in human medicine, incidental findings of gastrointestinal pneumatosis in veterinary medicine are rare, with only six cases reported in dogs overall—three with esophageal pneumatosis (EP) (7) and three with gastric pneumatosis (GP) (3, 19). These cases included dogs with mild or no clinical signs, some with clinical signs unrelated to gastrointestinal diseases, and some resolving without intervention or under mild conservative management.

Pneumatosis coli (PC) is uncommon in veterinary medicine and is rarely identified as an incidental finding. Clinical signs of PC are not specific and are often related to the underlying condition, including diarrhea, hematochezia, anorexia, tenesmus, abdominal pain, vomiting and other non-specific symptoms (2, 3, 9, 23-30). Treatment typically involves supportive therapies such as antibiotics, dietary management, and gastroprotective agents, as well as treatment of the underlying pathology, if it exists.

Cases of spontaneous resolution without treatment have also been described (27). Outcomes in reported cases vary, with many dogs showing clinical resolution; however, some reports show high mortality rates, reflecting the underlying causes, such as portal hypertension, colonic torsion, and other severe systemic or gastrointestinal conditions (2, 3, 24).

Radiographic information on gastrointestinal pneumatosis in veterinary medicine is exceedingly rare, with CT findings being particularly scarce. Existing CT imaging data primarily focuses on a small number of cases (2, 3, 7, 15, 17, 24, 26), with even fewer specifically addressing CT findings in dogs with PC (2, 24, 26).

This report describes a case of PC as an incidental finding in a dog undergoing diagnostic imaging for an intrahepatic congenital portosystemic shunt (ICPSS). This case contributes to the limited veterinary literature on incidental PC and highlights the need to reevaluate its clinical significance, particularly in asymptomatic patients.

CASE REPORT

A five-year-old spayed female Weimaraner was referred to the Hebrew University Veterinary Teaching Hospital (HUVTH) for a second-opinion consultation after being diagnosed with an intrahepatic congenital portosystemic shunt two months earlier. The dog initially presented to the referring clinic with neurological signs, including weakness and disorientation, and had been treated over the preceding two months with lactulose (0.25 mL/kg, PO, q12h; Avilac, Perrigo, Yeruham, Israel), omeprazole (1.3 mg/kg, PO, q24h; Omepradex, Dexcel, Or Akiva, Israel), and a liver supportive supplement (3 tablets, PO, q24h; Wepatic, Wepharm, Batalha, Portugal). Neurological signs improved shortly following initiation of treatment, however, the owners reported persistent soft stools following the initiation of lactulose therapy. Physical examination was unremarkable apart from a body condition score of 3/9.

Laboratory Analysis

A complete blood count (CBC) revealed mild neutrophilic leukocytosis, mild eosinophilia, and mild thrombocytopenia. Serum biochemistry abnormalities included mildly elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, mild panhypoproteinemia, low blood urea, fasting plasma hyperammonemia (276 μg/dL; reference interval [RI], 0–59 μg/dL), and increased preprandial serum bile acid concentrations (123 μmol/L; RI, <5 μmol/L).

Case Report

Benign Pneumatosis Coli in a Dog with a Congenital Intrahepatic Portosystemic Shunt: Case Report and Literature Review

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ABSTRACT

Pneumatosis coli (PC) is a rare condition characterized by intramural gas within the colonic wall. It is rarely reported in human medicine, and it is often reported as an incidental finding. It is even less frequently documented in veterinary medicine, where it is currently considered a clinically significant finding. This report describes a 5-year-old spayed female Weimaraner diagnosed and medically managed for an intrahepatic congenital portosystemic shunt (ICPSS). During diagnostic workup and follow-up imaging for the shunt, abdominal ultrasound (US) and computed tomography (CT) examinations revealed PC as an incidental finding. The dog received standard medical management for ICPSS, including antibiotics, gastroprotectants, and lactulose. A follow-up ultrasound performed three months later showed complete resolution of the PC, with no associated clinical signs. The incidental nature of PC in this case raises questions regarding its clinical relevance in veterinary patients. While the role of antibiotics in the resolution of PC herein remains unclear, this case highlights the need for further investigation into the significance of PC as a diagnostic finding in veterinary medicine, especially in asymptomatic patients.

Key words: Canine; Pneumatosis; Porto Systemic Shunt; Computed Tomography.

INTRODUCTION

Gastrointestinal pneumatosis describes the presence of gas within the subserosal or submucosal layers of the gastrointestinal tract (1-3). When gas accumulation is confined to the intestinal walls, it is termed pneumatosis intestinalis (PI), hence, intramural gas in the colonic walls is specifically termed pneumatosis coli (PC). In human medicine, PI is classified into primary and secondary forms which differ in radiologic appearance and clinical significance.

Primary PI (also known as benign PI or pneumatosis cystoides coli (PCC) when the colon is involved) accounts for approximately 15% of cases. It is idiopathic, often asymptomatic, and is considered benign (1, 4-6). Secondary PI (in the colon termed pneumatosis linearis coli [PLC]) is associated with a range of underlying critical conditions, including mesenteric ischemia, bowel necrosis, trauma, inflammatory bowel disease (IBD), malignancy, autoimmune disorders, infections, chronic obstructive pulmonary disease, and medications such as immunosuppressants, corticosteroids, and chemotherapeutic agents (1, 4-6). On a Computed Tomography (CT) scan, as reported in human medicine literature, Primary PI (including PCC) typically presents as well-circumscribed cysts or bubbles, most commonly located in the right colon. Secondary PI (including PLC) exhibits a linear or circumferential distribution of intramural gas (1, 4, 5).

The pathogenesis of gastrointestinal pneumatosis remains poorly understood, with various proposed pathophysiological mechanisms potentially acting as singular or combined contributing factors (1, 4, 6-10). The mechanical theory suggests that disruption of mucosal integrity, coupled with increased

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bacteria (42, 44). Similarly, in Türkiye, the uncontrolled use of antibiotics in animal husbandry, especially due to over-the-counter sales and inadequate regulations, contributes to the increase in antibiotic resistance. This situation highlights the need for stricter regulations at the local and global level to limit antibiotic resistance.

Our study demonstrates the presence of both ESBL production and the *mcr*-1 gene in *E. coli* strains isolated from broilers in Türkiye, indicating that these two important resistance mechanisms coexist. In particular, the dominance of the *bla*$_{\text{TEM}}$ genotype, the increasing prevalence of the *bla*$_{\text{CTX-M}}$ gene, and the detection of the *mcr*-1 gene at a rate of 12% indicate regional differences in the antimicrobial resistance profile in poultry farming in Türkiye. These findings, while being consistent with the data reported in the international literature, suggest that Türkiye's different geographical and production conditions make significant contributions to the dynamics of resistance spread. The increase in multiple antibiotic resistance and colistin resistance causes a decrease in the effectiveness of existing antibiotics, necessitating the development of new treatment strategies. These strategies include innovative approaches such as new antibiotic classes, bacteriophage treatments, and antimicrobial peptides. In addition, methods that reduce the risk of resistance, such as probiotics, can be evaluated among alternative treatment options. Given that multiple antibiotic resistance and colistin resistance limit current treatment options, the development and implementation of such alternatives is of critical importance.

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of these strains were resistant to broad-spectrum antibiotics. This confirms once again that the use of antibiotics in the livestock industry contributes to the development of resistance in bacteria (34). In particular, the widespread use of broad-spectrum antibiotics in animal farming accelerates the spread of antibiotic resistance.

The potential for transmission of ESBL-producing bacteria from animals to humans poses a serious threat to public health. A previous study emphasized that transmission of ESBL-producing *E. coli* strains commonly found in poultry has become a global problem (35). Our findings are consistent with this global trend and indicate that the poultry production chain plays a critical role in the spread of antimicrobial resistance (36). This situation highlights the need to develop effective strategies to ensure the safety of animal products and combat antimicrobial resistance.

In our study, the most common ESBL genotype was the *bla*$_{\text{TEM}}$ gene, with a prevalence of 53%; this prevalence is higher than the prevalence in a study conducted in Brazil (17%) (33) and lower than the prevalence in Egypt (100%) (37). The second most common ESBL genotype was the *bla*$_{\text{CTX-M}}$ gene, with a prevalence of 27%. This prevalence is higher than some studies in South America (38), but lower than other studies (39). In addition, the *bla*$_{\text{SHV}}$ gene was detected with a low prevalence of 8% in our study, whereas the prevalence of this gene in poultry farms in Brazil was reported as high as 45% (33). In Germany and Spain, *bla*$_{\text{SHV}}$-like enzymes have been reported to be common in retail poultry products (40). The prevalence of the rarely studied *bla*$_{\text{OXA}}$ gene was 5% in our study, while it was reported as 11% in the Egyptian study (37). These proportional differences may be due to various factors such as geographical variations and antibiotic utilization policies.

The antibiotic susceptibility results obtained in our study are parallel to the findings of a similar study conducted in Algeria (18). High resistance rates to antibiotics such as ampicillin (100%), tetracycline (100%), nalidixic acid (95%) and ciprofloxacin (87%) have been reported in avian pathogenic *E. coli* strains isolated in Algeria. Similarly, high resistance rates to these antibiotics were observed in our study. However, trimethoprim-sulfamethoxazole resistance was reported at a lower rate of 62% in the Algerian study, while it was 82% in our study. These findings are consistent with the study conducted in Algeria in 2023 (18) and emphasize the high prevalence of multiple antibiotic resistant strains. Differences in antibiotic resistance rates may be due to geographical, socio-economic factors and diversity in antibiotic use habits.

The risk of transmission of the *mcr*-1 gene from animals to humans through direct contact, contaminated food, and environmental sources is high (41). The spread of this gene threatens public health by causing infections that limit treatment options in humans and nosocomial infections (42). In our study, the mcr-1 gene was detected in 12% of ESBL-producing isolates; this finding is consistent with previous studies. In a study conducted in Argentina in 2019, the presence of the *mcr*-1 gene was confirmed in *E. coli* strains isolated from domestic animals and it was stated that this gene plays a critical role in the spread of resistance between humans and animals (38). The ability of the *mcr*-1 gene to be transmitted via plasmid allows rapid spread of resistance and poses a risk especially between poultry and domestic animals (42). Colistin is one of the antibiotics of last resort, and the presence of *mcr*-1-carrying strains severely limits treatment options (30, 41). The low rates of colistin resistance observed in our study indicate that careful management of antibiotics may be effective in limiting the development of resistance, but other environmental and genetic factors also play a role. In a recent study conducted in Algeria (18), 14% colistin resistance was reported, in line with the findings in this study. The rapid spread of resistance genes via horizontal gene transfer (conjugation, transduction, transformation) via plasmids is accelerated in environments with high antibiotic pressure, especially via conjugation. In environments where antibiotics are used intensively, the mobilization ability of plasmids and environmental stress factors promote this gene transfer (30). This situation may cause the rapid spread of resistant bacteria, especially in places such as hospitals and animal husbandry facilities.

Differences in antibiotic use among countries have a significant impact on the prevalence of ESBL-producing bacteria. While antibiotic use is strictly regulated in developed countries, uncontrolled and widespread antibiotic use is still a major problem in developing countries. For example, the ban on growth-promoting antibiotics in the European Union has reduced the spread of ESBL strains (43), while in countries such as India and Brazil, the easy accessibility of antibiotics and their uncontrolled use in animal husbandry have led to an increase in antibiotic resistance (43). Similarly, in countries such as the USA and China, the use of antibiotics in the animal husbandry sector contributes to the spread of resistant

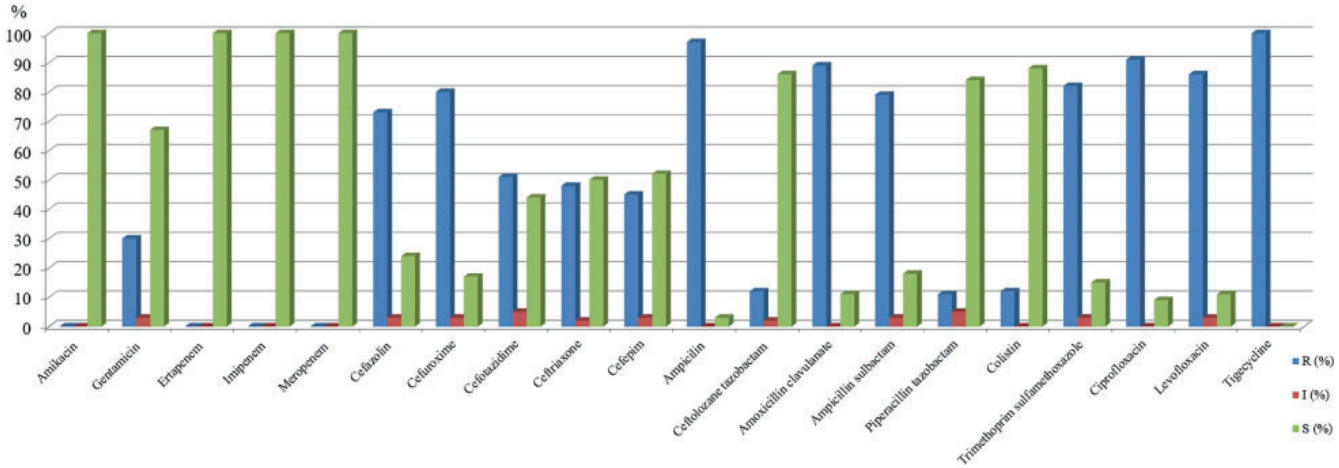

Figure 3. Antibiotic resistance rates of ESBL-producing *E. coli* isolates.
All ESBL-producing isolates (100%) were multi-antibiotic resistant.

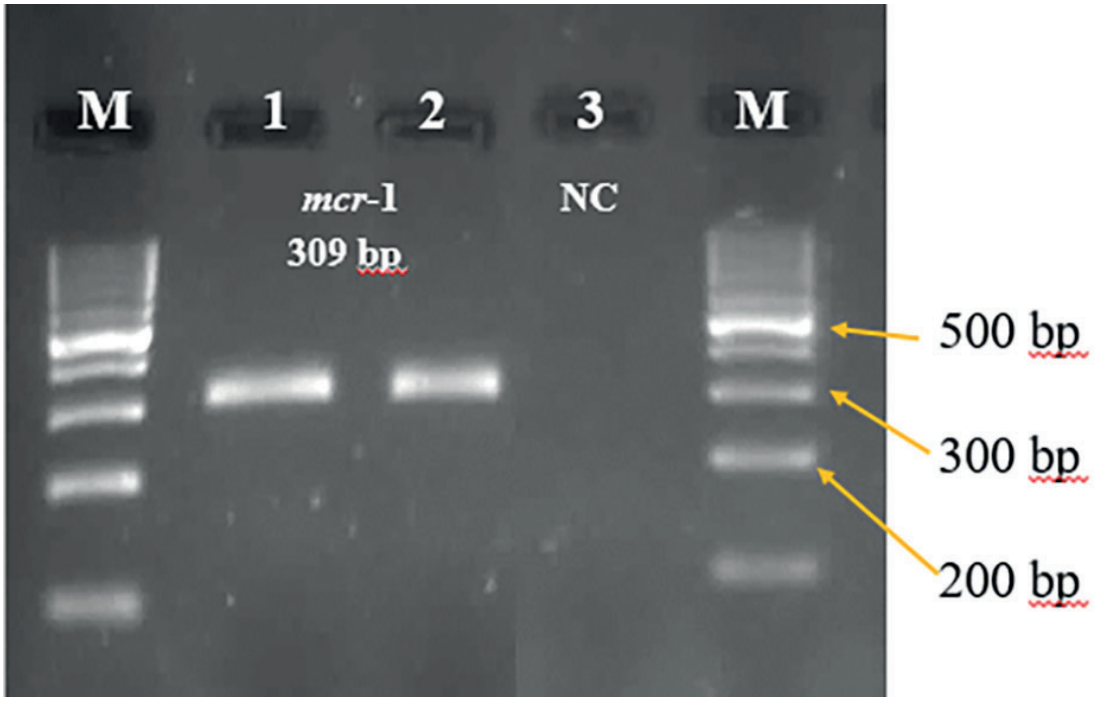

Figure 4. Gel electrophoresis for colistin resistance encoded by the *mcr*-1 gene: **1.** *mcr*-1 gene (309 bp, *E. coli* NCTC 13846); **2.** *mcr*-1 gene positive field isolate; **3.** NC (*E. coli* ATCC 25922); **M:** Marker (100 bp, Fermentas, USA).

producing and multidrug resistant *E. coli* strains isolated from broilers in western Türkiye were investigated. The findings indicate a possible zoonotic transmission risk and that these bacteria limit treatment options. In particular, the coexistence of two critically important resistance mechanisms, such as ESBL and colistin resistance, reduces the ability of bacteria to respond to treatment, posing a serious risk for both veterinary and public health.

In recent years, the prevalence of ESBL-producing *E. coli* strains has been increasing worldwide and this is a significant problem, especially in poultry farming (5). It has been reported that ESBL production is common in *E. coli* strains isolated from poultry in Brazil and that these strains have developed resistance to a wide range of antibiotics (33). Similarly, a high prevalence of ESBL-producing strains was detected in our study, and it was observed that the majority

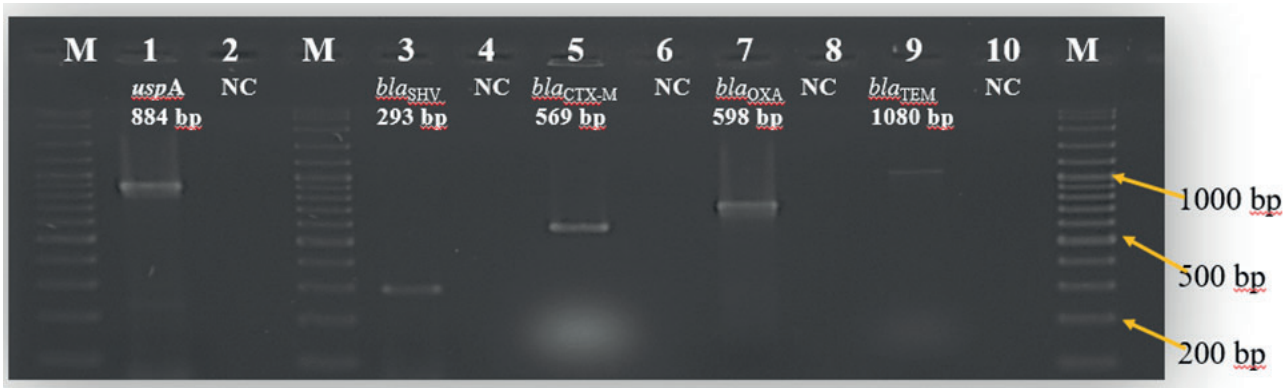

Figure 1. Agarose gel electrophoresis images of PCR products belonging to specific genes **1.** *usp*A gene (884 bp, *E. coli* ATCC 35150) **2.** NC (*S.* Typhimurium ATCC 14028) **3.** *bla*SHV gene (293 bp) **5.** *bla*CTX gene (569 bp) **7.** *bla*OXA (598 bp) **9.** *bla*TEM gene (1080 bp) **4, 6, 8, 10:** NC (DNA-free master mix). **M:** 100bp DNA Ladder (100 bp, Vivantis, Malaysia).

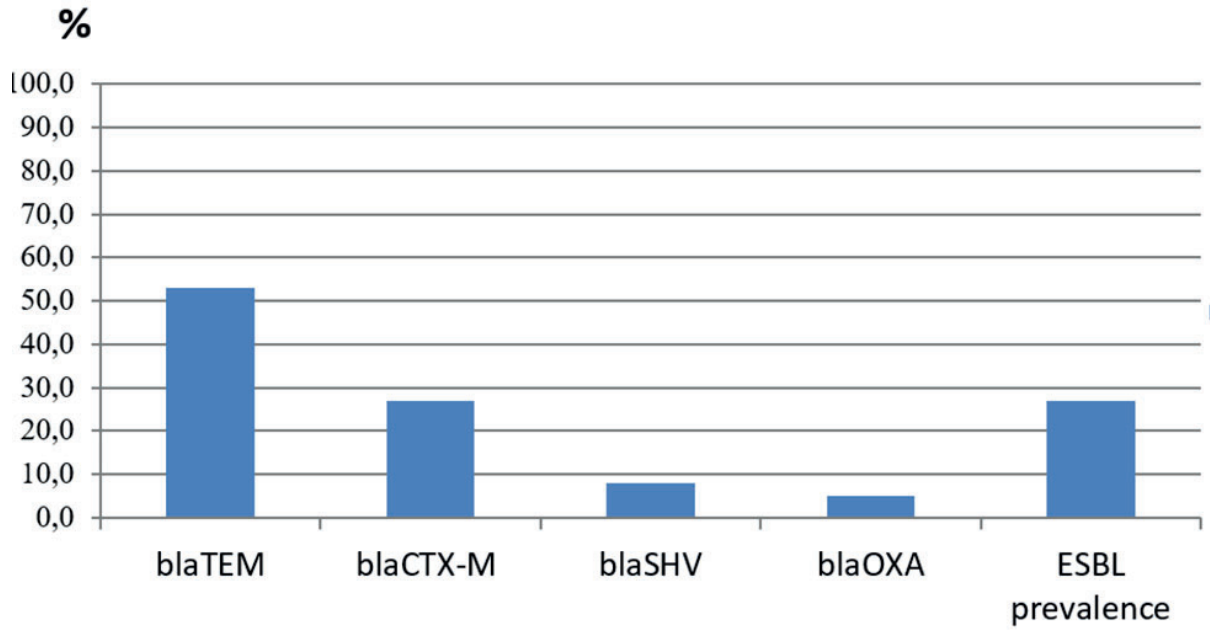

Figure 2. ESBL genotypes and prevalence.

Colistin resistance

In this study, the *mcr*-1 gene was detected in 12% of ESBL-producing isolates. It was determined that all isolates determined to be phenotypically resistant to colistin also carried the *mcr*-1 gene genotypically (Figure 4). These findings indicated that all phenotypically colistin-resistant isolates had the plasmid-mediated *mcr*-1 gene.

These results indicated that ESBL-producing *E. coli* strains developed resistance to a wide range of antibiotics and therefore treatment options should be carefully evaluated.

DISCUSSION

Detection of ESBL production is of great importance because ESBL-positive strains have been associated with higher mortality rates compared to ESBL-negative strains (1). ESBL-producing bacteria are often multidrug resistant, which significantly limits treatment options (31). The spread of these strains can occur both through direct animal contact and through consumption of contaminated animal products, which increases the risk of zoonotic transmission (32). In our study, the prevalence, antibiotic resistance profiles, and significant ESBL and colistin resistance genotypes of ESBL-

Table 2. Antibiotic resistance status of ESBL-producing *E. coli* isolates.

Antimicrobial Family-Antibiotic Name	ESBL positive isolates (n=66)			
	R	(%)	S	(%)
Aminoglycoside				
Amikacin	0	(0)	66	(100)
Gentamicin	20	(30)	44	(67)
Carbapenem				
Ertapenem	0	(0.0)	66	(100)
Imipenem	0	(0.0)	66	(100)
Meropenem	0	(0.0)	66	(100)
Cephem				
Cefazolin	48	(73)	16	(24)
Cefuroxime	53	(80)	11	(17)
Ceftazidime	34	(51)	29	(44)
Ceftriaxone	32	(48)	33	(50)
Cefepime	30	(45)	34	(52)
Penicillin				
Ampicillin	64	(97)	2	(3)
Beta Lactam				
CeftolozaneTazobactam	8	(12)	58	(86)
Amoxicillin Clavulanate	59	(89)	7	(11)
Ampicillin Sulbactam	52	(79)	14	(18)
Piperacillin Tazobactam	7	(11)	59	(84)
Lipopeptid				
Colistin	8	(12)	58	(88)
Folate				
Trimethoprim Sulfamethoxazole	54	(82)	12	(15)
Quinolone				
Ciprofloxacin	60	(91)	6	(9)
Levofloxacin	57	(86)	9	(11)
Tetracycline				
Tigecycline	66	(100.0)	0	(0.0)

while *bla*$_{\text{CTX-M}}$ (27%), *bla*$_{\text{SHV}}$ (8%) and *bla*$_{\text{OXA}}$ (5%) genes were also detected (Figure 2).

Antimicrobial resistance and multiple antibiotic resistance

In this study, antibiotic resistance profiles of 66 ESBL-producing *E. coli* isolates were evaluated using an automated microbiology system. All isolates were found to be susceptible to amikacin, ertapenem, imipenem, and meropenem, while they were found to be resistant to tigecycline (Table 2, Figure 3). It was determined that the isolates showed low-level resistance (11%-30%) to some antibiotics (gentamicin, ceftolozane-tazobactam, piperacillin-tazobactam, colistin), moderate resistance (31%-75%) to some antibiotics (cefazolin, ceftazidime, ceftriaxone, cefepime) and high-level resistance (76%-99%) to several antibiotics (cefuroxime, ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin).

Table 1. Primers used in the study.

Primer	Target Gene	Sequence (5'-3')	Amplicon size (bp)	Annealing (°C)	Referans
usp-F	*usp*A	CCGATACGCTGCCAATCAG	884	59	26
usp-R		ACGCAGACCGTAGGCCAGAT			
SHV-F	*bla*SHV	CGCCTGTGTATTATCTCCCT	293	57	27
SHV-R		CGAGTAGTCCACCAGATCCT			
CTX-M-F	*bla*CTX	CGCTGTTGTTAGGAAGTGTG	569	57	27
CTX-M-R		GGCTGGGTGAAGTAAGTGAC			
TEM-F	*bla*TEM	ATAAAATTCTTGAAGACGAAA	1080	49	28
TEM-R		GACAGTTACCAATGCTTAATCA			
OXA-F	*bla*OXA	ACCAGATTCAACTTTCAA	598	47	29
OXA-R		TCTTGGCTTTTATGCTTG			
*mcr*1-F	*mcr*-1	CGGTCAGTCCGTTTGTTC	309	55	30
*mcr*1-R		CTTGGTCGGTCTGTAGGG			

for 30 seconds, then annealing at temperatures dependent on the specific primers (Table 1) for 30 seconds and an extension step at 72˚C for 30 seconds, all steps for 30 cycles and a final extension at 72˚C for 10 minutes.

On electrophoresis, a 2% agarose gel stained with Safe View (100 ml/6 µl) (ABM, Richmond, Canada) was used and the gel was exposed to 100 volts for 45 minutes. After electrophoresis, the gel was placed in the chamber of the transilluminator device which was connected to the computer and photographed under UV light (Vilbert Lourmat, Collegien, France). When the amplified product formed a band of the expected size (Table 2.), it was assumed to carry the gene examined.

Molecular Identification of *E. coli* Isolates

In order to perform molecular verification of *E. coli* isolates at species level, the presence of the universal stress protein gene 'uspA' in the isolates was examined by PCR (26). In molecular studies, *E. coli* ATCC 35150 was used as positive control and *Salmonella* Typhimurium ATCC 14028 as negative control.

Sequencing of PCR Products

In phenotypically ESBL producing isolates, the presence of *bla*TEM, *bla*SHV, *bla*OXA, *bla*CTX genes was examined by PCR. In order to confirm the accuracy of the subtypes of the detected bla genes, the PCR product of a sample that gave a positive result from each gene subtype was purified and sent to Macrogen Company (Netherlands) for sequencing analysis. Sequencing results were analyzed using the BLAST program (www.ncbi.nlm.-nih.gov/BLAST). Sequences showing more than 97% homology were accepted as the detected gene types and used as positive control in PCR.

Genotypic Detection of Colistin Resistance

The presence of the *mcr*-1 gene, which is a plasmid colistin resistance gene, was examined in phenotypically colistin resistant isolates. In PCR procedures, *E. coli* NCTC 13846 strain was used as a positive control and *E. coli* ATCC 25922 strain was used as a negative control.

RESULTS

Bacterial isolates

In this study, 327 broiler samples suspected of colibacillosis were analyzed using an automated microbiology system (BD Phoenix, Becton-Dickinson, USA) and *E. coli* was identified in 247 (75%) of them. 27% of these isolates (66 isolates) were detected as ESBL positive with ChromagarTm ESBL agar. In all isolates, an amplification product of 884 bp was obtained in PCR analysis performed with uspA specific primers and the isolates were confirmed as *E. coli* (Figure 1). Then, ESBL resistance genes and antibiotic resistance profiles of 66 ESBL positive isolates were examined.

Characterization of ESBL-producing *E. coli* isolates

ESBL prevalence in isolates was detected as 27% (66 isolates). The most common ESBL gene was *bla*TEM (53%),

101347, Germany). After another 24 hours of incubation at 37°C, *E. coli* showing characteristic green metallic sheen colonies were collected. These colonies were subjected to biochemical tests (oxidase test, motility, citrate utilization, indole test, methyl red, etc.) (21). For the confirmation of bacterial identification, an automated system (BD Phoenix, Becton-Dickinson, USA) was used for evaluation according to the manufacturer's instructions. The isolates were stored in Brain Heart Infusion Broth (BHIB) containing 20% glycerol (Merck 110493, Germany) at -20°C.

ESBL-producing *E. coli* isolation

ESBL-producing *E. coli* isolates were identified using ChromagarTm ESBL agar (France, Chromagar, 201470) according to the manufacturer's instructions. Each *E. coli* isolate was incubated at 37°C for 18-24 hours under aerobic conditions. After incubation, bacteria forming pink (to burgundy) colonies on ChromagarTm ESBL agar were considered ESBL-producing *E. coli* strains. The colonies with pink colour were tentatively identified as ESBL producer. The ESBL producing isolates were further verified by Double-Disc Synergy Test (DDST) using ceftazidime (CAZ-30 µg) and ceftazidime with clavulanic acid (CAC-30/10 µg) as well as cefotaxime (CTX, 30 µg) and cefotaxime-clavulanate (CEC, 30/10 µg) discs. Briefly, *E. coli* isolates were inoculated into nutrient broth (Merck 105443, Germany) and incubated at 37°C. The bacterial culture with a turbidity equivalent to 0.5 Mac Farland standard unit was inoculated on to Mueller Hinton Agar (MHA) (Merck 103872, Germany) plates by spread plate method. The antibiotic discs were placed on the inoculated MHA plates at a distance of 20 mm apart and incubated overnight at 37°C. The inhibition zone diameter was measured for each antibiotic disc and its respective clavulanic acid containing discs. A difference of ≥5 mm in the presence of clavulanic acid when compared to its absence was considered as positive for the production of ESBL (22).

Antibiotic susceptibility test

Antibiotic susceptibility testing (AST) of the isolates identified as *E. coli* was performed using the BD Phoenix (Becton-Dickinson, USA) automated system with NMIC/ID 433 panels. The isolates were tested against 20 antibiotics belonging to nine different antimicrobial families (Lipopeptide: amikacin (AN), gentamicin (GM); Carbapenem: ertapenem (E), imipenem (IPM), meropenem (MEM); Cephem: cefazolin (CFZ), cefuroxime (CXM), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP); Penicillin: ampicillin (AMP); Beta Lactam: ceftolozane-tazobactam (CT), amoxicillin clavulanate (AMC), ampicillin sulbactam (AS), piperacillin-tazobactam (PT); Lipopeptide: colistin (COL); Folate: trimethoprim-sulfamethoxazole (TS); Quinolone: ciprofloxacin (CIP), levofloxacin (LF), Tetracycline: tigecycline (TIG)). The resistance status of the isolates against these antibiotics was examined. The results were evaluated according to the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (23). *E. coli* ATCC 25922 strains were used as quality control organisms.

Phenotypic detection of colistin resistance

During the study, colistin resistance in all isolates was examined using an automated system. The interpretation of colistin minimum inhibitory concentration (MIC) results was carried out using the EUCAST clinical breakpoints (susceptible ≤ 2 mg/L; resistant > 2 mg/L) (23).

Multidrug resistance (MDR)

MDR was defined as resistance to three or more antimicrobial classes (24).

DNA extraction

In this study, DNA extraction was performed using a commercial genomic DNA extraction kit (InstaGene™ Matrix, Biorad, Dubai) following the manufacturer's instructions. DNA purity and quantity were assessed using a nanodrop spectrophotometer (Maestrogen, Taiwan). An OD260/OD280 ratio of 1.6-2.0 indicated sufficient DNA purity (25).

Polymerase chain reaction

PCRs were performed in a volume of 25 µl. The final concentrations were adjusted as follows: 1x of 10xTaq enzyme buffer solution, 2 mM of 25 mM $MgCl_2$, 0.2 mM of 10 mM dNTP, 0.4 ρmol of each primer, and 1.5 U of Taq DNA polymerase (Fermentas, Massachusetts, USA). After preparing the master mixes, PCR tubes were numbered according to the number of samples, and 22 µl of master mix with 3 µl of DNA was added for each sample. PCR pre-denaturation was performed at 95°C for 5 minutes, denaturation at 95°C

INTRODUCTION

Extended-spectrum beta-lactamases (ESBLs) are enzymes that confer resistance to beta-lactam antibiotics, including third-generation cephalosporins and monobactams. These enzymes inactivate antibiotics by hydrolyzing the beta-lactam ring. ESBLs are found primarily in Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*, making infections caused by these bacteria difficult to treat (1). This resistance mechanism has become more widespread with increased contact between human and animal populations (2). This increases the risk of transmission of resistant bacteria to humans between humans and animals sharing the same environment (3).

To date, more than 350 ESBL variants are known, which have been classified into nine distinct structural and evolutionary families (TEM, SHV, CTX-M, PER, VEB, GES, BES, TLA, and OXA) based on amino acid sequence comparisons. The main types of ESBL variants include TEM, SHV, CTX-M, and OXA (4). The *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$ genotypes have traditionally been the most common, but the *bla*$_{\text{CTX-M}}$ genotype has been observed to be more widespread in recent years (5). The *bla*$_{\text{TEM}}$ gene was first isolated from a patient named Temoneira and generally confers resistance to broad-spectrum beta-lactam antibiotics such as ampicillin and piperacillin (6). The *bla*$_{\text{CTX-M}}$ gene, called cefotaximase-Munich, confers high-level resistance to third-generation cephalosporins (e.g., cefotaxime and ceftazidime) (7). *bla*$_{\text{SHV}}$ genes often produce enzymes called sulfhydryl variants. This gene confers resistance to beta-lactam antibiotics such as cephalosporins and penicillins (8). Oxacillinase genes (*bla*$_{\text{OXA}}$) often produce enzymes that confer resistance to carbapenems and broad-spectrum cephalosporins (9). Most genes encoding ESBLs are plasmid-borne and are often found in transposons and integrons, facilitating their mobilization by other resistance determinants. Therefore, genes encoding ESBLs can be easily transferred between bacteria (1).

It is known that poultry and poultry products are a potential source of antibiotic-resistant bacteria, including ESBL-producing *E. coli*, to humans (10). Therefore, the presence of ESBL-producing bacteria in poultry may pose a serious threat to public health. These bacteria can be transmitted to humans through direct contact or through contaminated food products (11). In Africa, 20% of ESBL-producing *E. coli* with *bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$ and *bla*$_{\text{TEM}}$ genes have been detected in chicken (12). Comparative analysis in Central Europe has shown genetic similarity of ESBL-producing *E. coli* from Mongolian migratory birds and clinical isolates from European hospitals (13). In Egypt, at least one ESBL phenotype/gene was phenotypically and genotypically identified in 47% of 120 chicken farms and in all human samples, and a high incidence of *bla*$_{\text{CTX-M}}$ gene was detected in chicken isolates (14).

In Türkiye, a limited number of studies have been conducted on the presence of ESBL-producing *E. coli* isolates in broilers, and these studies were conducted with samples taken from healthy birds. First, in 2017, the prevalence of ESBL in *E. coli* isolates obtained from cloacal swabs of healthy broilers was determined as 8.3%, and the *bla*$_{\text{CTX-M}}$ gene was reported in 80% of the isolates (15). In recent studies, ESBL-producing *E. coli* was reported in *E. coli* isolates obtained from chicken meat (16) and healthy pigeon cloacal swabs (17).

This study aimed to investigate the antibiotic resistance profiles of ESBL-producing *E. coli* isolates obtained from broilers with colibacillosis in western Türkiye, the main ESBL genotypes (*bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$, *bla*$_{\text{OXA}}$, *bla*$_{\text{CTX}}$), and the *mcr*-1 gene, which is one of the most important genetic determinants of colistin resistance.

MATERIALS AND METHODS

Materials

The liver samples of 327 broilers belonging to the Ross 308 breed, aged between 1 and 40 days, brought to the laboratory with suspicion of colibacillosis and obtained from farms belonging to the same broiler integration company were used as material in this study. The main clinical symptoms observed in these broilers were recurrent cough, anorexia, dyspnea, diarrhea, weight loss and lameness. The minimum sample size used in the study was calculated as 203 with an estimated prevalence of 75% (based on the studies of Aberkane and Seferoğlu) (18, 19), a confidence level of 90% and a margin of error of 5% (20).

Bacterial isolation and identification

Isolation was performed using standard bacteriological methods. Samples were streaked onto MacConkey Agar (Merck 105465, Germany) and incubated aerobically at 37°C for 24 hours. The next day, a single lactose-positive colony on MacConkey agar was sub-cultured onto EMB agar (Merck

Investigation of Broad-Spectrum Beta-Lactamase Production, Antibiotic Resistance and *mcr*-1 Gene in *Escherichia coli* Isolates from Broilers

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ABSTRACT

The detection of extended-spectrum β-lactamase (ESBL) production and mobilized colistin resistance gene (*mcr*) in *Escherichia coli* isolates reveals the potential for developing bidirectional resistance to both extended-spectrum β-lactamases and critically important colistin. The risk of transmission of these resistant bacteria from animals to humans can cause serious difficulties in terms of public health and veterinary medicine. This study was aimed to investigate the antibiotic resistance profiles of ESBL-producing *E. coli* isolates obtained from broilers with colibacillosis in the western part of Türkiye, the main ESBL genotypes (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{OXA}$, *bla*$_{CTX-M}$) and the *mcr*-1 gene, one of the important genetic determinants of colistin resistance. In the study, 247 (75%) *E. coli* isolates obtained by classical conventional methods from 327 colibacillosis suspected broiler livers brought to the laboratory for routine diagnosis within the scope of various studies during the last year were used. Bacterial identification was done by classical conventional methods. ESBL-producing isolates were phenotypically confirmed with CHROMagar™ ESBL. Genotypes and *mcr*-1 genes of ESBL-producing isolates were examined by polymerase chain reaction. Antibiotic resistance profiles of the isolates against 20 antibiotics belonging to nine antimicrobial families were evaluated by automated microbiology system (BD Phoenix, Becton-Dickinson, USA). Isolates resistant to at least one antibiotic from three or more antibiotic classes were considered as multidrug resistant (MDR). ESBL prevalence in isolates was determined as 27% (66 isolates). The most common ESBL gene was *bla*$_{TEM}$ (53%), and *bla*$_{CTX-M}$ (27%), *bla*$_{SHV}$ (8%) and *bla*$_{OXA}$ (5%) genes were also detected. All ESBL-producing isolates were determined to be MDR. All of the isolates were resistant to tigecycline, 97% to ampicillin and 91% to ciprofloxacin. The highest susceptibility was observed against amikacin, ertapenem, imipenem and meropenem (100%). In addition, the *mcr*-1 gene was detected in 12% of the ESBL-producing isolates. These results showed that ESBL production was high in *E. coli* isolates obtained from broilers, the *bla*$_{TEM}$ genotype was more dominant than other genotypes and the *bla*$_{TEM}$ genotype showed an increasing prevalence. The fact that all ESBL-producing isolates were MDR displayed the difficulty of treating these bacteria. The coexistence of ESBL and plasmid-mediated colistin resistance genes revealed that these bacteria pose a serious risk to public health.

Keywords: ESBL; *Escherichia coli*; broiler; antimicrobial resistance ESBL; *mcr*-1.

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GGC-3'; reverse primer: 5'- ATT CCA CCT ACC TCT CCC A-3' [37]. Program: 94°C 5' one cycle; 94°C-30"; 60°C-45"; 72°C- 1' 30 cycles; 72°C-1' one cycle; keep 10°C. PCR SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific, Rhenium, Modiin, Israel). PCR products were separated in 1% gel agarose; the *Helicobacter spp*. band size was estimated at 400 bp (46).

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Author Contributions

D.R. conceptualization, formal analysis, investigation and methodology; M.L.A. resources and revision; M.H. resources, revision and supervision.

Declaration of conflicting interests

The authors declare no conflict of interest.

Data availability statement

Full health monitoring data and history can be accessed at https://med.tau.ac.il/Health-Reports. Interested parties can contact Dr. Debora Rapaport debirapa@tauex.tau.ac.il and Dr. Michael Harlev mickey@tauex.tau.ac.il.

Ethics statement

Ethics for sentinel animal health evaluation at Tel Aviv University Animal Facilities were under the Institutional Animal Care and Use Committee approval number TAU-MD-IL-2307-150-2.

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on campus, demonstrating the importance of a barrier facility. Modern animal health monitoring is a dynamic process system that must evolve with advancements in technology, such as utilizing databases, such as REDCap, which integrate data from investigators and veterinarians, to promote best practices for improving experimental animal health (44).

Our rodent health monitoring system has certain limitations, as it relies on classical in-house microbiology protocols, including bacterial detection through culture and biochemical assays. Parasites are detected by indirect microscopy, while virus detection is outsourced to an external laboratory service. Additionally, the microorganism detection method, which is based on sentinels' soiled-bedding depends on the pathogen load present in the bedding during exposure and the agent's ability to remain viable and infectious (45). To modernize our methodology and increase assay sensitivity, our service is transitioning to an alternative health monitoring method using collection media filters. The validated system enhances pathogen detection by recovering nucleic acids from the media using PCR, providing a more efficient and reliable management platform for animal facilities.

SUPPLEMENTARY DATA

Material and Methods

Pathogens tested according to the Federation of European Laboratory Animal Science Associations (FELASA) recommendations and isolated bacteria not listed in FELASA panel are as follows:

Virology/Serology mouse FELASA panel (material blood). MHV, EDIM-ROTA-A, MVM, MPV-1, -2, -5, PVM, SEND, TMEV-GDVII, ECTRO, LCMV, FL-MAV-1, K87-MAV-2, MCMV, REO, NS-1, MNV, MPUL, CPIL.

Virology/Serology rat FELASA panel (material blood). HANT, H1, MAV1, MAV2, RPV, RMV, KRV, PVM, RCV/SDAV, REO, RTV, SEND, NS-1, PCAR/RRV, MNV, RCMV, CARB, MPUL, CPIL.

Microbiology FELASA panel (material nasopharynx and feces swabs). MPUL, *Bordetella bronchiseptica*, *Citrobacter rodentium*, *Clostridium piliforme*, *Corynebacterium kutcheri*, *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Pasteurella spp.*, *Pseudomonas aeruginosa*, *Salmonella spp.*, *Staphylococcus aureus*, *Streptococci* β-haemolytic, *Streptococcus pneumonia*, *Helicobacter spp.*, *Streptobacillus moniliformis*, Dermatophytes, *Corynebacterium bovis*, *Pneumocystis carinii.*

Microbiology isolated bacteria not listed in FELASA recommended panel (material nasopharynx and feces swabs). *Proteus mirabilis*, *Proteus vulgaris*, *Morganela morganii*, *Staphylococcus saprophyticus*, *Staphylococcus epidermis*, *Providencia rettgeri*, *Bacillus spp.*, *Pseudomonas stutzeri*, *Enterobacter cloacae*, *Enterobacter hormaechei*, *E. coli* "Shigella-like", *Enterococcus faecalis*, *Serratia marcescens*, *Staphylococcus xylosus*, *Mycoplasma genus.*

Parasitology. *pinworms and fur mites* (material feces and environmental swab cages): in quarantine group animals real-time PCR was tested by Charles River Laboratory Research Animal Laboratory (Diagnostic Service, Wilmington, Massachusetts, USA). Pinworms specification for *Aspiculuris tetraptera*, *Syphacia muris* (rat), *Syphacia obvelata* and mites specification for *Myobia musculi*, *Radfordia affinis*, *Radfordia ensifera*, *Myocoptes musulinus*.

Abbreviations. For mice, MHV, mouse hepatitis virus; EDIM-ROTA-A, mouse rotavirus; MVM, minute virus of mice; MPV-1,-2,-5, mouse parvovirus; PVM, pneumonia virus of mice; SEND, Sendai virus; TMEV-GDVII, Theiler's murine encephalomyelitis virus; ECTRO, ectromelia virus; LCMV, lymphocytic choriomeningitis virus; FL-MAV-1, K87-MAV-2, mouse adenovirus type 1,2; MCMV, mouse cytomegalovirus; REO, reovirus type 3; NS-1, generic parvovirus; MNV, murine norovirus; MPUL, mycoplasma pulmonis; CPIL, *Clostridium piliforme*. For rats, HANT, zoonotic hantaan virus; H1, Toolan's H1-rat parvovirus; MAV1, MAV2, rodent adenovirus strain 1, 2; RPV, rat parvovirus; RMV, rat minute virus; KRV, Kilham's rat virus-parvovirus; PVM, rodent pneumovirus; RCV/SDAV, rat coronavirus; REO, rodent reovirus; RTV, Rat theilovirus; NS-1, PCAR/RRV, *Pneumocystis carinii*; MNV, RCMV, rat cytomegalovirus; CARB, cilia-associated respiratory bacillus.

Helicobacter PCR. DNA was extracted from stool feces (3-4 pellets) using EZ-DNA (Biological Industries, Beit Ha'emek, Israel). For *Helicobacter spp*. identification, PCR analysis was used with the gene target 16S rRNA. Primers as followed: forward primer: 5'-CTA TGA CGG GTA TCC

whereas in Baghdad, almost a complete list of FELASA and other isolated bacteria not listed in the FELASA recommended panels were found in captured rats (38). Among these, the prevalence of *Escherichia coli* O157:H7, which requires Biosafety Level 3 laboratory working conditions, was 6.7%, as is typical for wild captured rodents. Similarly, in New York city, house mice (*Mus musculus*) were found to harbor high reservoirs of bacteria capable of causing gastrointestinal disease, including *Shigella spp*. and *Clostridium spp*. (10).

The most prevalent bacteria identified in our facilities were *Pasteurella spp.*, identified in 22% likely due to contamination from the supplier, followed by *Staphylococcus aureus* (8%). In the current study, the prevalence of *Klebsiella (pneumoniae, oxytoca)* and *Pseudomonas aeruginosa* was low. In comparison, a recent study conducted at Charles River Laboratories, reported higher prevalence rates for *Staphylococcus aureus* (38%), *Proteus mirabilis* (24%), *Klebsiella pneumonia* (5%), and *Klebsiella oxytoca* (3%) (35). These findings indicate that the pathogen levels in our facilities are relatively low. Furthermore, compared to the 9.4%, prevalence of *Klebsiella pneumoniae* among wild captured mice in New York City (10), the prevalence of this pathogen in our quarantined laboratory rodents was lower at 5.3%. Opportunistic isolated bacteria, such as *Proteus spp.*, *Morganella morganii*, and *Enterobacter cloacae*, were detected at even lower rates.

Helicobacter spp. is common both in wild rodents and laboratory animal facilities with a high prevalence ranging from 5% to 50% (35, 39) detected by PCR or multiplex DNA analysis for species identification, which is highly applicable in epidemiological studies (40). In our study, *Helicobacter spp*. was identified without species-specific identification and was routinely tested only in the companies' animal facilities, where a prevalence of 12% was observed.

Virology findings showed a low prevalence (less than 1%) of MHV and TMEV-GDVII in mice in our units compared to a prevalence of 2-3% reported for external clients in the Charles River Laboratories study (35). Rats in our conventional facilities, showed a high prevalence of rat Theilovirus (47%) and *Pneumocystis carinii* rat respiratory virus (15%), compared to a very low prevalence of 0.06% and 0%, respectively, reported in the Charles River Laboratories study (35).

Endoparasites attention is also essential for maintaining animal welfare. Animal facilities typically use direct microscopic examination for monitoring pinworms. Additionally, real-time PCR can be used to differentiate among *Syphacia obvelata*, *Syphacia muris* and *Aspiculuris tetraptera* by analyzing rDNA sequences spanning the internal transcribed spacer 1, the 5.8S gene, and internal transcribed spacer 2. This data was the basis for applying real-time PCR tests by fluorogenic 5' nuclease and target probes of 28S rRNA sequences on lysates from filter top media, pooled swabs and fecal pellets at Charles River Research Animal Diagnostic Services (41).

In our facilities the prevalence of pinworms was around 3%, and they were detected only in conventional units. Microscopic observations allowed us to distinguish between *Syphacia obvelata* and *Aspiculuris tetraptera*. Additionally, PCR was used in some cases to differentiate pinworms species, with *Syphacia obvelata* being more prevalent. In the Charles River Laboratories study, pinworms were detected in 1% of animals from external clients (35). In Argentinian laboratories, *Syphacia muris* was found in 39% of rats and *Syphacia obvelata* was present in 34% of mice (4). In a conventional animal facility in Malaysia, helminth types were significantly associated with mice strains, with *Syphacia obvelata* and *Aspiculuris tetraptera* more prevalent in ICR mice compared to BALB/c mice (22). Pinworm infections were detected in 8-30% of cases. The study also revealed that the perianal tape test is optimal for identifying *Syphacia obvelata*, while the fecal flotation technique is more effective for detecting *Aspiculuris tetraptera* (22). Fecal samples from wild rodents captured in Chile showed an 90% prevalence of endoparasites with various helminth egg types. Among the detected parasites, *Syphacia sp*. accounted for 3.7% of cases in live rodents compared to 36.4% of cases in post-mortem examinations (42).

At our center, mites were detected in only two vivaria at a very low frequency (0.31%). Interestingly, a pilot study conducted in animal facilities in Finland revealed that mites were absent from the animal rooms but were present in 25% of samples taken from staff room chairs and storage areas. The spread was attributed to contamination via animal food or bedding (43).

The prevalence of pathogens is highly associated with the cleanliness standards maintained in the tested facility. In our units, high pathogen levels were initially detected in biological cabinets by the Lumitester-ATP technique, but these levels decreased following the implementation of a cleaning protocol.

In conclusion, we have successfully lowered microbiological counts in our SPF veterinary service compared to the conventional animal units, despite their close proximity

Table 4. The prevalence of selected pathogens in SPF facilities compared to Group 1 facilities and quarantine facilities

Units (Groups)	SPF		Group 1[a]		Quarantine		P-value (*t*-test) SPF vs Group 1	SD SPF vs Group 1	P-value (*t*-test) SPF vs Quarantine	SD SPF vs Quarantine
	N=494	n (%)	N=140	n (%)	N=133	n (%)				
Pathogens bacteria FELASA panel										
Klebsiella pneumoniae	0	(0)	0	(0)	7	(5.26)	0	0	0.04*	0.05
Klebsiella oxytoca	2	(0.4)	2	(1.43)	1	(0.75)	0.22	0.01	0.25	0.02
Pasteurella spp.	109	(22.1)	40	(28.6)	25	(18.8)	0.18	0.16	0.39	0.12
Staphylococcus aureus	35	(7.09)	13	(9.28)	12	(9.02)	0.25	0.04	0.19	0.08
Pseudomonas aeruginosa	0	(0)	5	(3.57)	0	(0)	0.04*	0.03	0	0
Other isolated bacteria not listed in FELASA recommended panel										
Proteus mirabilis	1	(0.2)	1	(0.71)	2	(0.015)	0.28	0.01	0.11	0.01
Proteus vulgaris	2	(0.4)	0	(0)	0	(0)	0.17	0.006	0.17	0.007
Morganella morganii	0	(0)	10	(7.14)	1	(0.008)	0.006*	0.05	0.17	0.016
Staphylococcus saprophyticus	3	(0.61)	2	(1.43)	8	(6.01)	0.21	0.016	0.04*	0.06
Enterobacter cloacae	8	(1.62)	6	(4.29)	5	(0.034)	0.12	0.034	0.26	0.05
Enterobacter hormaechei	0	(0)	1	(0.71)	0	(0)	0.17	0.007	0	0
Escherichia coli "Shigella-like"	0	(0)	0	(0)	1	(0.008)	0	0	0.17	0.016
Serratia marcescens	0	(0)	1	(0.71)	0	(0)	0.17	0.009	0	0
Virology/other pathogens FELASA panel										
Mouse hepatitis virus (MHV)	0	(0)	0	(0)	0	(0)	0	0	0	0
Mycoplasma pulmonis (MPUL)	0	(0)	0	(0)	0	(0)	0	0	0	0
Gross pathology	22	(4.45)	15	(10.7)	2	(1.5)	0.06	0.05	0.1	0.04
Parasitology										
Endoparasites (pinworms)	0	(0)	13	(9.29)	1	(0.75)	0.012*	0.06	0.17	0.02
Ectoparasites (mites)	0	(0)	2	(1.43)	0	(0)	0.17	0.01	0	0

Abbreviations: FELASA, the Federation of European Laboratory Animal Science Associations; SD, standard deviation

[a] Group 1 included the conventional housing of the School of Zoology (9 sentinel mice), Felsenshtein Medical Research Center (36 sentinel mice), Sheba Medical Center (10 sentinel mice) and biotechnology companies (85 sentinel mice)

* $P<0.05$ (statistically significant)

despite the proximity of both units, demonstrating the importance of a barrier facility. To reduce or eliminate the potential of introducing biological pathogens into the facility, it was essential to monitor critical control points that pose safety risks. Key factors to consider include the entry of animals, the use of biological materials (e.g., cells, parasites, viral stocks, proteins, antibodies, non-pathogenic bacteria), cleaning, disinfection, and sterilization processes, as well as housing and husbandry practices (water, food, air and bedding quality) and it must be ensured that personnel must be carefully trained and managed.(36) Additionally, facility construction and animal services must adhere to animal facility standards (2).

Pathogens are present in low levels in animal facilities worldwide and generally do not affect biomedical research. In this study, the prevalence of isolated pathogens was below the permitted ratio, ensuring our animal facilities met the FELASA standards.

It is well documented that the composition of gut microbiota is dynamic and influenced by factors such as host genetics, environment and geographical location. Wild mice exhibit significant differences in microbiome composition compared to laboratory animals (37). For example, in Argentina, the most frequently isolated bacteria in animal facilities were *Pseudomonas aeruginosa* and *Proteus spp.* (4),

Table 3. The prevalence of selected pathogens in Group 1 versus quarantine facilities

	Group 1[a]		Quarantine		P-value (*t*-Test)	Standard deviation
	N=140	n (%)	N=133	n (%)		
Pathogens bacteria FELASA panel						
Klebsiella pneumoniae	0	(0)	7	(5.26)	0.04*	0.05
Klebsiella oxytoca	2	(1.43)	1	(0.75)	0.4	0.02
Pasteurella spp.	40	(28.6)	25	(18.8)	0.3	0.17
Staphylococcus aureus	13	(9.3)	12	(9.02)	0.29	0.09
Pseudomonas aeruginosa	5	(3.57)	0	(0)	0.04*	0.03
Other isolated bacteria not listed in FELASA recommended panel						
Proteus mirabilis	1	(0.71)	2	(1.50)	0.24	0.02
Proteus vulgaris	0	(0)	0	(0)	0	0
Morganella morganii	10	(7.14)	1	(0.75)	0.02*	0.05
Staphylococcus saprophyticus	2	(1.43)	8	(6.01)	0.07	0.05
Enterobacter cloacae	6	(4.29)	5	(3.75)	0.45	0.05
Enterobacter hormaechei	1	(0.71)	0	(0)	0.17	0.007
Escherichia coli "Shigella-like"	0	(0)	1	(0.75)	0.15	0.016
Serratia marcescens	1	(0.71)	0	(0)	0.17	0.009
Virology/other pathogens FELASA panel						
Mouse hepatitis virus (MHV)	0	(0)	0	(0)	0	0
Mycoplasma pulmonis (MPUL)	0	(0)	0	(0)	0	0
Gross pathology	15	(10.71)	2	(1.5)	0.003**	0.05
Parasitology						
Endoparasites (pinworms)	13	(9.29)	1	(0.75)	0.04*	0.06
Ectoparasites (mites)	2	(1.43)	0	(0)	0.17	0.01

Abbreviations: FELASA, the Federation of European Laboratory Animal Science Associations

[a] Group 1 included the conventional housing of the School of Zoology (9 sentinel mice), Felsenshtein Medical Research Center (36 sentinel mice), Sheba Medical Center (10 sentinel mice) and biotechnology companies (85 sentinel mice)

* P<0.05,

** P<0.005 (statistically significant)

statistically significantly higher in G1 compared to QUA (Table 3). Since G1 comprised external facilities, an expected significantly higher prevalence of endoparasites was found in 13 of 140 cases (9.29%), compared to one case among 133 rodents (0.75%) in QUA.

Next, we examined if the prevalence of pathogens in the SPF unit is lower compared to G1 and QUA (Table 4). The prevalence of *Klebsiella pneumoniae* and *Staphylococcus saprophyticus* was statistically significantly higher in QUA compared to the SPF group (P=0.04 for both pathogens). The prevalence of *Pseudomonas aeruginosa* and pinworms was statistically significantly higher in G1 compared to the SPF group (P=0.04 and P=0.012, respectively).

Environmental monitoring of microorganisms. Analysis of the water showed that the pH was within normal range (2.8 to 3.2). The Lumitester-ATP average was at an acceptable level of 8.50 RLU. Surfaces showed an average level of 182.25 RLU. High levels of ATP-bioluminescence (1500-1800 RLU) were found in biological safety cabinets. These levels were reduced after protocol cleaning, until they reached less than 500 RLU.

DISCUSSION

Our five-year analysis showed that we were able to define safe and lower microbiological counts in our SPF veterinary service unit compared to the conventional husbandry unit,

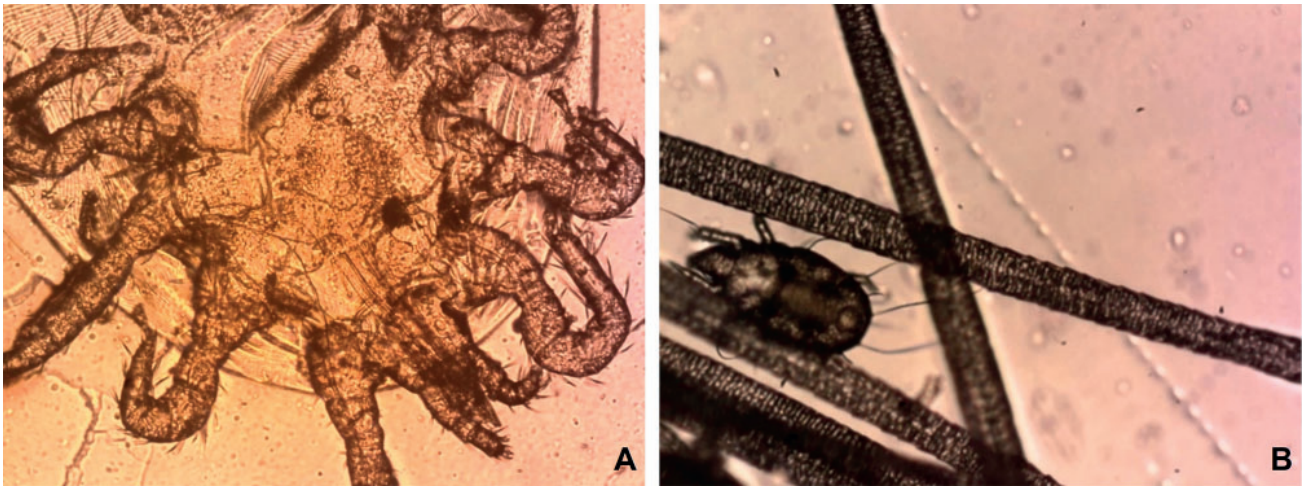

Figure 2. Acariasis images.
(**A)** Mites identified as *Myocoptes musculinis* by morphology; (**B**) Ectoparasite in fur hair. Magnification 100X (B); 400X (A).

and ectoparasites were detected only in conventional units at a prevalence of 8.0% and 0.53%, respectively; however no statistically significant difference was observed between husbandry types.

G1 and QUA were considered at high risk for pathogen contamination. Between these two groups, the prevalence of *Klebsiella pneumonia, Pseudomonas aeruginosa, Morganella morganii,* gross pathology findings and pinworms was

Table 2. The prevalence of selected pathogens in SPF versus conventional facilities

	SPF		Conventional units[a]		P-value (*t*-test)	Standard deviation
	N=494	n (%)	N=188	n (%)		
Pathogens bacteria FELASA panel						
Klebsiella oxytoca	2	(0.41)	0	(0)	0.04*	0.005
Pasteurella spp.	109	(22.06)	35	(18.62)	0.33	0.146
Staphylococcus aureus	35	(7.1)	18	(9.6)	0.34	0.095
Other isolated bacteria not listed in FELASA recommended panel						
Proteus mirabilis	1	(0.2)	0	(0)	0.041*	0.002
Proteus vulgaris	2	(0.4)	2	(1.06)	0.42	0.02
Morganella morganii	0	(0)	4	(2.13)	0.2	0.039
Staphylococcus saprophyticus	3	(0.61)	1	(0.53)	0.5	0.02
Enterobacter cloacae	8	(1.62)	3	(1.6)	0.28	0.023
Virology/other pathogens FELASA panel						
Mouse hepatitis virus (MHV)	0	(0)	6	(3.2)	0.14	0.071
Mycoplasma pulmonis (MPUL)	0	(0)	5	(2.66)	0.25	0.074
Gross pathology	22	(4.45)	5	(2.66)	2.24	0.044
Parasitology						
Endoparasites (pinworms)	0	(0)	15	(8)	0.065	0.105
Ectoparasites (mites)	0	(0)	1	(0.53)	0.289	0.036

Abbreviations: FELASA, the Federation of European Laboratory Animal Science Associations
[a] Medicine, Life Sciences, Psychology
* P<0.05 (statistically significant)

	Number of positive-tested mice/rats by unit (n)										
	SPF	Conventional facilities								Total	
	N=494	Medicine N=94	Life Sciences N=38	Psychology N=56	Zoology[a] N=9	Felsenshtein[a] N=36	Sheba[a] N=10	Quarantine N=133	Companies[a] N=85	n/N	(%)
Mouse rotavirus (EDIM-ROTA-A)	0	0	0	0	0	0	0	0	0	0/955	(0)
Minute virus of mice (MVM)	0	0	0	0	0	0	0	0	0	0/955	(0)
Mouse parvovirus (MPV)-1,-2,-5	0	0	0	0	0	0	0	0	0	0/955	(0)
Pneumonia virus of mice (PVM)	0	0	0	0	0	0	0	0	0	0/955	(0)
Sendai virus (SEND)	0	0	0	0	0	0	0	0	0	0/955	(0)
Virology/other pathogens FELASA panel N=955											
Theiler's murine encephalomyelitis virus (TMEV) GDVII strain	0	0	0	0	2	2	0	0	0	4/955	(0.42%)
Ectromelia virus (ECTRO)	0	0	0	0	0	0	0	0	0	0/955	(0)
Lymphocytic choriomeningitis virus (LCMV)	0	0	0	0	0	0	0	0	0	0/955	(0)
Mouse adenovirus type 1,2 (FL-MAV-1, K87-MAV-2)	0	0	0	0	0	0	0	0	0	0/955	(0)
Mouse cytomegalovirus (MCMV)	0	0	0	0	0	0	0	0	0	0/955	(0)
Reovirus type 3 (REO)	0	0	0	0	0	0	0	0	0	0/955	(0)
Generic parvovirus (NS-1)	0	0	0	0	0	0	0	0	0	0/955	(0)
Murine norovirus (MNV)[c]	+	+	+	+	+	+	+	+	+		
Mycoplasma pulmonis (MPUL)	0	0	0	5	0	0	0	0	0	5/955	(0.52%)
Clostridium piliforme (CPIL)	0	0	0	0	0	0	0	0	0	0/955	(0)
Virology FELASA panel (Rat) N=47											
Rat zoonotic hantaan virus (HANT)	NT[d]	0	0	0	NT[d]	NT[d]	NT[d]	NT[d]	0	0/47	(0)
Toolan's H1-rat (H1)	NT[d]	0	0	0	NT[d]	NT[d]	NT[d]	NT[d]	0	0/47	(0)
Rat minute virus (RMV)	NT[d]	0	0	0	NT[d]	NT[d]	NT[d]	NT[d]	0	0/47	(0)
Kilham's rat virus-parvovirus (KRV)	NT[d]	0	0	0	NT[d]	NT[d]	NT[d]	NT[d]	0	0/47	(0)
Rat coronavirus (RCV/SDAV)	NT[d]	0	0	0	NT[d]	NT[d]	NT[d]	NT[d]	0	0/47	(0)
Rat theilovirus (RTV)	NT[d]	2	6	10	NT[d]	NT[d]	NT[d]	NT[d]	4	22/47	(46.81%)
Pneumocystis carinii (PCAR, 'RRV')	NT[d]	0	2	9	NT[d]	NT[d]	NT[d]	NT[d]	0	7/47	(14.89%)
Rat cytomegalovirus (RCMV)	NT[d]	0	0	0	NT[d]	NT[d]	NT[d]	NT[d]	0	0/47 (0)	(14.89%)
Pathology N=955											
Gross pathology	22	2	0	3	1	1	0	2	13	44/955	(4.61%)
Parasitology N=955											
Endoparasites (pinworms)	0	8[e]	6	1	1	1[e]	4	1[f]	4	26/955	(2.72%)
Ectoparasites (mites)	0	0	1	0	2	0	0	0	0	3/955	(0.31%)

Abbreviations: FELASA, the Federation of European Laboratory Animal Science Associations; NT, not tested

[a] Included in Group 1 (G1)

[b] *Helicobacter spp.* was tested only for rodents from companies' facilities

[c] MNV was not tested in the current study and was considered positive (+) in all cases due to its high prevalence previously tested in our facilities

[d] Rat pathogens were not tested because there were no rats in these housing.

[e] Pinworms were diagnosed as *Syphacia obvelata* by PCR

[f] Pinworms were diagnosed as *Aspiculuris tetraptera* by PCR

Table 1. Pathogen prevalence by health monitoring program

	Number of positive-tested mice/rats by unit (n)										
	SPF	Conventional facilities								Total	
	N=494	Medicine N=94	Life Sciences N=38	Psychology N=56	Zoology[a] N=9	Felsenshtein[a] N=36	Sheba[a] N=10	Quarantine N=133	Companies[a] N=85	n/N	(%)
Number of health monitoring/year	4	2	2	2	2	2	2	1	1		
Bacteria FELASA panel N=955											
Bordetella bronchiseptica	0	0	0	0	0	0	0	0	0	0/955	(0)
Citrobacter rodentium	0	0	0	0	0	0	0	0	0	0/955	(0)
Corynebacterium kutcheri	0	0	0	0	0	0	0	0	0	0/955	(0)
Klebsiella pneumoniae	0	0	0	0	0	0	0	7	0	7/955	(0.73%)
Klebsiella oxytoca	2	0	0	0	1	0	0	1	1	5/955	(0.52%)
Pasteurella spp.	109	12	7	16	0	7	2	25	31	209/955	(21.88%)
Pseudomonas aeruginosa	0	0	0	0	0	1	1	0	3	5/955	(0.52%)
Salmonella spp.	0	0	0	0	0	0	0	0	0	0/955	(0)
Staphylococcus aureus	35	7	1	10	1	2	1	12	10	79/955	(8.27%)
Streptococci b-haemolytic (not group D)	0	0	0	0	0	0	0	0	0	0/955	(0)
Streptococcus pneumoniae	0	0	0	0	0	0	0	0	0	0/955	(0)
Streptobacillus moniliformis	0	0	0	0	0	0	0	0	0	0/955	(0)
Dermatophytes (skin)	0	0	0	0	0	0	0	0	0	0/955	(0)
Corynebacterium bovis	0	0	0	0	0	0	0	0	0	0/955	(0)
Pneumocystis carinii (Nude lung)	0	0	0	0	0	0	0	0	0	0/955	(0)
Helicobacter spp.[b]	NT	NT	NT	NT	NT	NT	NT	NT	10	10/85	(11.76)
Other isolated bacteria not listed in FELASA recommended panel N=955											
Proteus mirabilis	1	0	0	0	1	0	0	2	0	4/955	(0.42%)
Proteus vulgaris	2	2	0	0	0	0	0	0	0	4/955	(0.42%)
Morganella morganii	0	2	1	1	0	0	0	1	9	14/955	(1.47%)
Staphylococcus saprophyticus	3	0	0	1	0	0	0	8	2	14/955	(1.47%)
Staphylococcus epidermis	0	0	0	0	0	0	0	0	0	0/955	(0)
Providencia rettgeri	0	0	0	0	0	0	0	0	0	0/955	(0)
Bacillus spp.	0	0	0	0	0	0	0	3	0	3/955	(0.31%)
Pseudomonas stutzeri	0	0	0	0	0	0	0	0	0	0/955	(0)
Enterobacter cloacae	8	2	0	1	0	2	0	4	4	21/955	(2.20%)
Enterobacter hormaechei	0	0	0	0	0	0	0	0	1	1/955	(0.10%)
Escherichia Coli "shigella-like"	0	0	0	0	0	0	0	1	0	1/955	(0.10%)
Enterococcus faecalis	0	0	0	0	0	0	0	0	0	0/955	(0)
Serratia marcescens	0	0	0	0	0	0	0	0	0	0/955	(0)
Staphylococcus xylosus	0	0	0	0	0	0	0	0	0	0/955	(0)
Mycoplasma genus	0	0	0	0	0	0	0	1	0	1/955	(0.10%)
Virology/other pathogens FELASA panel N=955 *(cont. on next page)*											
Mouse hepatitis virus (MHV)	0	1	5	0	0	0	0	0	0	6/955	(0.63%)

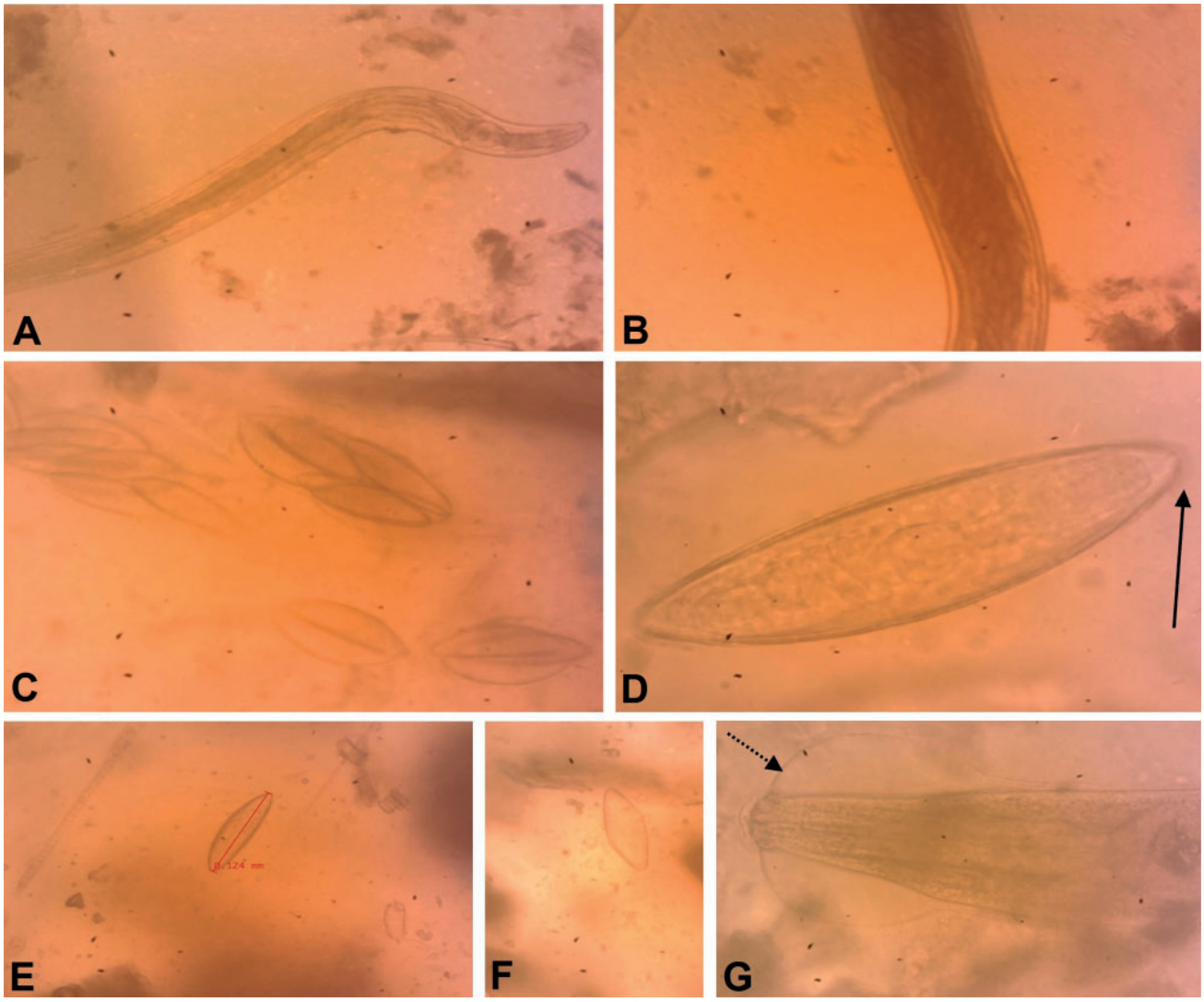

Figure 1. Nematodes identified in rodents housed in conventional facilities images. (**A**) Adult *Syphacia obvelata*, identified by morphology showing a cephalic end with lips followed by pharynx esophagus, bulb and intestine. (**B**) Gravid *Syphacia obvelata* female carrying eggs embryonated in uteri. (**C**) "Banana-shaped" eggs. (**D**) Egg overview with operculum (arrow). (**E**) Egg measurement 0.124 mm length. (**F**) *Aspiculuris tetraptera* with round football-shaped ova. (**G**) Female head halo (dashed arrow). Magnification 40X (A, B); 100X (C, E, F, G); 400X (D).

Ectoparasites (mites) were found in 3 out of 955 cases (0.31%), detected only in two conventional units (LS, ZOO) (Table 1). Figure 2 illustrates a *Myocoptes musculinus* adult (Figure 2A) and Acariformes mites spp. (Figure 2B) according to morphology parameters (36). Treatment was applied in units in which parasites were detected.

Comparison of pathogen prevalence among husbandry types. Comparison of the prevalence of pathogens between SPF and conventional units (Table 2) showed that among the bacteria tested, *Pasteurella spp.* was the most common bacteria, with a prevalence of 22.06% among SPF animals and 18.62% among rodents housed in conventional units, with no statistically significant difference between the two husbandry types. *Staphylococcus aureus* was also a very common pathogen in both SPF and conventional units (7.1% and 9.6%, respectively) with no statistically significant differences between the two husbandry types. *Klebsiella oxytoca* was found in two of 494 mice in SPF (0.41%) but not in animals housed in the conventional units (P=0.04 for the prevalence difference between the units). *Proteus mirabilis* (an opportunistic isolated bacteria not listed in FELASA recommended panel), was also found in one SPF mouse (0.20%) but not in animals housed in the conventional units (P=0.041). Endoparasites

water, up to 200 RLU for smooth and hard surfaces, and up to 500 RLU for fragile surfaces. If the read exceeded 500 RLU, a disinfection protocol with ethanol, Virusolve (Amity International, South Yorkshire, United Kingdom), hydrogen peroxide and Biocide solution (Airsurdis, Robaix, France) was applied until RLU levels reached the normal ranges.

Statistical analysis. Statistical analysis was performed using Excel (Microsoft, Redmond, WA, USA). Mouse and rat data were combined for simplification, as previously analyzed by Albers *et al*. (35). The number and frequency of each pathogen tested were summarized per housing unit and in total. To understand if there is a correlation between the presence of specific pathogens and the husbandry type, the prevalence of identified pathogens was compared by *t*-test between pairs of housing types (SPF versus all conventional facilities [CM, LS and PS]; G1 versus QUA; SPF versus G1; and SPF versus QUA). The samples were not normalized since there was no control group. P-values <0.05 were considered statistically significant.

RESULTS

Pathogen prevalence in animal facility units. A total of 955 mice and rats were tested over the five-year study period (Table 1).

Bacteria. The most commonly isolated bacterial strains using the FELASA panel were *Pasteurella spp*. (22%) and *Staphylococcus aureus* (8%). The prevalence of *Klebsiella pneumoniae* and *Klebsiella oxytoca* was 0.73% and 0.52%, respectively. *Mycoplasma pulmonis*, showed very low prevalence, in 0.52% of tested animals – all of them rat samples from the PS unit. *Mycoplasma genus* was found in only one quarantined mouse, indicating an overall prevalence of 0.1%. Other isolated bacteria not listed in the FELASA recommended panel, such as *Enterobacter cloacae, Morganella morganii*, and *Staphylococcus saprophyticus* showed prevalence rates of 2.2%, 1.47%, and 1.47%, respectively. The prevalence of *Proteus mirabilis* and *Proteus vulgaris* was low at 0.42%. *Helicobacter spp.,* which was tested exclusively in animals from companies, showed a frequency of 11.76% (10/85 rodents) (Table 1).

Viruses. In mice, the most prominent pathogens were mouse hepatitis virus (MHV) and Theiler's murine encephalomyelitis virus (TMEV-GDVII) with a prevalence of 0.63% and 0.42%, respectively. In rats, the highest prevalence was observed for rat theilovirus (46.81%) and *Pneumocystis carinii* rat respiratory virus (14.89%). Murine norovirus (MNV) was considered positive in all units, since its prevalence was very high in previous findings in our facilities (Table 1).

Gross pathology. During sentinel mice necropsy health monitoring, abnormal signs were observed and considered as gross pathology in 4.61% of cases (Table 1). The range of signs included ovarian cysts, hydrometra, bilateral hemorrhagic ovaries, alopecia, skin lesions, internal hemorrhagic organs or tissues, abscesses and abnormal mass of tissue representing potential incidental tumors, necrotic tumors or blocked ducts. The most common sign was ovarian cysts in sentinel female mice, which could be attributed to the age of the tested females (8-9 weeks old).

Alopecia was found in patches around the face, concentrated in one facial area, whereas the skin was healthy and in a few cases the whiskers or eyelashes might be missing. This "barbering" sign was caused by overgrooming by animal cage mates or the mice themselves, which can represent a compulsive grooming disorder. No medical treatment was applied; however, a benefit was observed when environmental enrichment was increased. Abscesses were rare; however, they were associated with bite wounds.

Parasitology. Among the 955 tested rodents, 26 (2.7%) had pinworms in feces samples, including *Syphacia obvelata* adult specimens, gravid females and eggs. *Syphacia sp*. was identified (33) based on the presence of a muscular oesophagus ending within an oesophageal bulb (Figure 1A, B) and distinctive ellipsoidal eggs. These eggs were found embryonated in uteri and measured 0.120–0.139 mm in length (mean 0.129±0.001 mm). The eggs were asymmetrical with one flattened banana-shaped side, and operculated on the convex side. (Figure 1C, D, E). PCR tests confirmed the presence of nine cases of *Syphacia obvelata* and one case of *Aspiculuris tetraptera* (Table 1). Figure 1F shows a characteristic egg from *Aspiculuris tetraptera* featuring a symmetrical ovoid ellipsoid shape resembling a "football". Pinworms were found in all conventional units but not in animals housed in the SPF unit.

Germany) and animal sterilized bedding (Sani-Chips 7090, Harlan-Teklad, Madison, WI, USA).

The facilities in which the rodents were housed included: 1) an SPF unit (494 sentinel mice), which was built according to practices and guidelines for animal housing and husbandry of the National Institutes of Health.[1] This facility is located at Tel Aviv University. 2) The conventional housing of the Faculty of Medicine (CM, 94 sentinel mice and rats), Faculty of Life Sciences (LS, 38 mice and rats) and the School of Psychology Sciences (PS, 56 mice and rats), which were also located at Tel Aviv University campus. 3) The conventional housing of the School of Zoology (ZOO, 9 sentinel mice), Felsenshtein Medical Research Center (FEL, 36 sentinel mice), Sheba Medical Center (TEL, 10 sentinel mice) and biotechnology companies (Companies, 85 sentinel mice), which were all grouped as "Group 1" (G1) and included 140 rodents altogether. All G1 housing were external facilities to the Tel Aviv University main campus but are under the care of the university's Animal Facility staff. 4) Quarantine facilities (QUA) located at Tel Aviv University's Animal Facility, which included imported animals (133 mice). G1 and QUA were considered at high risk for pathogen contamination.

Health monitoring. Rodents housed in SPF facilities were monitored quarterly, while those in all other facilities were monitored biannually. Pathogens were tested according to FELASA guidelines and recommendations (See Supplementary Data) (7). Post-mortem sample collection was conducted following euthanasia of sentinel animals with carbon dioxide followed by a clinical examination of external and internal organs.

Serology. A total of 20 µl of blood was collected from a facial vein puncture using the HemaTIP Microsampler (Charles River Laboratories, Wilmington, MA, USA). Viruses were identified by serology antibodies using multiplexed fluorometric immunoassays and immunofluorescence assays at Charles River Research Animal Laboratory (Diagnostic Service, Wilmington, MA, USA).

Bacteriology. Standard microbiological procedures were applied under Biosafety Level 2. These procedures included a systematic inoculation of the nasopharynx by a gel swab introduced into the respiratory tract, and collection of fecal specimens from different sources from the rodents' gut duodenum, cecum and colon. The specimens were then plated in differential agar plates (HyLaboratories, Rehovot, Israel) followed by analysis with a series of chromogenic media and biochemical differentiation for enteropathogenic bacteria (HyLaboratories, Rehovot, Israel) (28, 29). FELASA bacterial panels were used according to FELASA recommendations (7). Other isolated bacteria not included in the FELASA recommendations were included in the analyses as output microbiology results followed by selective and differential media. *Pasteurella spp.* (30) identification was confirmed by oxidase strip test (HyLaboratories, Rehovot, Israel). *Helicobacter spp.* was identified by PCR analysis using the gene target 16S rRNA (31) (detailed in Supplementary Data). Bacterial characterization was confirmed by MALDI-TOF (32) by the Authority for Biological and Biomedical Models at the Hebrew University of Jerusalem (Jerusalem, Israel).

Gross pathology. Clinical examination following euthanization of sentinel animals included external and internal inspection to identify abnormalities. Externally, the skin, eyes, ears, teeth and genital area were observed for lesions, discoloration, or deformities. Internally, organs such as the heart, lungs, liver, spleen, pancreas, kidneys, uterus, ovaries and gastrointestinal tract were examined for signs of disease.

Parasitology. Direct exams and PCR tests were applied for parasite screening. Feces from the duodenum, cecal and proximal colon and fur hair tape were collected with forceps, mounted on slides and inspected for pinworms, mites and eggs under a microscope (Nikon TS-2-S-SM, Nikon Instruments Inc., Melville, NY, USA). When pinworms, mites and eggs were observed, they were counted and classified by morphology parameters (33, 34). Fresh feces and environmental cage swabs of quarantined imported animals, were tested by real-time PCR at Charles River Laboratory Research Animal Laboratory (Diagnostic Service, Wilmington, Massachusetts, USA). Detection of pinworms and mites by PCR is described in the Supplementary Data.

Monitoring of environmental microorganisms. Water and working surfaces were monitored for the presence of microorganisms using the Lumitester-ATP System (Kikkoman Biochemifa Company, Tokyo, Japan). The normal range values were 0-10 relative light units (RLU) for

Classical health surveillance includes serologic tests, bacterial cultures and parasitology examinations. Molecular diagnostics have been developed for precise bacteria and pinworms identification using real-time polymerase chain reaction (PCR), DNA sequencing, 16S ribosomal DNA (rDNA) and 18S ribosomal RNA (rRNA) sequencing. The latest development in the veterinary field is the use of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) as a reliable tool for identifying anaerobic bacteria (9-16). This technology enables the rapid identification of these microorganisms using well-characterized isolates in animal facilities. However, gram positive anaerobic cocci remain under-represented in available databases (15). Environmental monitoring of areas in the facilities is also considered to be part of a routine including frequency of sampling, time, duration and sample size per surface area controlled by different techniques as contact and settle plates, swabbing, active monitoring of air volume and ATP-based methods to detect the presence of live or dead organic material (17, 18).

Direct examination for parasites, including pinworms and mites, remains classical in animal facilities. Oxyurina order pinworms, *Syphacia obvelata* and *Aspiculuris tetraptera* are the most common parasites found in laboratory mice, transmitted through the ingestion of embryonated eggs (19-21). Helminths are opportunistic pathogens, and are generally expected at low levels in laboratory mice, rarely causing clinical signs, unless there is a heavy infection. The prevalence of helminths infection is associated by factors such as host age, strain, health status, stocking density and environmental conditions (22). *Syphacia muris* is commonly detected in rats (23, 24).

The two most frequently observed ectoparasites are fur mites, *Myocoptes musculinus* and *Myobia musculi*. While low infestations are typically subclinical, heavy infestations can cause irritation, pruritus, hair loss and scabs (22). In addition to detecting mites through microscopic examination of fur and skin and PCR assays, a metagenome of *Myocoptes musculinus* was derived by sequencing fur plucks of an infected mouse. *Myobia spp*. and *Demodex spp*. are particularly found in immunodeficient or transgenic laboratory mice (25).

Specific pathogen-free (SPF) colonies are integral to modern biomedical research, ensuring the reliability and reproducibility of experimental outcomes by providing a consistent and controlled baseline for experiments. SPF animals are housed in barrier facilities to prevent exposure to pathogens. These facilities include features such as filtered air, sterilized food and bedding, and strict protocols for cleaning and staff access. Entry of new animals or materials is closely monitored, often requiring quarantine and testing. Regular health monitoring and testing are performed to confirm the SPF status of the colony (26).

While research mice are commonly maintained as SPF colonies to ensure they are free of defined infectious agents, many academic and research institutions continue to house laboratory animals in conventional units (11). The specific pathogens in conventional animal facilities compared to SPF units, as well as imported animals in quarantine, have been poorly investigated. In this study we aimed to identify and describe the pathogens in our animal facilities and to compare them across units.

MATERIAL AND METHODS

Study design. This observational study analyzed pathogens identified in rodents housed in various facilities at Tel Aviv University based on comprehensive health monitoring tests during a five-year period (2019-2023). Our center functions according to the FELASA recommendations (7). The study was approved by the Institutional Animal Care and Use Committee of Tel Aviv University (approval number TAU-MD-IL-2307-150-2) (27).

Animals. The animals included in two-month-old sentinel female ICR strain mice (CD-1 outbred) and Sprague-Dawley (SD) rats both purchased from Harlan Laboratories (Jerusalem, Israel), male and female quarantine mice aged 1-3 months, which were C57BL/6-based transgenic mice from various institutes and universities in Israel and other countries. The animals were confined in designated rooms with restricted personal access for four weeks, followed by health monitoring tests and allocation to SPF or conventional units. Only imported quarantined mice, negative for endoparasites and ectoparasites tested by PCR enter the facilities. SPF and conventional sentinel animals were from the same strain, age, sex and originated from the same distributor.

Housing facilities. The animals were housed in pairs in individually ventilated cages provided with filtered and acidified sterile reverse osmose water, sterilized food ad libitum (Irradiated Rodent Diet, Cat. # 1318, Altromin, Lage,

Challenges in Maintaining Microbial Status in Specific Pathogen-Free (SPF) and Conventional Animal Housing

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ABSTRACT

Routine microbiology, virology and parasitology monitoring of rodent colonies in animal facilities is essential for evaluating the health status of animals used in research. Over a five-year period, we examined the presence of selected microbial infections and parasitology contaminations in various types of animal facilities at Tel Aviv University, including specific pathogen-free (SPF), conventional and quarantine facilities. Animal health monitoring followed the Federation of European Laboratory Animal Science Associations (FELASA) recommendations. A total of 955 rodents (mice and rats) were monitored during the study. The most common bacterial strains found in both conventional and SPF units were *Pasteurella spp.*, followed by *Staphylococcus aureus*, *Klebsiella* (*pneumoniae, oxytoca*) and *Pseudomonas aeruginosa*. Other isolated bacteria, not included in FELASA recommended panels, such as *Proteus spp.*, *Enterobacter cloacae and Morganella morganii* were less prevalent. Pinworms and mites were not found in SPF rodents and showed a prevalence of 0.5-8% in the conventional facilities. The rodents housed in the SPF unit had a statistically significant lower prevalence of specific pathogens compared to those in conventional units, emphasizing the critical role of microbiological barriers established by SPF health monitoring standards. This study demonstrates that despite the physical proximity of the SPF and conventional facilities, their distinct microbiological status can be maintained long-term through rigorous health monitoring, strict management and well-designed facility infrastructure.

Keywords: Animal Facility; Health Monitoring; Infectious Diseases; Parasites Rodents

INTRODUCTION

The presence of contaminated pathogens in research animals, even in cases of subclinical microbial outbreaks, may compromise research outcomes, as animals that are sick or stressed do not yield results as reliable as those obtained from healthy and unstressed animals (1). Therefore, ensuring animal health by minimizing microbial variability is vital for research integrity, supporting the reduction in animal use and advancing the ethical principles of the 3Rs: replacement, reduction, and refinement (2).

Health monitoring and continuous diagnosis of infectious pathogens, in experimental research animals and breeding colonies, are essential for assessing the prevalence of infections and for maintaining the sanitary and environmental conditions of the facilities. Environmental and rodent microbiological monitoring programs have been published in different countries (3-6). Institutional Health Monitoring programs and testing laboratories can be accredited by the Federation of European Laboratory Animal Science Associations (FELASA) (7) or by the National Research Council (US) Committee on Infectious Diseases of mice and rats (8).

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even in winter and create an environment that favors the growth of bloodsucking flies, it is necessary to increase the control of stinging flies and research on EHDV infections in different animal species.

The results obtained in this study demonstrated the presence of EHDV in camels, goats, and sheep in the Aydin region. However, it is still unquestionably unknown if EHDV triggers what form of clinical symptoms and pathogenesis in camels, goats, and sheep. Therefore, these species should not be ignored in the epidemiology of EHDV. Further research, including molecular detection analysis and next-generation sequencing, is required to understand the role of sheep, goats, and camels in the epidemiology of EHDV.

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Ethical statement

Aydın Adnan Menderes University Animal Experiments Local Ethics Committee approved the study with Decision No. 050.04/2011/123.

Conflict of interest

The authors declared that there is no conflict of interest.

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re-isolated. While only a small amount of viral RNA was found in the blood of two sheep, their antibody response after inoculation of sheep with the virus from that outbreak was conflicting. The authors reported that the sheep did not show any symptoms. Therefore, they concluded that the infection did not lead to a productive infection. However, some researchers have isolated EHDV from sheep (8, 20). Also, there is evidence of EHDV infection with clinical symptoms in sheep in Türkiye. Yavru *et al.* (8) isolated EHDV from three sheep showing clinical symptoms in Aydin province. The results of this study and the findings of Yavru *et al.* (8) support the hypothesis that sheep could be natural hosts of EHDV.

Recently, a few studies have been published about the sero-prevalance of EHDV infection in cattle, goats, and sheep (21, 22). In a study in Zimbabwe, the median seroprevalences of EHDV antibodies in cattle and sheep were found to be 62% and 0%, respectively (21). No EHDV antibodies were detected in an epidemiological study in goats and sheep in Kazakhstan (22). Hampy (23) detected antibodies against EHDV in three sheep in Texas, USA. Odiawa *et al.* (13) found the highest seropositivity in goats and sheep in the American state of Georgia. They detected 29% of sheep (n=286) and 7% of goats (n=433) were seropositive for EHDV. Cêtre-Sossah *et al.* (2) investigated 276 cattle, 142 sheep, and 71 goats on Reunion Island. They observed that the EHDV seroprevalence rate in cattle was 63.77%, 5.63% in goats, and 3.70% in sheep. They suggested that EHD widely occurs in cattle rather than in goats and sheep. Mahmoud *et al.* (24) studied the seroprevalence of EHDV infection in goats, cattle, and sheep in Libya. They found it to be 4% as the overall seroprevalence rate (small ruminants and cattle) of EHDV. Of the 555 sheep tested, 32 were found positive; none of the 135 goats were positive; and only 1 out of 165 cattle was found to be positive (24). In a field study conducted in Indonesia, antibodies against EHDV serotype 5 using seroneutralization were found to be 24% (150/620), 1.8% (1/62), and 0% (0/20) in cattle, goats, and sheep, respectively (14). In line with the previous study, we discovered a high seropositivity rate in goats. It is possible that goats are susceptible to EHDV and could act as natural reservoirs. Further studies are needed to understand the susceptibility and the role of goats as hosts for EHDV.

There are a few reports of various serotypes of epizootic hemorrhagic disease virus infecting camels (25, 26). Wernery *et al.* (26) showed that 29% of dromedary camels from the United Arab Emirates have antibodies to EHDV. Cossedou *et al.* (25) observed 63 out of 86 camels (73%) seropositivity for EHDV in Mauritania. This seropositivity rate was the highest in the world. However, EHDV infection was not found in camels in seroepidemiological studies conducted in Tunisia and Morocco (15, 27, 28). There are no detailed studies on the clinical appearance of EHDV in camels. In this study, the rate of EHDV-specific antibodies was detected to be 7.8% in camels. This finding supports reports that camels may be natural hosts of EHDV infection and could play a role in epizootiology.

The Aydin locality is one of the regions where vector diseases such as akabane, bluetongue, and ephemeral fever are frequently seen. This region has suitable ecological and geographical conditions for the biological circulation of blood-sucking mosquitoes as biological vectors, especially *Culicoides spp.* The samples investigated in the study were collected during the period when EHDV infection was clinically prevalent. In the study, although EHDV infection seropositivity was observed in camels, goats, and sheep, clinical findings were found only in sheep. In contrast to previous studies, this study revealed the infection with solid evidence of the serologic presence of EHDV in camels, goats, and sheep. The clinical findings in sheep in the Aydin region (8) supported the results obtained in this study. The infection in these animals may depend on the serotype or topotype of the virus and epizootiological factors such as animal species, breed, geographic location, climate, etc. in the region. Therefore, further arthropodological, epizootiological, and molecular virological studies should be performed in the region. In addition, experimental pathogenesis and clinical studies with serotypes and topotypes of regional EHDV need to be carried out in these animals through further investigations.

CONCLUSIONS

EHDV might be causing sporadic atypical symptoms and subclinical infections that result in complications in the differential diagnosis of various viral infections. Thus, periodic controls should be carried out on camels, goats, and sheep and prevention and control strategies should be developed accordingly. Especially in regions such as Aydın, where the average temperature and humidity remain relatively high

since they could not identify any signs of EHDV infection in sheep. It is not yet clear how goats serve as hosts for the EHDV virus (5). Goats are not considered susceptible to infection. However, some researchers consider that a proportion of goats, as in sheep, may develop a low level of viremia (12). EHDV antibodies have been detected in goats in a few countries under field conditions (2, 13, 14). The infection has been reported in camels as well (15), but there are few studies on the EHDV infection in camels. No serological evidence of EHDV infection in camels or goats has been reported in Türkiye so far, although clinical cases of EHD were reported in sheep flocks around Aydin province (8).

The objective of this study was to investigate the serological presence and distribution of EHDV infection in camels, goats, and sheep using an Enzyme-Linked Immunosorbent Assay (ELISA). Thus, this preliminary study aimed to help understand the epizootiology of EHDV in Turkey. Furthermore, the results from this study are hoped to be useful in larger future studies aimed at preventing and controlling the infection.

MATERIALS AND METHODS

This retrospective study investigated EHDV infection in serum samples from 40 goats, 55 sheep, and 68 camels. The samples were collected from sheep and goats in 2012 and camels in 2011. The tested samples were collected randomly from animals in Aydin province. The 68 blood samples were collected from dromedary camels (*Camelus dromedarius*) that were brought to Incirliova Municipal Slaughterhouse, to the Faculty of Veterinary Clinics of Aydin Adnan Menderes University for medical examination and bred on local farms in Aydin province. The camels brought to slaughter or those to be bred were appeared healthy at the time of blood collection. The animals that were brought to the clinics also did not show any clinical signs of systemic disease. The age of the animals varied between one and 20 years.

The blood samples were collected in polystyrene tubes containing kaolin. Serum was obtained by centrifugation of blood samples collected into tubes containing kaolin and stored at -20°C until testing. All samples were tested with ELISA in the same year of blood collection.

The presence of EHDV-specific antibodies (against VP7) in blood serum samples was tested using a commercially available blocking ELISA (LSIVet, Lissieu, France). The assay was optimized to eliminate cross-reactivity. The test was performed following the manufacturer's instructions. The positive samples were tested in duplicate, in order to confirm the results.

Table 1. Numbers of tested animals and seropositivity rates against EHDV in camel, goat, and sheep

	Tested Animals	EHDV Ab positive	%
Camel	68	5	7.3
Goat	40	7	17.5
Sheep	55	4	7.3
Total	163	16	9.8

RESULTS

In this study, antibodies against EHDV were detected in five of the 68 camels (7.3%), seven of the 40 goats (17.5%), and four of the 55 sheep (7.3%) investigated (Table 1). In total, 16 of the 163 animals were seropositive (9.8%). The findings of this study suggest that natural EHDV infection is present and may be circulating in camels, goats, and sheep in western Türkiye. These animals may contribute in the epidemiology of EHDV infection since EHDV may induce subclinical or unfamiliar symptoms. In addition, the virus may cause outbreaks from time to time, resulting in economic losses in the Aegean region. To our knowledge, this is the first report of serological evidence of natural EHDV infection in camels and goats in Türkiye.

DISCUSSION

There are few reports of EHDV infection in camels, cattle, goats, and sheep in the existing literature. In general, it has been determined that EDHV infection in sheep and goats either doesn't exist or occurs very infrequently (9, 10, 11). It has been observed that giving goats and sheep direct virus inoculation or material containing EHDV did not result in any clinical signs or viremia (9, 11, 16, 17, 18, 19). Temizel *et al.* (7) isolated EHDV in cattle in Türkiye but did not report any evidence of infection in sheep or goats. Kedmi *et al.* (11) claimed that there is no evidence for the involvement of sheep in the epidemiology of cattle.

Eschbaumer *et al.* (16) detected 12 East Frisian sheep with a virulent EHDV-7, isolated from an outbreak in Israel. After the inoculation of sheep with the virus from that outbreak, the viremia was not detectable; the virus could not be

Retrospective Serological Investigation of Epizootic Haemorrhagic Disease Virus in Sheep, Goats and Camels in the Aydin Province, Türkiye

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ABSTRACT

Epizootic haemorrhagic disease virus (EHDV) causes a systemic viral infection primarily affecting deer but has also led to significant economic losses in cattle. In Türkiye, the infection was first reported in Mugla province in 2007 and has caused fatalities in Aydın province as well. The disease is characterised by systemic blood circulation disorders and death. Data on the epizootiological, clinical, and pathological characteristics and virulence of EHDV in sheep, goats, and camels in Aydın province are very limited. The role of these animals as hosts for the virus is unknown. This study investigated EHDV infection in serum samples collected from 40 goats, 55 sheep, and 68 camels. Samples were tested for EHDV-specific antibodies using a commercial enzyme-linked immunosorbent assay. Antibodies against EHDV were detected in five of the 68 camels (7.3%), seven of the 40 goats (17.5%), and four of the 55 sheep (7.3%). These findings suggest that natural EHDV infection is present and may still be circulating in camels, goats, and sheep in the region. Since EHDV may cause subclinical or atypical symptoms, these animals could play a role in the epidemiology of EHDV infections. Additionally, the virus may occasionally cause outbreaks, resulting in economic losses in the Aegean region. To our knowledge, this is the first report of serological evidence of natural EHDV infection in camels and goats in Türkiye. Results from this preliminary study may be useful in larger future studies aimed at preventing and controlling the infection.

Keywords: Antibody; Camel; Epizootic Haemorrhagic Disease Virus; Goat; Sheep

INTRODUCTION

Epizootic hemorrhagic disease virus (EHDV) is a member of the *Orbivirus* genus within the *Sedoreoviridae* family (1). It has a double-stranded segmented RNA genome. Officially, seven serotypes of EHDV (EHDV-1, -2, -4, -5, -6, -7, and -8) have been recognized (1, 2, 3, 4). However, novel EHDV strains from South Africa, Japan, and China have been proposed as possible serotypes (4). The virus is transmitted between ruminants by midges of the genus *Culicoides spp.* (5).

Epizootic hemorrhagic disease (EHD), sometimes referred to as deer disease, can cause significant mortality events in deer populations but has also been linked to severe clinical symptoms, major loss of productivity, and death in cattle (6). The infection in white-tailed deer leads to serious illness. Although it is thought that EHDV rarely causes disease in domestic ruminants, the infection was first seen in the form of severe clinical symptoms in cattle and outbreaks in Israel and Türkiye in the years 2006-2007 (6, 7, 8).

Data on the prevalence and virulence of the virus and the clinical and pathological findings in camels, goats, and sheep are very limited in literature. It is assumed that sheep are susceptible to EHDV infection but rarely develop clinical symptoms (9, 10). In an outbreak in Israel, Kedmi *et al.* (11) concluded that sheep had no part in the epizootiology

Letter to the Editor

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Caenorhabditis elegans, a worm to which the whole mankind should be grateful!

One year has gone by since the achievement of the Nobel Prize in Physiology or Medicine by Katalin Karikó and Drew Weissman for the messenger RNA (mRNA) technology, which has made feasible the production of the revolutionary anti-SARS-CoV-2 vaccines that have globally saved hundreds of millions of lives during the CoViD-19 pandemic (1).

In 2024 the central stage has once again been taken by RNA, since the Nobel Prize in Physiology or Medicine has been awarded to Victor Ambros and Gary Ruvkun for their discovery of "micro-RNAs" (miRNAs) (2). These are short, non-coding RNA sequences orchestrating human and animal genes' activities, which may result either stimulated or silenced by them. And, while their current applications in human as well as in veterinary medicine concern the diagnostic field, with several miRNAs being employed as disease biomarkers (3), their potential use in human and animal cancer therapy appears to be promising, alongside the cure of cardiovascular and neurodegenerative disorders as well as of infectious diseases in people and animals. Still of interest, miRNAs were originally discovered thanks to the investigations carried out on a tiny, one-millimetre-long roundworm termed *Caenorhabditis elegans* and made up of a thousand cells, the number of which does not change throughout its life. This is the reason why the Scientific Community has developed during the last 60 years a growing interest into the aforementioned nematode, with special emphasis on the biological events and mechanisms underlying cell death, cell regeneration and cell differentiation. Thanks to these efforts, the key process of "programmed cell death" – alias "apoptosis" – was identified, with the pioneering investigations on the genes regulating it having been performed in *C. elegans*, fifty years ago, by Sydney Brenner, who in 2002 shared the Nobel Prize with Robert Horvitz and John Sulston. In conclusion, two highly deserved Nobel Prizes in Physiology or Medicine have been achieved throughout the last 22 years (alongside two additional ones, awarded for RNA interference- and green fluorescent protein-related investigations, respectively), thanks to the studies carried out on C. *elegans*, a minute worm to which the whole mankind should be forever grateful!

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Editorial

Dear Readers of the IJVM,

The March edition of the IJVM has a number of very exciting articles relevant to a variety of fields in our profession. I am sure that you, our readers will be able to glean a lot of important information relating to the work in your field of Veterinary Medicine.

Thank you, Prof. Giovanni Di Guardo for your interesting letter to the editor. I highly recommend our readers examining this interesting outline appraisal of the valuable contribution of the worm *Caenorhabditis elegans* for important medical research.

Dr. Rapaport from the University of Tel Aviv has described the challenge of maintaining disease free laboratory animals for research. This has been carried out by comparing the status of rats and mice kept under difference hygienic and quarantine conditions. Individuals working in this field of laboratory animals will find this article of interest.

Dr. Kachtan and his associates have presented a clinical case of a rare condition "Pneumatosis Coli in a dog. This condition is characterized by intramural gas within the colonic wall. It is also rarely reported in human medicine, and it is often reported as an incidental finding. This is the first description of this condition in a dog in Israel, with only a few cases been reported world wide.

Two articles from Turkey dealing with animal health are very welcome. Prof. Turkyilmaz and her team of bacteriologists continues to describe very interesting aspects of antibiotic resistance. In this article she places emphasis on *E. coli* with special emphasis on the effect of vaccination on the pathogenicity of this bacteria.

Prof. Dr. Nural Erol, presents an interesting virological serology article on Epizootic Haemorrhagic Disease Virus in Sheep, Goats and Camels in Turkey. The article frequently mentions the outbreak of the disease in Israel and the similarities found.

Dr. Sarah Weyl Feinstein and colleagues presents an encompassing article on nitrate intoxication in grazing cattle caused by intoxication caused by grazing *Silybum marianum* plants. The authors emphasize the importance of pasture management and proper knowledge relating to potential hazardous plants. The natural grazing environment cannot be ignored, rather addressing the cows and the pasture as one holistic eco-system.

Sincerely,

Dr. Trevor (Tuvia) Waner

Editor-in-Chief, Israel Journal of Veterinary Medicine

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Contents

Front Cover: **ZEBRAS**

Zebras are African equines with distinctive black-and-white striped coats. There are three living species: Grévy's zebra (*Equus grevyi*), the plains zebra (*E. quagga*), and the mountain zebra (*E. zebra*). Zebras share the genus *Equus* with horses and asses. Zebra stripes come in different patterns, unique to each individual. Several theories have been proposed for the function of these patterns, with most evidence supporting them as a deterrent for biting flies. Zebras inhabit eastern and southern Africa and can be found in a variety of habitats such as savannahs, grasslands, woodlands, shrublands, and mountainous areas. Zebras are primarily grazers and can subsist on lower-quality vegetation. The International Union for Conservation of Nature (IUCN) lists Grévy's zebra as endangered, the mountain zebra as vulnerable and the plains zebra as near-threatened. Zebras may travel or migrate to wetter areas during the dry season. Plains zebras have been recorded travelling 500 km (310 mi) between Namibia and Botswana, the longest land migration of mammals in Africa. When migrating, they appear to rely on some memory of the locations where foraging conditions were best and may predict conditions months after their arrival. A zebra's diet is mostly grasses and sedges, but they will opportunistically consume bark, leaves, buds, fruits, and roots. Compared to ruminants, zebras have a simpler and less efficient digestive system. Nevertheless, they can subsist on lower-quality vegetation. Gestation is typically around a year. A few days to a month later, mares can return to oestrus. Usually, a single foal is born, which is capable of running within an hour of birth.

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