

Diagnostic Tips for the Equine Practitioner

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COLLECTION CONTAINERS – THE ESSENTIALS

1. Blood collection tubes

- Red top
- Purple top EDTA

2. Sterile leak-proof containers

- Red and white top tubes work for small volume
- Fecal cup with gasket to prevent spillage for large volume

3. Bacterial transport media

- Aerobic (Amies)
- Anaerobic transport media

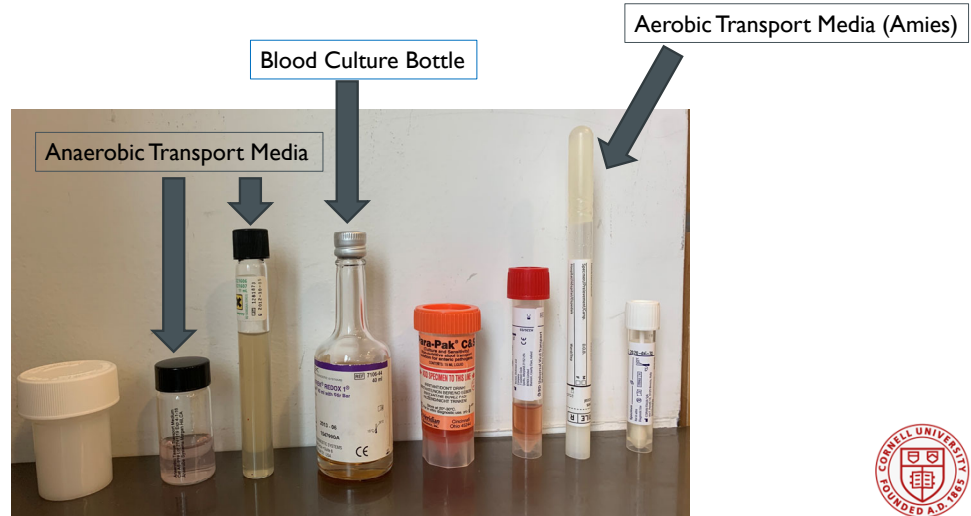


Bonus: Blood culture bottles, especially if you work with neonates or might work-up a septic joint or meningitis



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THE CORNUCOPIA OF BACTERIAL TRANSPORT MEDIA WHICH TO CHOOSE?



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BACTERIAL TRANSPORT MEDIA

Amies aerobic transport media

- Not for anaerobes, even if the label indicates otherwise
 - Human hospitals that plate specimens quickly can use Amies for anaerobes, *not vets!*

With or without charcoal?

- Without charcoal
 - Works for most aerobic organisms
 - Keeps bacteria alive for 72hrs
- With charcoal
 - Required for CEM cultures
 - Charcoal eliminates metabolic products of bacterial growth – Campylobacter, fastidious growers



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BACTERIAL TRANSPORT MEDIA

Anaerobic Transport Media (ATM) – *Best kept secret!*

Can be used for aerobic, anaerobic and fungal culture

- 2 different brands
 - ATM
 - BD BBL Port-A-Cul
- 2 different sizes
 - Tube for swab
 - Vial for specimen
- Liquid specimens should be injected directly into the tube or vial through the rubber septum of the cap



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THE SECRET SUPERPOWERS OF ANAEROBIC TRANSPORT MEDIA

Invisible hydrogen sulfide gas cap inside, heavier than O₂

- Keeps O₂ out to maintain anaerobic environment
- Must hold upright during inoculation so gas cap doesn't 'spill out'

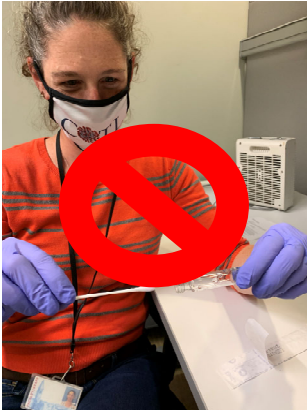
Helpful Tips:

- Do not tip tube horizontally during inoculation
- Store at room temperature, don't refrigerate
- Deliver to lab within 72hrs



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COMMON ATM MISTAKES



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BLOOD CULTURE MEDIA

Separate bottle for aerobic and anaerobic culture

Also good for culture of fluids of low cellularity

- Joint fluid, CSF

Specimen must be collected using aseptic technique

- Sterile gloves, sterile handling of needle/syringe, sterile prep of collection site required (just like a joint injection)
- Very easy to contaminate blood culture media



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BLOOD CULTURE MEDIA

Helpful tips to avoid contamination:

- Clean top of vial with alcohol and let dry prior to inoculation, then cover with sterile gauze while collecting specimen
- Change needle on collection syringe prior to inoculation and between bottles
- Maintain at room temperature prior to submission – do not refrigerate



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OTHER TRANSPORT MEDIAS:

BD eSwab™ in liquid Amies media

- Non-enteric aerobic, anaerobic and fungal culture
- Maintains for 48hrs at room temperature
- Labeled for PCR, but not validated for this purpose in most veterinary diagnostic labs

Para-Pak® Fecal Transport Medium

- Contains buffered solution for pH maintenance
- *Salmonella*
- *Shigella*
- *Yersinia*



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PLASMA

- Clotting factors present in plasma

Serum

- No clotting factors in serum

The lab can't decipher serum vs. plasma when transferred to a red/white top tube, PLEASE LABEL 😊

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RED TOP TUBE

- No additives and sterile
- Plastic red tops are coated with clot activator
- Separating serum avoids hemolysis and artifact on chem panels
 - Serum sitting on cells too long → ↑K ↓glucose
 - Certain testing can not be performed accurately on hemolyzed serum samples

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PURPLE TOP EDTA TUBE

- EDTA stands for Ethylenediaminetetraacetic acid.
- EDTA functions by binding calcium in the blood and keeping the blood from clotting

Common uses:

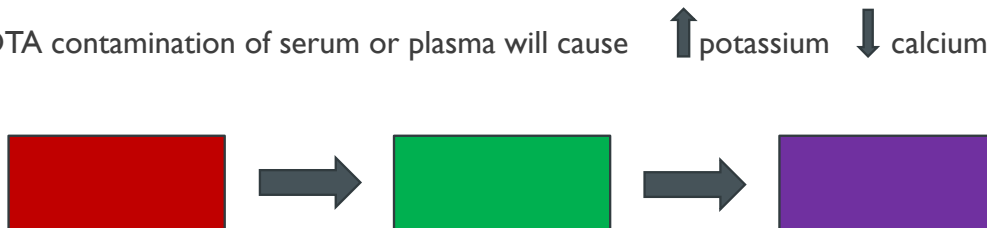
- CBC
- Preserve cells for cytology**
 - ie. pleural, pericardial, peritoneal, CSF, tracheal wash, bronchoalveolar lavage or joint fluid cytology*
- Endocrinology testing
- PCR on whole blood



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PURPLE TOP EDTA TUBE

- Draw purple tube **after** red top and green heparin tubes used for chemistry analysis to avoid chemistry artifacts
- EDTA contamination of serum or plasma will cause \uparrow potassium \downarrow calcium

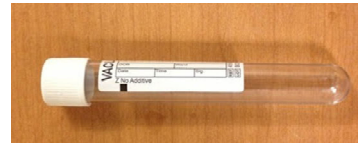


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STERILE LEAK-PROOF CONTAINERS

Red and white tops

- No additive
- Uses:
 - Transfer of EDTA/Heparinized plasma
 - Sterile samples (fluid or swab for PCR)



Sterile cup with gasket to prevent spills



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MINIMUM DATABASE OF SPECIMENS

Specimen	Container	Storage
EDTA Whole Blood	Purple top tube	Refrigerate
Serum (separated)	Red top tube	Refrigerate
Manure	Leak proof sterile container	Refrigerate
Nasal swab or nasopharyngeal wash	Nasal swab: Red top tube with 0.5ml saline Nasopharyngeal wash – sterile leak-proof container like red top tube	Refrigerate
Nasal swab	Bacterial transport media	Refrigerate



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CASE-DEPENDENT - BONUS SPECIMENS

*VERY FRESH MANURE = CAUGHT WHILE PASSED, BEFORE IT HITS THE GROUND IDEALLY

Bonus Specimen	Container	Test	Storage
Very fresh manure*	Sterile leak-proof container	Clostridium toxin testing	Frozen
Rectal mucosal swab	Anaerobic transport media (ATM)	Anaerobic culture for <i>Clostridium difficile</i>	Room temperature
Whole blood	Aerobic and anaerobic blood culture bottles	Blood culture	Room temperature
Fluids <ul style="list-style-type: none"> • Tracheal wash • BAL • Joint fluid • Peritoneal fluid • CSF 	<ol style="list-style-type: none"> 1. EDTA Purple top tube 2. 2-3 unstained air-dried slides 3. Red top tube 4. Anaerobic and anaerobic bacterial transport media 	Cytology (1,2) PCR (3) Cultures (4)	Refrigerate



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CONSIDERATIONS FOR SPECIFIC WORK-UPS

- Abortion
- Respiratory
- Diarrhea
- Neurologic
- Fever of Unknown Origin
- Hepatitis



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ABORTION

Common differentials – bacterial/fungal infection, equine viral arteritis, EHV-1, Leptospirosis

Samples

- Dam blood – red and purple top tubes
- Set of tissues
 - Formalin fixed – ok to comingle in jars if contents labeled
 - Package fresh tissues individually
 - Placenta, lung, liver, kidney, stomach contents



Practice Tip: Collect fetal heart blood and fetal effusion

- Used for serology
- Effusion = pleural, pericardial, peritoneal



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AHDC EQUINE ABORTION FETAL TISSUE DIAGNOSTIC PLAN

Tests Performed	Test Code	Samples Needed
(3) Aerobic Bacterial Culture	AER	<ul style="list-style-type: none"> • Submit 3 fresh tissue samples: placenta, lung, and stomach contents - labelled and individually bagged; for individual aerobic culture
Equine Arteritis Virus (EAV) FA	EVAFA	<ul style="list-style-type: none"> • Fresh tissue: placenta, liver, lung, kidney - labelled and individually bagged
Equine Herpesvirus PCR Panel	EHVNL	<ul style="list-style-type: none"> • Fresh tissue: lung (preferred), placenta - use only if lung not available
Histopathology	HISTO	<ul style="list-style-type: none"> • Formalin-fixed tissue: placenta, liver, lung, brain, adrenal, heart, thymus, small intestine, kidney, and fetal skin
(2) Leptospira PCR	LEPTPCR	<ul style="list-style-type: none"> • Submit 2 fresh tissue samples: placenta and fetal kidney preferred; stomach contents acceptable

Notes

Collect fetal heart blood, pleural fluid or abdominal fluid and place in red top tube for possible antibody serology testing.



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ACUTE NEUROLOGIC WORK-UP

Presentation:

- +/- Fever
- Ataxia



Questions to ask yourself:

*Lack of fever doesn't rule out infectious etiologies

1. Vaccine status – Rabies, EEE, WN, Tetanus, Botulism?
2. Mosquito exposure? Other seasonal risks?
3. Muscle wasting? If yes, symmetric or asymmetric?
4. Other body systems affected? (Respiratory, GI, Hepatic, Renal)?

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FEBRILE ACUTELY NEUROLOGIC HORSE

Thankfully, the preferred diagnostics for most infectious differentials do not require CSF

Serum

- Eastern Equine Encephalitis (EEE) – IgM ELISA
- West Nile Virus (WNV) – IgG/IgM ELISA
- Chemistry
 - Myopathies can sometime be difficult to decipher from other primary neurologic conditions

EDTA whole blood

- EHVI PCR
- *Anaplasma phagocytophilum* PCR (pre-oxytetracycline)
- CBC

Nasal Swab in red top with 0.5ml saline

- EHVI PCR



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AFEBRILE ACUTELY NEUROLOGIC HORSE

So many differentials

- Rabies and many infectious ddx shouldn't be ruled out without testing

Also consider:

- Infectious – EPM, neuroborreliosis, aberrant parasite migration
- Acquired and Degenerative – eNAD, cauda equina, neoplasia, THO, cervical OA
- Congenital – cervical vertebral stenotic myelopathy, cervical vertebral malformation
- Trauma
- Other systemic diseases with neurologic sequelae
 - Renal disease with uremic encephalopathy
 - Liver disease with hepatic encephalopathy
 - GI disease with hyperammonemia (PHF, Coronavirus, *Salmonella spp*)

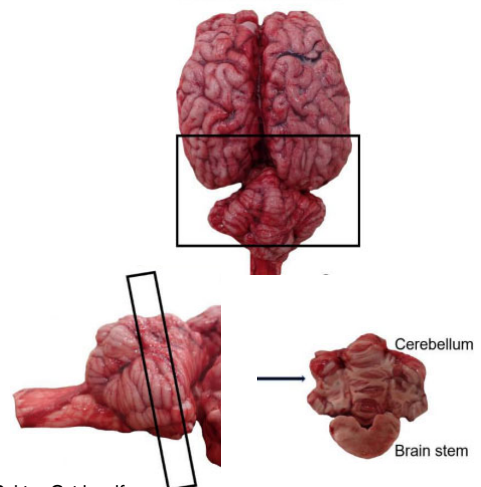


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RABIES

Rabies Fluorescent Antibody Test

- Fresh tissue (not fixed!) in rigid container
- **Cross section of cerebellum and brainstem**
- Inconclusive result if:
 - Sagittal section – lesions can be focal
 - Smooshed specimen
- Send whole head if necessary – let the lab perform brain extraction



<https://www.vdl.ndsu.edu/wp-content/uploads/2019/10/Rabies-Guide.pdf>

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ACUTE DIARRHEA WORK-UP

Common differentials

- *Salmonella* spp.
- *Clostridium difficile* and *C. perfringens*
- Potomac Horse Fever (*Neorickettsia risticii*)
- Beta coronavirus
- Cyathostomiasis (small strongyles)

- Additional considerations for foal and weanlings/yearlings
 - Rotavirus (A and B)
 - *E. coli* septicemia
 - *Cryptosporidium* spp.
 - *Strongyloides westeri*
 - *Lawsonia intracellularis*



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ACUTE DIARRHEA WORK-UP

- *Salmonella* spp
 - PCR vs Enriched culture with susceptibility?
- *Clostridium difficile* and *C. perfringens*
 - PCR vs anaerobic culture vs toxin ELISA?
- Potomac Horse Fever
 - PCR – whole blood vs feces, or both?
 - Are both *Neorickettsia* spp detected on PCR?
 - When is serum IFA appropriate?
- Rotavirus
 - Not all labs test for both types A and B

viruses

Article

Identification of a Ruminant Origin Group B Rotavirus Associated with Diarrhea Outbreaks in Foals

Tirth Uprety ^{1,†,‡}, Chithra C. Sreenivasan ^{1,†,‡}, Ben M. Hause ^{2,‡}, Ganwu Li ³, Solomon O. Odemuyiwa ⁴, Stephan Locke ^{5,†}, Jocelynn Morgan ^{5,†}, Li Zeng ^{5,†}, William F. Gilsean ⁶, Nathan Slovis ⁷, Laurie Metcalfe ⁸, Craig N. Carter ^{2,†}, Peter Timoney ^{1,†}, David Horohov ^{1,†}, Dan Wang ^{1,†,‡}, Erdal Erol ^{5,*,†}, Emma Adam ^{3,*,†} and Feng Li ^{1,*,†}

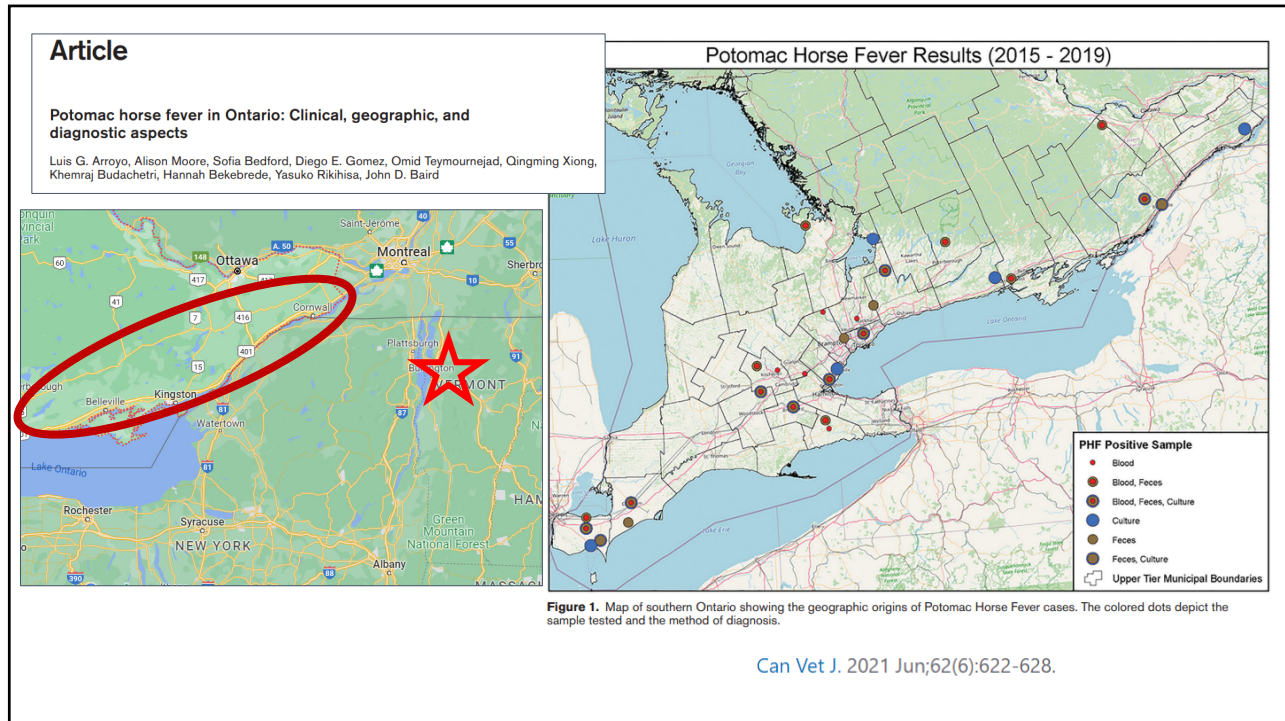
Journal of Clinical Microbiology CLINICAL VETERINARY MICROBIOLOGY

Check for updates

Real-Time PCR Differential Detection of *Neorickettsia findlayensis* and *N. risticii* in Cases of Potomac Horse Fever

Khemraj Budachetri,* Mingqun Lin,* Qi Yan,* Rory C. Chien,* Laura D. Hostnik,* Gillian Haanen,* Mathilde Leclère,* Warren Waybright,* John D. Baird,* Luis G. Arroyo,* Yasuko Rikihisa*

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Special Section

Fecal PCR testing for detection of *Clostridium perfringens* and *Clostridioides difficile* toxin genes and other pathogens in foals with diarrhea: 28 cases

K. Gary Magdesian,¹ Samantha Barnum, Nicola Pusterla

Journal of Veterinary Diagnostic Investigation
2022, Vol. 34(3) 396-401
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DOI: 10.1177/10406387211047529
jvdi.sagepub.com

Clostridium spp. causes hemorrhagic diarrhea and necrotizing enterocolitis in foals

Species	Virulent Toxin genes
<i>Clostridium perfringens</i> Type A found in normal and sick foals Type C found in sick foals	Type A – alpha*, B2, NetE/F*, <i>cpe</i> (enterotoxin), <i>cpb2</i> Type C – beta toxin
<i>Clostridium difficile</i>	Toxin A* Toxin B*

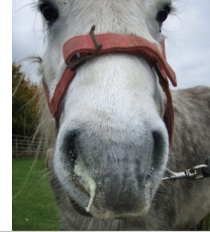
***Practice Tip**
Equine diarrhea PCR panels are available directly on feces that include *C. perfringens* alpha and NetE/F toxin genes and *C. difficile* toxin A and B genes

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ACUTE RESPIRATORY WORK-UP

Common differentials diagnosed with PCR of nasal swab or nasopharyngeal wash:

- *Streptococcus equi* subsp *equi* – 'strangles'
 - Culture and/or PCR?
 - Pinnacle MLV detection on PCR - Genotyping
- Equine Influenza A
- EHV-1, EHV-4
 - EHV-2 (?)
- Equine adenovirus types 1 and 2
- Equine rhinitis virus types A and B
- Equine Arteritis Virus



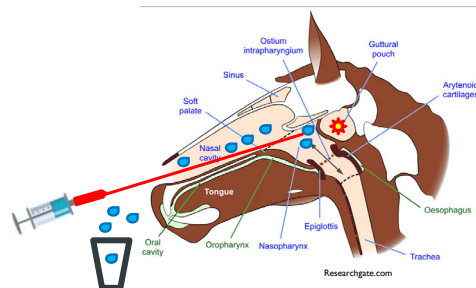
Other considerations– bacterial pneumonia, EHV-5 (EMPF), neoplasia, shipping fever

- Tracheal wash or BAL (EMPF)
 - Cytology and culture

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STRANGLES

Nasopharyngeal Wash



Practice Tip

- Nasopharyngeal wash is more sensitive than nasal swab for the detection of *Strep equi* subsp. *equi*. If the horse has been recently vaccinated with Pinnacle (within 2-4wks) the PCR may detect vaccine.
 - Genotyping from a cultured bacterial isolate is required to differentiate vaccine strain from wildtype *Strep equi* subsp. *equi*

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EQUINE HEPATITIS VIRUSES – CORRECTING THE CONFUSION

Since 2011, 4 viruses were described in the context of equine hepatitis:

- ~~Equine pegivirus (Pegivirus E)~~
- ~~Theiler's disease associated virus (TDAV, Pegivirus D)~~
- Equine parvovirus hepatitis (EqPV-H) has been revealed as the cause of Theiler's disease and mild acute hepatitis
- Equine hepacivirus (EqHV) has been implicated in cases of mild acute and severe chronic hepatitis

Practice Tip:

Only investigate parvovirus and hepacivirus in cases of equine hepatitis

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EQUINE HEPATITIS VIRUSES

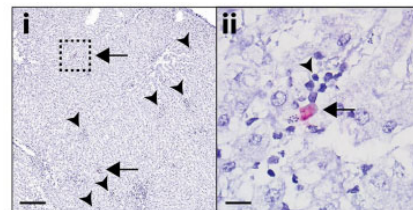
- PCR on **serum** is sensitive for screening
 - Equine parvovirus
 - Equine hepacivirus
- Liver biopsy is required to determine if equine hepatitis virus is incidental finding or related to active hepatitis
- **Equine parvovirus in-situ hybridization (ISH) on formalin fixed liver biopsies at Cornell AHDC**
 - Clarifies the relationship between liver lesions and equine parvovirus

Equine Viral Hepatitis: Clinical Conditions and Epidemiology

Joy Tomlinson, DVM, DACVIM (LAIM), PhD

Liver enzyme activity in horses with hepatic necrosis due to equine parvovirus hepatitis

	Biologic ³	Non-biologic ¹¹	Experimental ¹³	Reference intervals
AST (U/L)	1,187 (770–3,426)	2,925 (1,239–6,177)	542 (310–1,068)	222–489
GGT (U/L)	134 (68–314)	116 (97–185)	49 (15–233)	8–33
Total bilirubin (mg/dl)	15.1 (9.6–24.3)	20.1 (8.7–21.7)	1.9 (1.4–4.3)	0.5–2.1
Bile acids (μmol/L)	128 (111–171)	90 (76–176)	11 (6–148)	2–10



Jager et al. *Virology Journal*

(2022) 19:175

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FEVER OF UNKNOWN ORIGIN

Practice Tip – The AHDC equine FUO panel

Tests Performed	Test Code	Samples Needed
Anaplasma phagocytophilum PCR	EHRE	• EDTA whole blood in lavender top tube or spleen
Coronavirus PCR, Beta	BCOR	• Fresh feces, colon or colon contents in leak-proof container
Equine Herpesvirus 1 PCR	EHV1PCR	• EDTA whole blood in lavender top tube or spleen
Equine Herpesvirus 4 PCR	EHV4PCR	• EDTA whole blood in lavender top tube or spleen
Equine Respiratory PCR Panel	ERPNI	• Nasal swab or nasopharyngeal swab or oropharyngeal swab or tracheal wash or bronchoalveolar lavage or lung tissue
Potomac Horse Fever PCR	EHRR	• EDTA whole blood in lavender top tube or spleen

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 Jvznsj#ijst{naxjx%#esi#
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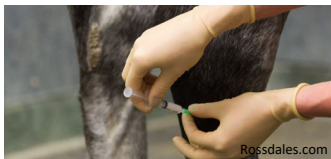


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Influence of Exogenous Steroid Administration on ACTH

- Joint Injections
 - Variable duration of effect on baseline ACTH from 2- 60 days
- Long-term systemic steroid administration
 - Ex. Equine Asthma or IBD cases treated with dexamethasone or prednisolone
 - Stop administration for 2 days minimum or 1 week ideally for baseline ACTH

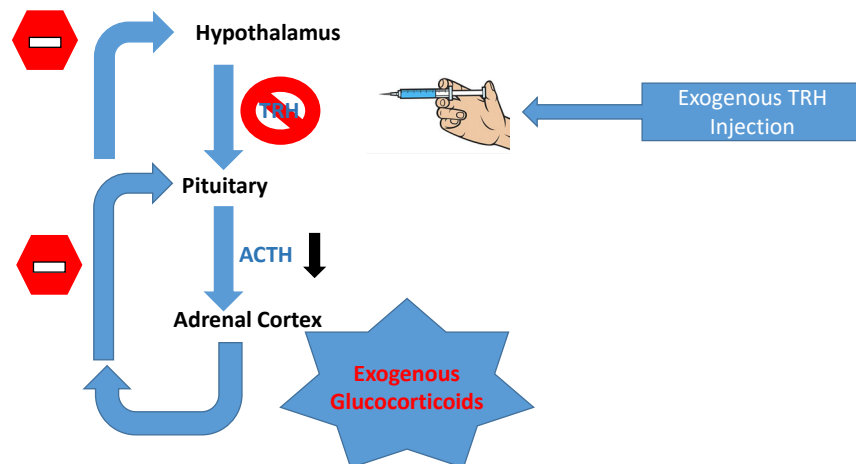


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Influence of Steroid Administration on ACTH

- Use TRH Stim Test to override the negative feedback loop
 - Horse can still respond to TRH with ACTH production



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Influence of Stress on ACTH

- ACTH rises during stressful scenarios:
 - Trailering/Travel
 - Veterinary/farrier procedures
 - Sedating a needle-shy horse
- Don't forget about the 'internalizer' – stressed by life, herd dynamic
 - Always interpret your results within the context of the clinical signs and personality of your patient
- The half life of ACTH is 8min



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Influence of Stress on ACTH

- Wait at least 30min after stressful event before sampling ACTH baseline
- Example:
 - Fjord mare presents to Cornell's Equine Hospital for history of hoof soreness
 - She was stressed by her trailer ride and has ongoing stress in new environment

	1hr After Hospital Arrival	4hrs After Hospital Arrival	Normal Ref. Interval
Baseline ACTH	87.6 pg/ml	47.2 pg/ml	9-35 pg/ml

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Does this horse really need Prascend?

- Scenario: Horse started on Prascend without baseline testing.
 - Maybe bloodwork was borderline, or horse was stressed during initial testing
- Withhold Prascend for 1 week, then perform TRH response testing to assess PPID status



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Withdrawal of Pergolide Prior to Parturition

- **Pergolide** is a dopamine agonist and inhibits prolactin synthesis by lactotrophs in the anterior pituitary → **inhibits lactation**
- Withdraw pergolide 2-4 weeks prior to parturition
 - Personal communication with Boehringer Ingelheim

