Protocol

1. **DO NOT** administer rabies vaccine or immunoglobulin to a patient with rabies. This practice has never worked and may cause adverse outcomes.
   - RIG delays development of rabies antibodies in CSF, essential for survival.
   - Preliminary evidence favors detrimental survival times after rabies vaccine in bat rabies.
   - We have administered beta-interferon to a few rabies patients with poor prognostic epidemiology, with evidence for a beneficial peripheral effect on viral load. This can be considered in particular for dog rabies, where CSF responses are often poor. It appears to stabilize peripheral rabies disease and “buy” an additional week for serological response to develop.

2. Maintain patient in isolation.
   - There has never been a laboratory-documented case of human-human transmission of rabies (other than by transplantation of corneas or solid organs).
   - Patients can be removed from isolation when saliva is negative by RT-PCR on 3 occasions in the presence of serum neutralizing antibodies > 0.5 IU/ml by RFFIT, FAVN or other test for neutralizing antibodies.

3. Transfer patients with laboratory-confirmed rabies to a tertiary care facility capable of critical care including intracranial pressure monitoring.
   - Institutions in developing countries can handle rabies if they treat head trauma and/or tetanus within critical care units.

4. Treatment requires access to a rabies reference lab
   - Transport needs to be prioritized. There can be delays in transporting samples to rabies reference laboratories and in their analysis and reporting that compromise patient care. Reporting should be done by telephone or email as quickly as possible in addition to through standard reporting channels.
   - Depending on logistics of transport, treatment with the Milwaukee Protocol may need to begin if patient is approaching day 5 without a diagnosis. Sedation for 7 days is less dangerous than untreated rabies.
   - Consider use of Bio-Rad Platelia Rabies II Kit (human) #355-1180 for rabies anti-glycoprotein antibody, that is ELISA based and for which comparative studies and precedent for use in humans exist. This can be done locally with fast turnaround and reference laboratory backup confirmation.

5. Treatment also requires access to a rehabilitation facility

6. Treatment generally requires 2 lines of communication:
   - A small group of physicians with confidential communications. This has been done by email and by text-messaging applications such as WhatsApp.
   - A larger group of public health and laboratory authorities charged with epidemiology, public health response, logistics and drug procurement, and public relations. This has been done by conference calls and email.
7. Count hospital days (HD) starting at HD0 from the first day of admission to inpatient care. This is more accurate than counting days of prodromal symptoms for predicting complications.

8. Prevent fatal dysautonomia (20% of rabies patients die from dysautonomia) during days 0 to 7. Aggressive sedation is essential in the first week of hospitalization:
   - Minimize stimulation. DO NOT perform interval neurological examinations.
   - Recommend use of ketamine at 0.5-1.0 mg/kg/h to prevent fatal dysautonomia in the first 7 days of hospitalization. Patients with rabies have very high levels of quinolinic acid, an excitatory agonist of the NMDA receptor from time of diagnosis. Ketamine blocks these agonists.
   - Ketamine is best balanced by a benzodiazepine, typically midazolam, sufficient to minimize vascular reactivity during endotracheal suction or turning.
   - Sedation is directed at cardiac dysautonomia, not abnormal movements
   - Propofol tends to over-sedate rabies patients (to isoelectric EEG). But can be used carefully with EEG or BIS monitoring.
   - Barbiturates are contraindicated until the immune response to rabies is sufficient for viral clearance (0.5 IU/ml in blood, 1.0 IU/ml in CSF) due to their immunosuppressive properties.
   - Opiates and central alpha-adrenergic agonists have been used but not enough for us to comment.
   - Sedation can be monitored by EEG or BIS monitor. We DO NOT recommend titration to burst suppression. Sedation should be held temporarily if the EEG is suppressed.

9. Reduce sedation aggressively starting HD8. Attempt to wean every 12 hours. Sedation should be off by HD12 if possible.
   - The vagus nerve is no longer functional at this point; atropine ceases to be effective.
   - Tolerate abnormal movements, particularly of the face. These are not seizures, are common during recovery, and do not respond to usual sedatives.
   - Consider addition of clonidine or dexmedetomidine rather than repeat increases in benzodiazepines or ketamine when additional sedation is needed.
   - DO NOT aggressively taper if there is cerebral edema.

10. Place a central venous catheter, urinary catheter and NG tube. A NJ tube is recommended for nutrition during the brief (5 day) period of ileus encountered in rabies in the second week.

11. Maintain normovolemia and serum sodium > 145 mEq/L.
   - Use of isotonic solutions is strongly recommended for the first 2 weeks due to salt wasting encountered at 5 days of hospitalization.
   - Administer fludrocortisone 100 mcg (child) – 200 mcg (adult) to maintain normal serum sodium during the first 2 weeks of illness. Otherwise salt wasting may be very difficult to control despite administration of hypertonic saline.
   - When fludrocortisone is not available, consider a physiological dose of hydrocortisone (1X not 3X stress dosing; 15 mg/day divided Q8-12h in adults; 8 mg/m2/day divided Q8h in children). Hydrocortisone risks mild immunosuppression.
There is an unusual form of cerebral edema in rabies, along with cerebral artery spasm on hospital days 6-8 and 13-15. Cerebral edema from hyponatremia exacerbates these processes.

   - The tetrahydrobiopterin (BH₄) deficiency repeated measure in human rabies is predicted to abrogate low-pressure autoregulation. It may also contribute to modest pulmonary hypertension.

13. Administer low-dose insulin drip (1 U/h regular insulin in adults; 0.025 U/kg/h in children) with sufficient enteral and intravenous nutrition to maintain euglycemia.
   - Complications in rabies are associated with biochemical markers of catabolism (gluconeogenesis and Ketogenesis measured in CSF). Promotion of anabolism appears to improve the survival curve by about a week.
   - Insulin may also minimize toxic alcohol metabolites and lactic acidosis associated with propylene glycol stabilizers in benzodiazepine sedatives.

14. Prophylaxis against DVT is recommended.

15. Precautions against pressure ulcers are recommended.

16. General targets:
   - Maintain head of bed elevated 30 degrees
   - mean arterial pressure > 80 mm (adult)
   - central venous pressure 8-12 mm Hg
   - O₂ saturation > 94%
   - PaCO₂ 35-40 mm Hg. Avoid low PaCO₂.
   - Hemoglobin > 10 mg% (historical observation)
   - Serum sodium 145-155 mEq/L. Avoid Na < 140.
   - Serum glucose 70-110 mg% with low dose insulin infusion. This IS NOT intended to be tight control.
   - Maintain diuresis > 0.5 cc/kg/h with hydration; AVOID DIURETICS given rapid evolution of salt wasting on day 5 and possible diabetes insipidus in second week

17. Maintain core temperature 35-37°C. Patients are poikilothermic.
   - Antipyretics generally have no effect in rabies
   - Ambient temperature has a major effect in rabies.
   - Patient temperature affects heart rate and blood pressure

18. Amantadine is given because of its use in the original protocol.
   - There is biochemical evidence of high quinolinic acid in CSF in human rabies, an agonist of the NMDA glutamate receptor. Amantadine in neuroprotective by this mechanism.

19. Ribavirin is NOT RECOMMENDED because of its immunosuppressive effects.

20. Vasospasm and clinical exacerbations are regularly encountered on days 6-8 and 13-15 of first hospitalization.
   - Early, effective use of fludrocortisone appears to minimize vasospasm. These are effectively monitored by transcranial Doppler and can be evident by EEG or BIS monitor.
• Vitamin C (250 mg daily for child and 500 mg for adult, IV or enterally)
• Sapropterin (Kuvan (Merck) 5 mg/kg/day enterally) with vitamin C (250-500 mg total/day IV or PO), and L-arginine (0.5 gm./kg/day IV or enterally) are preferred over nimodipine when available. DO NOT use nimodipine and sapropterin together.
• Nimodipine is recommended at half to full dose for prophylaxis against vasospasm if fludrocortisone or physiological hydrocortisone is not used. Reduce dose to avoid hypotension.
Laboratory monitoring:

1. Serum sodium twice daily.
   - Obtain urine sodium when serum sodium abnormal or difficult to control
   - Consider serum/urine uric acid as second marker of tubular salt wasting
2. Arterial blood gases twice daily or more frequently as needed
3. Serum magnesium daily on hospital days 5-8 and 12-15 to avoid hypomagnesemia during periods of high risk for vasospasm
4. Serum zinc once weekly (inflammatory state, no body stores)
5. MRI or CT in the second and third week twice weekly until CSF titers stabilize.
   - MRI and CT are poorly sensitive for increased intracranial pressure present before seroresponse.
   - IN BAT RABIES, IMAGING IS PARTICULARLY CRITICAL DURING THE SECOND WEEK TO DETECT CEREBRAL EDEMA.
6. Transcranial Doppler ultrasound daily on days 4-8 and days 12-15 after first hospitalization to monitor for degree of vasospasm.
   - TCDs on days 9-11 may detect progressive cerebral edema if no intracranial pressure monitoring is undertaken.
7. Dog rabies: ECG daily HD 5-14 to measure PR interval and assess for heart block
   - MRI or CT in the second and third week twice weekly until CSF titers stabilize.

Virological Monitoring (clinical samples) with transport twice weekly to the reference laboratory

8. Saliva (0.5-1.0 ml, frozen for PCR) every other day (twice weekly minimum) until 3 negatives obtained sequentially.
   - Avoid collection after chlorhexidine mouth care.
   - More frequent testing removes patient from isolation faster
9. Serum (2 ml, frozen for serology) every other day (twice weekly minimum) for first 2 weeks, then 1-2 times weekly.
   - More frequent testing better anticipates complications related to immune response.
10. CSF (2 ml, frozen for serology) twice weekly. Consider ventricular or lumbar drain.
11. CSF twice weekly for cells, chemistry including lactate
12. After a number of incidents, we now strongly recommend:
   - splitting samples to maintain local backup samples (frozen -20/c or -80C) to avoid loss or thawing of samples during transport.
   - Local use of Bio-Rad Platelia rabies II or ADTEC lateral flow assays for more timely reporting and patient management. Rabies titers are essential to rabies management.
Timeline for complications

Timeline seems most exact based on analyses when timed from first hospital admission.

First 3 days after first hospitalization (HD 0-HD 3): Dysautonomia

1. Dehydration, electrolyte disturbances, ketosis.
   - volume replacement, isotonic fluids, low-dose insulin drip
2. Increased intracranial pressure (20-35 cm water)
   - Radiologically subtle but can lead to herniation
   - Associated with increased N-acetylaspartate in CSF (? overlap w/ Canavan’s disease)
   - Consider intracranial pressure monitoring. Ventricular or lumbar drain provides therapeutic and diagnostic advantages over mechanical monitors (bolts or electrodes).
3. Sudden death from asystole or tachyarrhythmias.
   - minimize stimulation and neurological exams
   - sufficient sedation to avoid changes in heart rate with nursing care
   - consider pacer at bedside
   - asystole responds to increased sedation
4. Cardiac stunning from catecholamine storm
   - consider milrinone and beta-blockers

HD 5: Salt wasting

5. Salt wasting, hyponatremia and dehydration
   - fludrocortisone prophylaxis. Hydrocortisone at 1X physiological if fludrocortisone is not available.
   - CVP monitoring
   - frequent measures of serum sodium
   - hypertonic saline replacement; watch free water from medications
   - enteric sodium (23%; 1 g in 5 ml water) is more efficacious than 3% IV hypertonic saline
   - patients are often over-nourished and over-hydrated, given lack of movement, poikilothermia and mechanical ventilation

HD 6-8 (within 1 day of hyponatremia): Cerebral artery spasm, generalized

6. Type 1 vasospasm, coma, declining EEG or BIS, within 24 hours of salt wasting. Self limited.
   - Prophylaxis with fludrocortisone, serum sodium > 145, normal CVP.
   - Prophylaxis with sapropterin (5 mg/kg/day), vitamin C and 0.5 g/kg/day arginine if available
   - Alternatively, nimodipine prophylaxis x 14 days. Reduce nimodipine dose to avoid hypotension.
   - Baseline TCDs on HD 4-HD5, then daily on HD 6-8 and HD13-15
Milwaukee Protocol, version 5.2 (updated 17 april 2017)

HD 5-14: Neurometabolic effects of catabolism; bat/dog rabies-specific complications

7. Progression of rabies in second week correlate with increasing lactic acidosis in CSF, possibly related to metabolism of excipients in IV sedatives or to the immune response by astrocytes or to decreased lactate consumption by neurons
   • Taper sedation aggressively after 7 days, particularly to maintain EEG or BIS activity. Target removal of all sedation by HD 12.

8. Complications of rabies associated with increased branched chain amino acids and glycine
   • Use low-dose insulin (1U/h in adults, 0.025 U/kg/h in children) with sufficient nutrition to maintain euglycemia.

9. Bat rabies: immune-potentiated cerebral edema
   • Monitor serum rabies titers at least twice weekly
   • Monitor by MRI or CT twice weekly in the second or third weeks
   • Methylprednisolone 30 mg/kg/day or dexamethasone 6 mg/kg/day in a 5-day pulse once serological response > 1 IU/ml
   • If patient received rabies vaccine, then risk for cerebral edema is much higher. Administer intravenous immune globulin (IVIG) 1 g/kg over 12-24 hours along with corticosteroid pulse.

10. Dog rabies: third degree conduction block
    • Pacing effective
    • Consider xanthines (adenosine inhibitors). Caffeine base 2.5 mg/kg daily (approximately 1-1.5 cc/kg of expresso coffee).
    • Atropine ineffective after 7 days from vagal denervation
    • CAUTION: Isoproterenol dilates intracranial arteries, increasing ICP (relative contraindication)

11. Diabetes insipidus
    • Tends to be episodic or cyclical. True DI is biphasic, so be prepared.
    • Vasopressin drip, cc/cc replacement over physiological losses; DDAVP may be too long-lasting but is effective.

12. Increased inflammatory markers (CRP, WBC with left shift, high platelets)
    • Confounded by poikilothermia
    • Correlates with detection of rabies antibody in serum
    • Correlates with “ratty appearance” (clearance) by rabies DFA from skin biopsy
    • Empirical use of antibiotics should be limited to 3 days without culture evidence

HD 13-15: Cerebral artery spasm, generalized

13. Type 2 vasospasm (often catastrophic). Ominous when associated with loss of EEG activity, autonomic instability, onset of DI, renal failure. Appears dependent on severity of antecedent Type 1 vasospasm.
   1. Therapeutics unclear. Consider induced hypertension/hypervolemia.
   2. This may be an optimal period for induced hypothermia (days 12-14) as a neuroprotective strategy, because the immune response is already present
Milwaukee Protocol, version 5.2 (updated 17 april 2017)

**HD 15+: Recovery**

Associated either with clinical recovery or progression to death.

14. Death in rabies associated with presumed ketogenesis (acetone > increased CSF isopropanol, presumed via alcohol dehydrogenase).

3. Use low-dose insulin (1U/h in adults or 0.025 U/kg/min in children) with sufficient nutrition to maintain euglycemia.

15. Type 2 vasospasm is followed by pressure-passive, chaotic arterial velocities by TCD. In long term, may see laminar necrosis by MRI.

4. Chaotic TCD flow appears anecdotally improved by use of xanthines (see #10).

16. Futility defined as diabetes insipidus, isoelectric EEG, CSF lactate >4 mM and CSF protein > 250 mg/dl after HD10.
   - It is NOT clear that these criteria still apply with later versions of the protocol using mineralocorticoid and insulin that have pushed survival out further.