Current Developments in Laboratory-based Diagnostics for Canine & Feline Infectious Diseases

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CONFLICT OF INTEREST

- I am not an employee, paid consultant or member of advisory board for Biomed Diagnostics

- I have not received any compensation for this presentation to the Chicago Vet 2019, from Biomed Diagnostics, who a Sponsor and Exhibitor at the *Chicago Vet Show.*

- I have used Biomed Diagnostics products, as part of *Diagnostic Testing & Research*

OUTLINE

I. Disruptive technologies/advances in clinical laboratories for microbial ID

II. Routine submissions received for microbial detection & recommendations

III. Current Trends (input from D-LABs @ GA, IL, OK, MI, PA, NC, LA)

- Fungal- Dermatophytosis & Systemic Mycoses (Blastomyces)
- Rapidly Growing Mycobacterium infections & Tularemia
- UTI testing (constitute ~25% Bacteriology submissions for culture & sensitivity)
- Canine Infectious Respiratory Diseases-Flu, Canine Distemper, Mycoplasma

IV. Antimicrobial Resistance & Antimicrobial Stewardship

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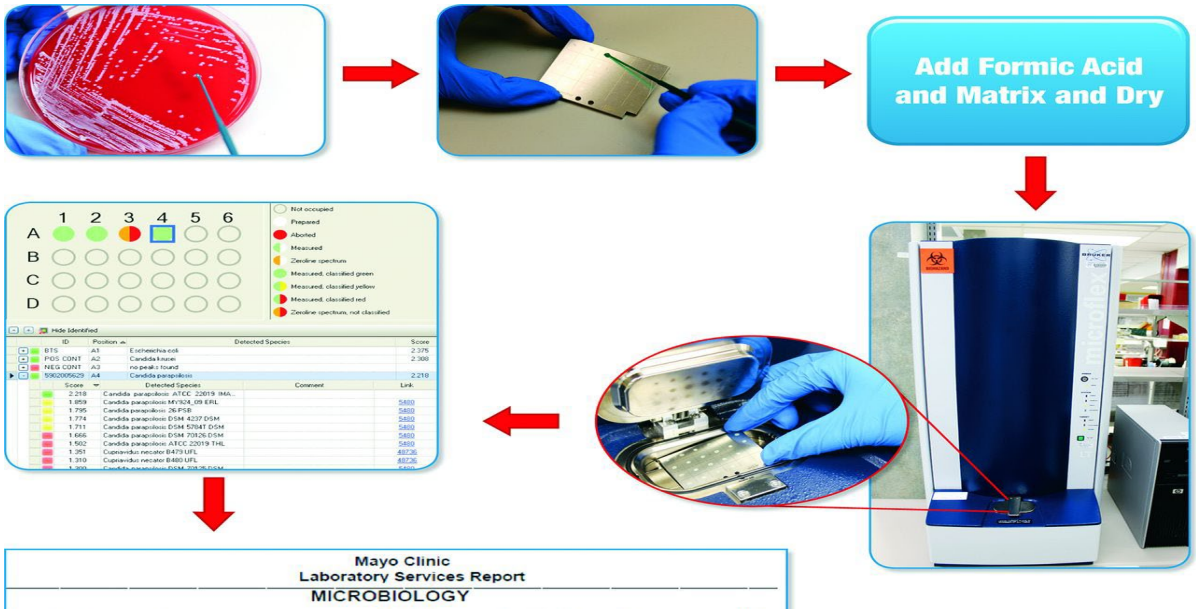
Major Advances in Clinical Microbiology

- **MALDI-TOF MS based microbial detection**
  - Matrix Assisted Laser Desorption/Ionization-Time Of Flight Mass Spectrometry

- **Next Generation Sequencing (NGS)**

MALDI-TOF: Accelerated Bacterial Identification Typical Work Flow



MALDI-TOF uses a unique “protein fingerprint” from the bacteria of interest, and compares it to a library of known spectra to produce an ID

Courtesy: Mayo** clinic **image from web

Performance of MALDI-TOF-MS for Bacterial Identification

- Several studies demonstrated successful application to ID human pathogens and veterinary pathogens compared to commercially available conventional test systems

- MALDI-TOF MS testing yields a numeric score

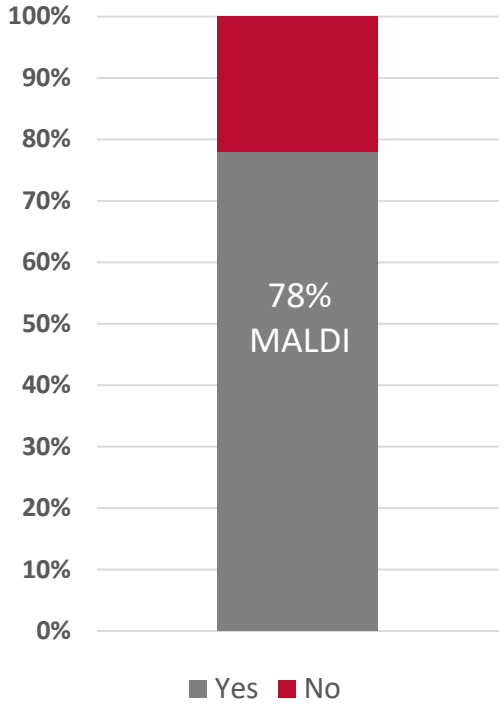
SCORE	Accuracy (genus)	Accuracy (species)
> or =2.0	99.5%	99%
> 1.7 to 2.0	95%	89%

MALDI-TOF MS based microbial identification (bacteria & fungi) in veterinary diagnostic labs

- **Best Practice**
  - 4/5 D-Labs in US & Canada have implemented
- **Faster and Accurate Results**
  - 5 - 10 minutes vs 24 – 48 hours
  - > 90% agreement compared to current methods
  - Antimicrobial susceptibility test (AST) results in 8 hours
- **Increased Efficiency and Traceability**
  - Detects > 1000 bacterial species in minutes
  - Requires less technical resources: load and walk away
  - Seamless LIMS integration for added efficiency
- **Cost Effective**
  - Costs USD$1.00 per test vs. $4 for commercial conventional methods

BOTTLE-NECK: ~$250,000 initial capital cost & $25,000 annual PM service

~ 4 out of 5 labs have MALDI-TOF MS in Micro sections



Next Generation Sequencing (NGS)

Targeted NGS for multiple pathogen detection

- vector-borne disease agent detection panel
- bacterial & viral disease, food-borne pathogen detection panel
- Toxins, antimicrobial resistance genes, virulence genes

Specimen types: cultured isolates, blood, tissues, feces, body fluids

Next Generation Sequencing (NGS)

Whole Genome Sequencing

- Sequencing and analyzing entire genome of a bacterium, virus, or other microbes without requiring bacterial culture
- Tracking disease outbreaks
- Sequencing thousands of microbes in parallel with NGS

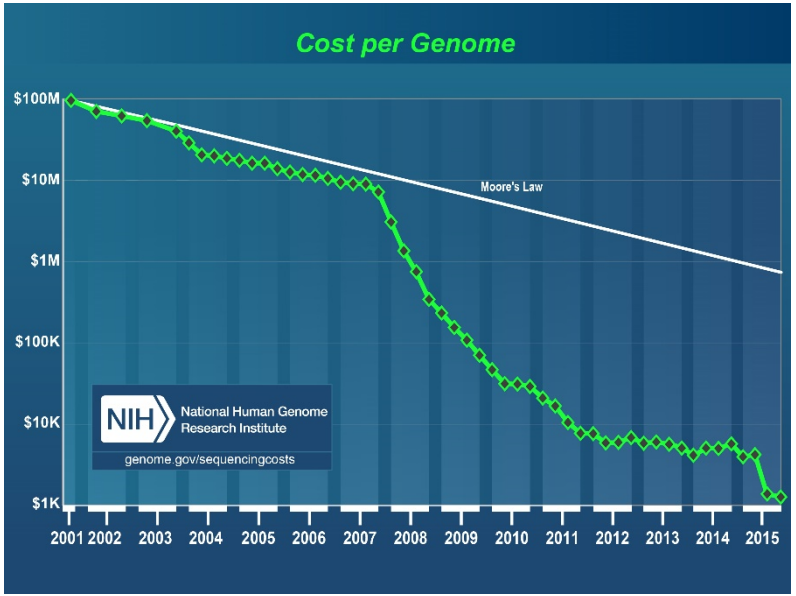
MiSeq

Metagenome Sequencing

- Comprehensively sequence all genes in all organisms present in a given complex sample.
- Enables microbiologists to evaluate bacterial diversity and detect the abundance of microbes in various environments.

Challenges with Next Generation Sequencing

- Initial cost of investment: > $100 K and upward
- Requires Bioinformatics support
- Operational costs per run are very high
- Requires to batch samples to make it cost effective
- Targeted NGS or WGS per sample test cost:$200- $400
- Current turn-around-times: 2 to 4 weeks



WITH TIME, TECHNOLOGY WILL BECOME MORE AFFORDABLE

In 2001, Human genome sequencing cost $100 million vs. Today: $1000

MULTIPLEX qPCR PANELS OFFER RAPID, ACCURATE, AFFORDABLE DIAGNOSTIC SOLUTIONS

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- Canine Parvovirus infections, Feline Parvovirus infections, Giardia & Feline Trichomonas
- Rapidly Growing Mycobacterium infections
- Fungal-Dermatophytes & systemic (Blastomyces & others)
- UTI

IV. Antimicrobial Resistance & Antimicrobial Stewardship

Routine submissions received for microbial detection & recommendations for Bacteriology testing

- **Live animal sample submissions**
  - Urine- *cysto, free catch, swab (avoid free catch-contaminants); pre-enrichment*
  - Skin swab/hair, crust
  - Wound swab/ aspirate
  - Ear swab
  - Ocular swab
  - Isolates/ cultured plates (presumptive culturing at clinic)
  - Nasal swab
  - Feces/fecal swab
  - Vaginal/ uterine swab
- **Necropsy submissions**
  - whole animal
  - tissues

INCLUDE CULTURETTE SWABS and SWABS in RED TOP TUBES FOR PCR (if needed)-chilled

QUALITY IN- QUALITY OUT or GARBAGE IN- GARBAGE OUT

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- Urinary Tract Infections (UTI)
- Respiratory: Canine Infectious Respiratory Diseases- Mycoplasmas, Dog Flu, K9 Distemper

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Fungal- Dermatophytosis/ Ringworm

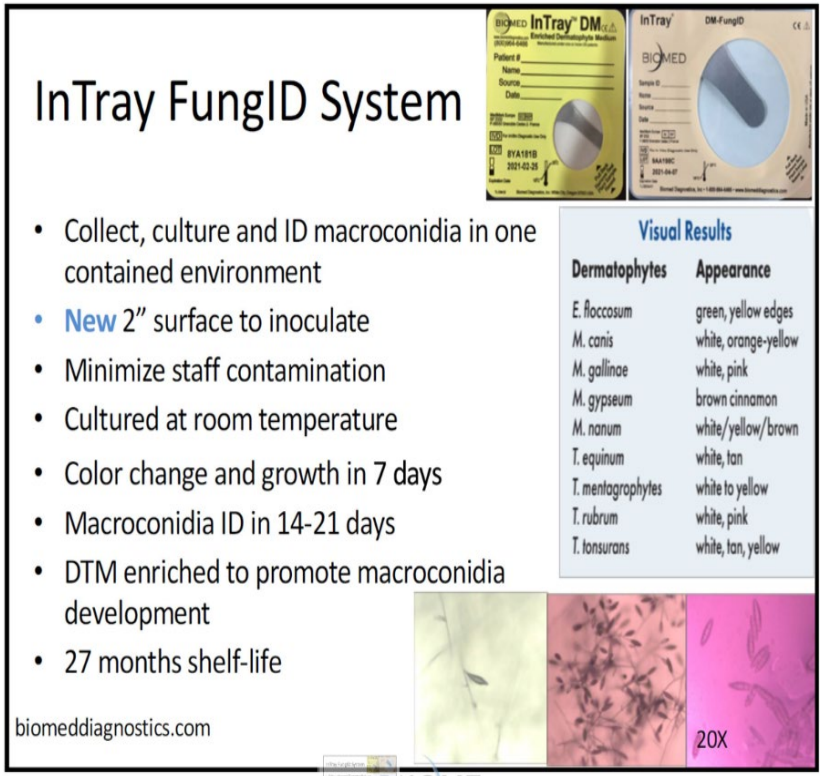
- Dermatophytes are fungi that require keratin for growth.
- Humans & animals (skin, nail, hair); spread by direct contact & indirect (fomites)
- Ringworm in dogs mainly caused by:
  - *Microsporum canis* (~70% of cases)
  - *Microsporum gypseum* (~20% of cases)
  - *Trichophyton mentagrophytes* (~10% of cases)

Small Animal Dermatology 4th Ed 2017

- Ringworm in cats: 98% are caused by *Microsporum canis*
- Common in young kitten, focal alopecia, scaling, crusting around the ears and face or on the extremities
- Lesions in dogs are classically alopecic, scaly patches with broken hairs

Diagnosis of Dermatophytosis

- KOH Microscopy “hyphae”
- Fungal Culture of hair, skin scrapings, crust: DTM agar, Saboraud’s agar for 2-3 weeks at room temperature
- PCR for dermatophytes on hair, crust offers greater sensitivity and specificity & quickest turn-around-time of 1-2 days.
- Sequencing
- InTray FungID system (in-clinic testing) for presumptive diagnosis



Fungal In-Tray as a collection & transport media and for **Presumptive diagnosis**

- Collect, transport, incubate and result in one device
- View directly on the microscope stage
- Rapid TAT
- No re-plating required
- FDA Registration and Euro CE Mark* [*on select products]
- Extended 6 - 27 month shelf life [varies depending on product]
- Reduces contamination risk and lab materials


Label, and then peel back
to reveal inner seal

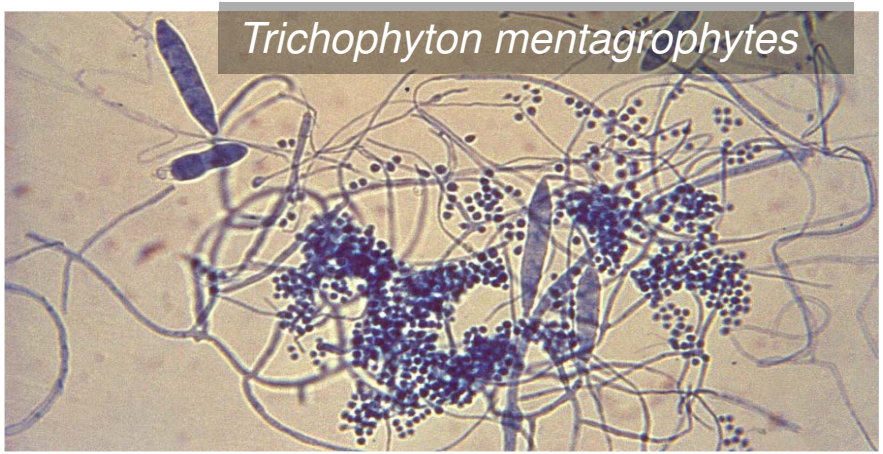

Discard inner seal

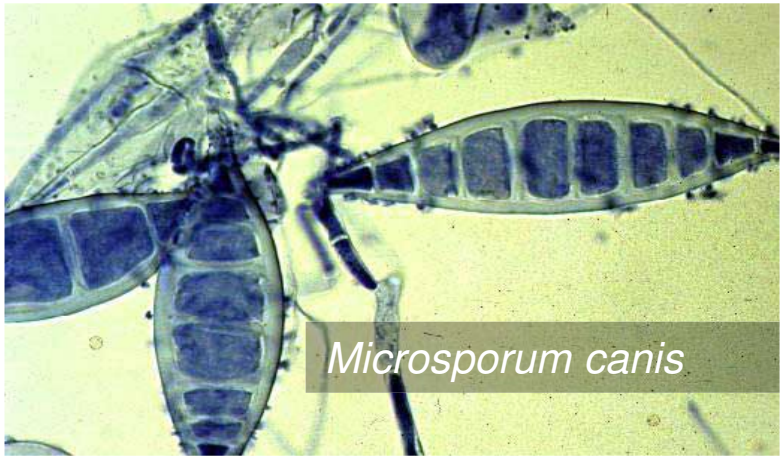

Inoculate using standard techniques


Ensure tension and reseal outer label.
Incubate as instructed.


InTray™
BIOMED
Sample ID
Name
Source
Date
MediMark Europe
BP 2332
F-38033 Grenoble Cedex 2 France
For In Vitro Diagnostic Use Only
LOT
Expiration Date
ECS/TL/GEN/02
Made in USA
Pull Back
Remove Seal
Plant Sample
Reseal Label
Biomed Diagnostics, Inc. • 1-800-964-6466 • www.biomeddiagnostics.com


Microsporum gypseum

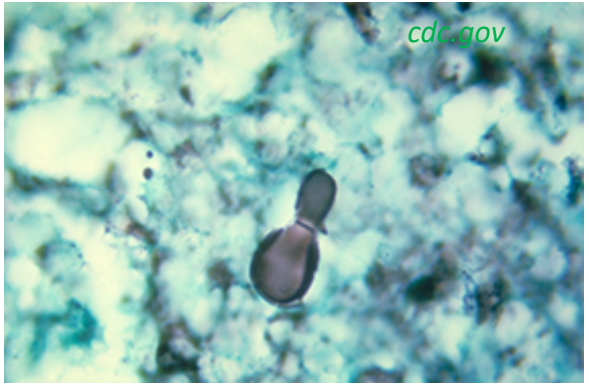

Trichophyton mentagrophytes


Microsporum canis

SYSTEMIC MYCOSIS

Blastomycosis:

- *Caused by Blastomyces dermatitidis*
- Commonly diagnosed in dogs and humans
- Dimorphic fungi: infective phase “mycelial”; non-infective phase “yeast”
- Infective (Mycelial)-found in sandy acidic soil, moist soil, transmitted by spore inhalation
- Endemic or hyperendemic in midwestern, south-central, and southeastern United States



Diagnoses of Blastomycosis

- **Antigen detection**: Enzyme immunoassay (EIA) is preferred screening test; urine has highest sensitivity followed by serum, bronchoalveolar lavage fluid. Cross-reactions can occur with histoplasmosis and other fungal diseases (MIRAVISTA LABS)

- **Antibody tests (serum)**: antibody tests such as EIA, immunodiffusion (AGID) and complement fixation (CF) are available, but have low sensitivity and specificity

- **Cytology/ Histopath/Radiograph**: effective for detection of yeast in tissue/ respiratory secretions

- **Polymerase chain reaction (PCR):** *Blastomyces* PCR can be used to confirm culture or histopathologic identification and on blood to detect disseminated disease.

- **Signalment**: non-specific (fever, anorexia, lethargy, failed Rx to antibiotics), H/O Travel

- **Culture**: the gold standard for diagnosing blastomycosis- slow, 2-4 weeks.

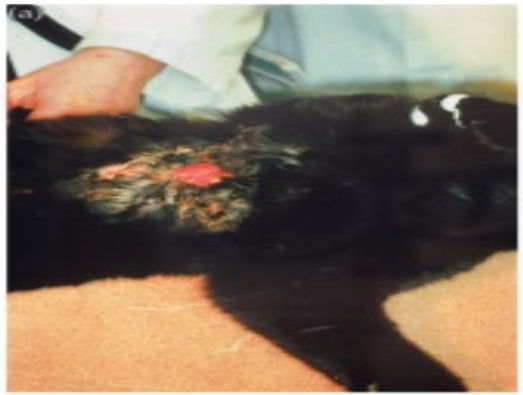
Other Systemic Mycoses : Histoplasmosis, Coccidioides

- Histoplasmosis: caused by *Histoplasma capsulatum*
  - Urine Antigen test: is the preferred screening tool, followed by serum, CSF, BAL, other body fluids
  - Serum Antibody test may be useful in cases where urine Ag test gives false negative
  - Cross-reactivity with *Blastomyces dermatitidis*. However, differentiation is not required as Rx & monitoring are very similar

- Coccidioides: Preferred screening tool is the serum Antibody AGID test. A titer of > 1:8 is supportive of active infection

Rapidly Growing Mycobacterium (RGM) infections

- **Cats** with chronic, nodular, fistulous, pyogranulomatous, draining cutaneous & S/C, non-healing wounds, not responding to antimicrobial Rx
- **Confirmatory diagnosis:** demonstrating mycobacteria through culture or visual identification of mycobacterial agents in cytologic or histologic samples.
- Differential diagnosis: high lipid bacteria: *Corynebacterium, Nocardia, Rhodococcus* sp.



Axillary Panniculitis

Inguinal Panniculitis

Malik etal J. Fel Med & Surg

Two types of RGM in cats & dogs

- *M. smegmatis*
- *M. fortuitum*
- Rapidly grows in 7 days
- Acid-Fast Bacilli
- Long-term antimicrobial Rx for 3-6 months
- Effectively cured

Tularemia in cats

- Causative agent: *Francisella tularensis (Gram negative rods, transmitted by ticks)*
- Domestic cats are very susceptible;
- Mainly cats are infected by rabbits
- Rabbits and rodents are susceptible; often die in large number during outbreaks
- Clinical signs of infection in cats: High fever, lethargy, death

https://criticalcaredvm.com/tularemia-cats/

- **Zoonotic transmission:**
  - Tick and deer fly bites
  - Skin contact with infected animals
  - Drinking contaminated water
  - Inhaling contaminated aerosols or agricultural and landscaping dust
  - Exposed as a result of bioterrorism (SELECT AGENT)
  - Laboratory exposure

cdc.gov

Tularemia Cases at OADDL: 2014-2018

Over the last four years, OADDL has diagnosed tularemia in several cats and rabbits. The first two cases of tularemia in 2018 occurred earlier this month (April).

Tularemia is endemic in Oklahoma (Fig 1). Confirmed cases of feline tularemia at OADDL have involved outdoor and indoor/outdoor cats ranging from 6 weeks to 10 years of age, with both sexes equally affected. Common clinical signs have included high fever and lethargy, followed by death. Infected cats often had the additional history of contact with a dead rabbit prior to onset of illness.

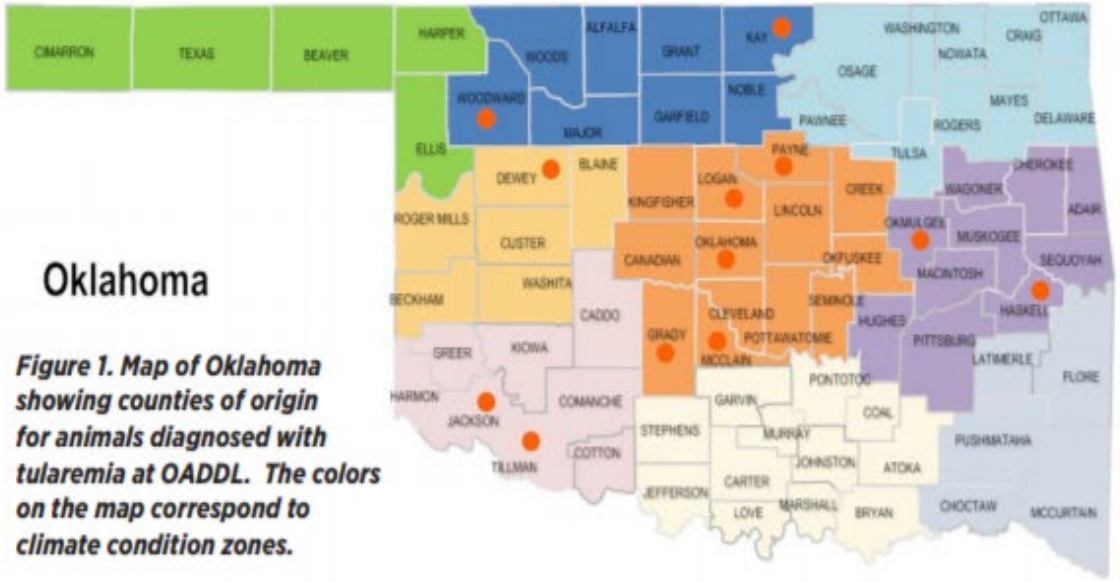


Figure 1. Map of Oklahoma showing counties of origin for animals diagnosed with tularemia at OADDL. The colors on the map correspond to climate condition zones.

The most characteristic necropsy lesion has been ***multifocal necrosis in the spleen*** and lymph nodes.

Tularemia is caused by *Francisella tularensis* and is a significant zoonotic disease. Extreme caution must be taken when handling infected cats and tissues so as to minimize human exposure.

– Dr. A. Ramachandran

Urinary Tract Infections (UTI)

- Urine cultures typically ~ 30% times significant growth
- 25-30% times no growth (thioglycollate enrichment to improve recovery)
- “Cysto-collected urine” is the most relevant sample

- Most common bacterial sp. isolated from UTI cases of dogs and cats:

  - *E. coli (*dogs and cats +++)
  - *Staphylococcus* spp. (dogs +++, cats ++)
  - *Enterococcus* spp. (dogs ++; cats +++).
  - Other bacteria: *Proteus* spp., *Klebsiella* spp. *Enterobacter* spp (dogs+ & cats +)

Is there a need to enhance Urine Culture for improved Pathogen Detection in UTI cases?

New enhanced technique for urine culture known as "Enhanced Quantitative Urine Culture (EQUC)" method

EQUC detected significantly more pathogens than standard urine culture in women with symptoms of UTI
(Price et al ASM Microbe 2017, JCM 2016)

EQUC method uses higher volumes of urine (1, 10, or 100 µL) and different growth media than the standard protocol (urine volume = 1 µL).

NOTE: Utility in the clinical setting has not been established

In-clinic Presumptive Identification of Urinary Tract Pathogens

- Urine culture on a single chromogenic agar plate can detect and differentiate Gram + and Gram – pathogenic bacteria vs. culturing on multiple different media plates

- InTray Colorex Screen (Biomed)

HiChrome UTI agar

- Antimicrobial susceptibility testing (AST(can be performed directly from primary isolates on Chromogenic agar medium without need for subcultures
- Reference Diagnostic Labs can confirm ID and perform AST

Manjusree etal (2017) IJCMAS

Canine Infectious Respiratory Diseases (CIRD)/ Kennel Cough

Multiple bacterial & viral pathogens are involved sequentially/synergistically to cause illness

Common pathogens:

- ***Bordetella bronchiseptica***
- **Canine Adenovirus 1 & 2**
- **Canine Distemper virus**
- **Canine Parainfluenza**
- Canine Herpes virus
- Mycoplasma spp.
- Influenza A

Emerging pathogens:

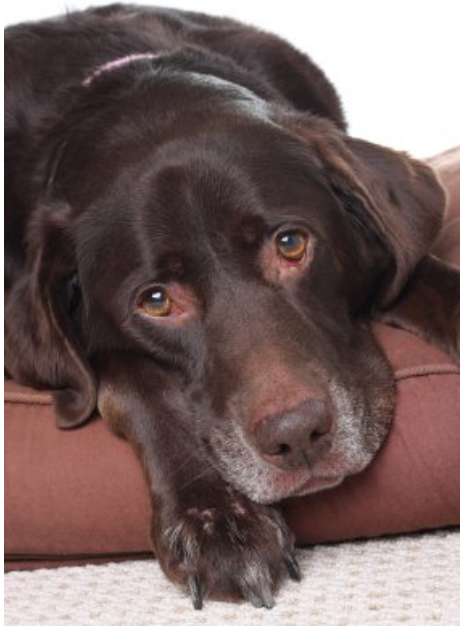
- Canine *Mycoplasma cynos*
- *Mycoplasma canis*
- Canine Coronavirus
- *Streptococcus equi subsp. zooepidemicus*

Opportunistic: *E.coli, Klebsiella, Pasteurella, Pseudomonas*

CIRD: New insights into the etiol. & epidemiol. of asso. pathogens

Maboni *etal* (Plos One April 25, 2019): n=478 dogs: PCR study

- CPIV, *M. canis*, and *M. cynos* were the most commonly detected pathogens (24%-29%)
- Influenza A, *Bordetella bronchiseptica* , Coronavirus, CAV, and CDV were detected (2-11%)
- All samples were negative for *Streptococcus equi subsp.zooepidemicus*
- CIRD pathogens were detected from all age groups
- Puppies were commonly infected with *Bordetella bronchiseptica and CDV*
- CoV was more prevalent in adults.
- Influenza A was less common in puppies



https://vcahospitals.com/know-your-pet/canine-influenza-the-dog-flu

CIRD: New insights into the etiol. & epidemiol. of asso. pathogens

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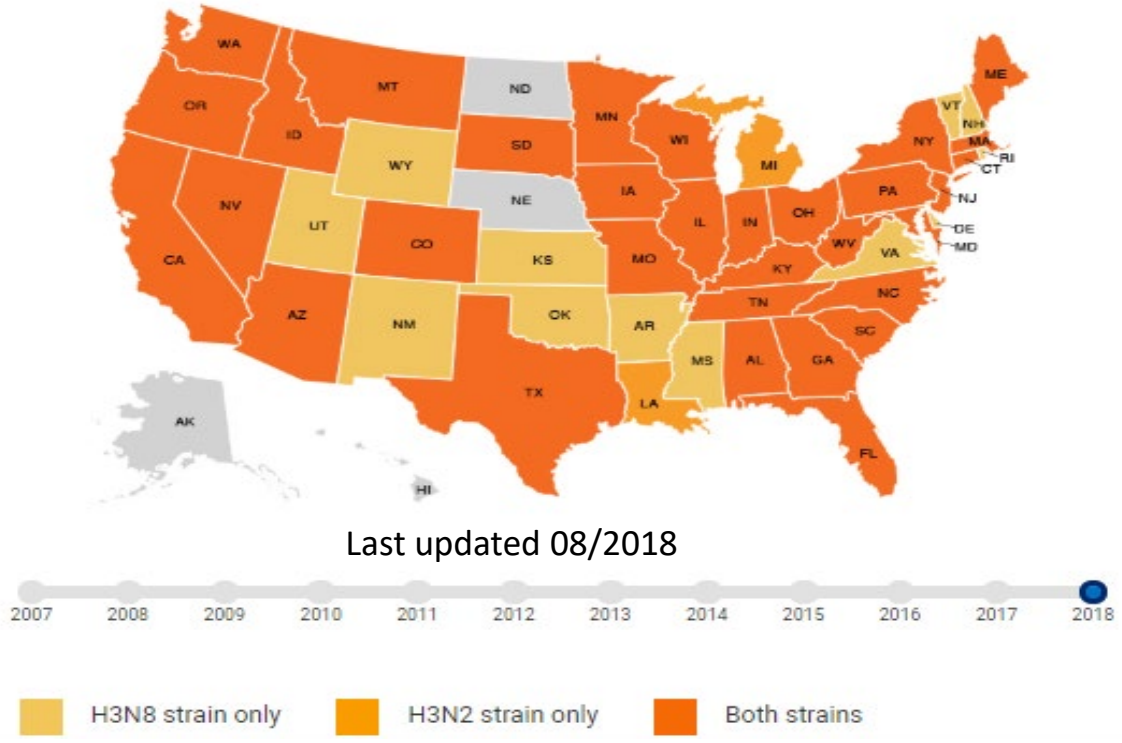
- All pathogens were commonly detected from animals with clinical signs vs. asymptomatic
- *Mycoplasma canis* found more often in clinical vs. asymptomatic dogs
- Disease occurrence was reduced in dogs vaccinated against classical CIRD pathogens
- *Mycoplasma cynos* is an emerging bacteria implicated in CIRD
- Young age was the most significant predictor of severe clinical signs
- Presence of co-infections & young age were associated with the severity of clinical signs
- *M. cynos*, CPIV, *M. canis* and *B. bronchiseptica* were most common co-infections

Canine Infectious Respiratory Diseases: Dog Flu, Canine Distemper, Mycoplasma



Dog Flu (Canine Influenza):

- Specific Type A influenza virus
- contagious resp. disease in dogs
- No human cases with dog flu
- Two different influenza A Dog flu viruses: H3N8 and H3N2
- Canine influenza A(H3N2) viruses differ from seasonal flu A(H3N2) viruses in people
- Vaccines available for both types

https://www.dogflu.com/

NO MAJOR ACTIVITY OF CANINE INFLUENZA

Canine Distemper

- CDV-highly contagious systemic viral disease of dogs
- Re-emergence of CDV inspite of vaccinations in US
- Sequenced 59 CDV + samples (collected from dogs from different regions and states from 2014 to 2017)
- 12 different lineages detected worldwide
- 3 main CDV lineages differ from the historically identified lineages in the US
- Identified lineages differ from America-1 lineage, which contains the majority of the vaccine strains
- Continuous surveillance is required for monitoring circulating CDV strains in the US, to prevent potential vaccine breakthrough events

Distribution of 3 main CDV lineages currently circulating in the US

Anis etal 2018 Virology J

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- Canine Parvovirus infections, Feline Parvovirus infections, Giardia & Feline Trichomonas

IV. Antimicrobial Resistance & Antimicrobial Stewardship

Veterinary Nosocomial Infections & Antimicrobial Resistance (AMR)

- Nosocomial infections and AMR are life-threatening problems for veterinary patients, especially surgical patients.

- *Enterococcus* spp. contamination was reported in a study of 10 private veterinary hospitals with 20% of the isolates having AMR (Kukanich 2012 JAVMA).
- *Staphylococcus pseudintermedius*: 44% of dogs with pyoderma were reported in a study to be infected with resistant isolates; most frequently isolated *Staphylococcus spp.* in cats and dogs*(Eckholm Vet Derm 2013).*
- *Acinetobacter baumannii:* AMR strains isolated from cases such as canine pyoderma, feline necrotizing fasciitis, UTI. *(Vander Kolk Glob Antimicrob Resist. 2019).*

- Increased prevalence of AMR *E.coli,* AMR *Pseudomonas* ear infections and AMR Salmonella in gastrointestinal & UTIs.

How to address spread of AMR?

- Proper hand hygiene, surface disinfection, & surgical etiquette are essential in minimizing risk; lead to positive outcomes for patients

- Judicially administer antibiotics only when needed
  - avoid antibiotics during biopsies (unless infection exists prior to biopsy)
  - ensure correct dosage & duration
  - use narrow spectrum & avoid broad spectrum antibiotics when possible
  - use topical antiseptics to avoid use of antibiotics when possible
  - Recommend Bacterial isolation & antimicrobial susceptibility testing/PCR testing prior to use of antibiotics

- Educate pet owners of zoonotic risks & imp. of proper hand hygiene
  - Examples: MRSA transmissions from humans to pets & reverse
  - Risk of Campylobacter transmission via fecal-oral route from pets

Multidrug-Resistant *Campylobacter jejuni* outbreak linked to Puppy Exposure- United States, 2016-2018

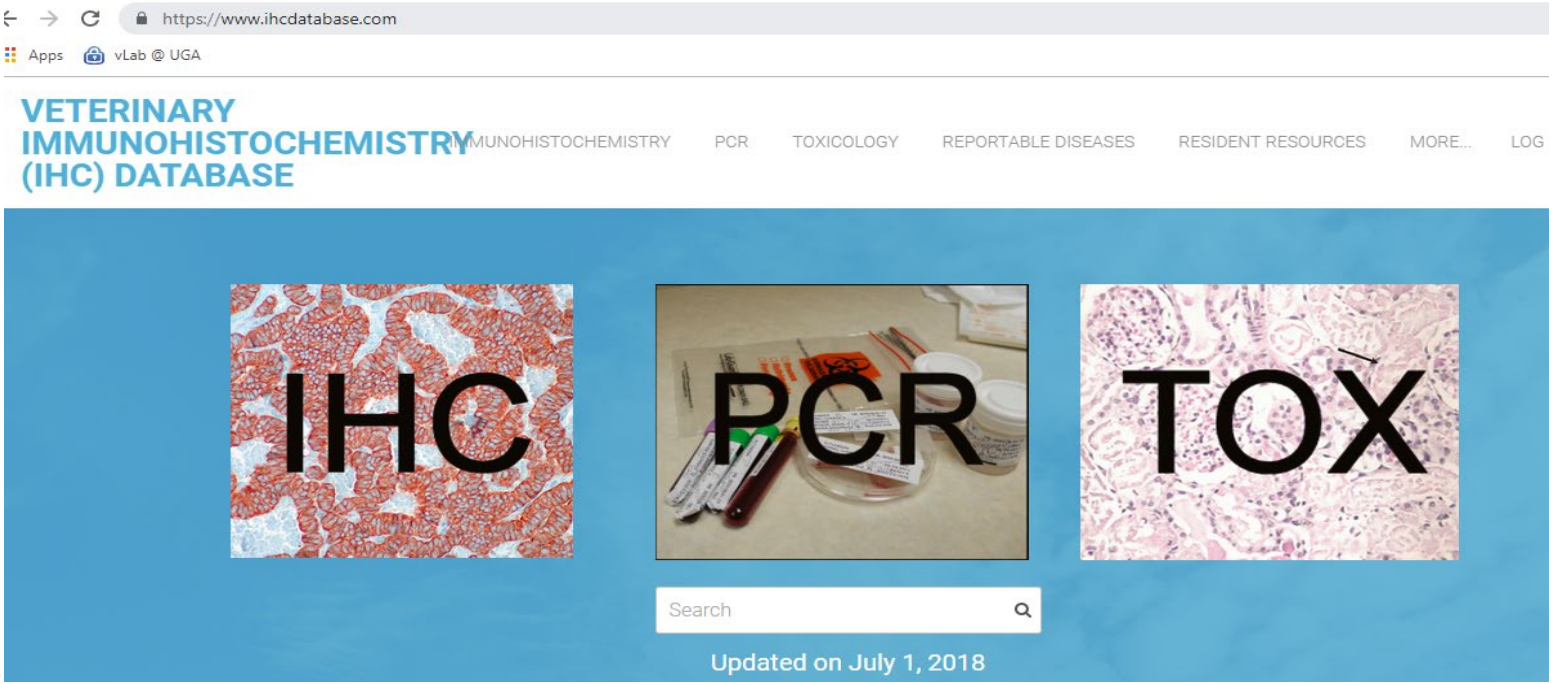
- N=118 persons including 29 pet store employees were infected
- 105 persons reported dog exposure
- 26 persons were hospitalized
- No mortality
- Indicated that puppies got infected with *Campylobacte*r before reaching pet stores

Montgomery etal (Sept. 2018) Morbidity and Mortality Weekly Report, USDHHS/CDC

INDEXING OF TEST RESOURCES: IHC DATABASE, PCR DATABASE, TOXICOLOGY DATABASE

https://www.ihcdatabase.com/

Maintained by Dr. Laura Bryan, DVM, Dip. ACVP



Thank You!