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Equine Dysautonomia

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Abstract

Equine dysautonomia (ED, *syn* Equine Grass Sickness) is a neurological disease of unknown etiology which primarily affects grazing horses. The clinical signs reflect degeneration of specific neuronal populations; predominantly within the autonomic and enteric nervous systems, with disease severity and prognosis determined by the extent of neuronal loss. This review is primarily focused on the major clinical decision making processes in relation to ED; namely (a) clinical diagnosis, (b) selection of appropriate ancillary diagnostic tests, (c) obtaining diagnostic confirmation, (d) selection of treatment candidates, and (e) identifying appropriate criteria for euthanasia.

Key Points

- Despite the adopted nomenclature, Equine Dysautonomia (ED; syn Equine Grass Sickness) is a multi-system neuropathy. Although the clinical phenotype is largely reflective of autonomic (including enteric) nervous system dysfunction, neuronal degeneration occurs at other non-autonomic neuroanatomic locations [1].

- ED primarily affects grazing equids and a number of other disease-associated risk factors have been identified.

- The etiology remains undetermined; although the currently favored etiological hypotheses include *Clostridium botulinum* toxicoinfection and mycotoxicosis [2-4].

- Disease phenotype is largely determined by neuronal degeneration in the autonomic and enteric nervous systems and prognosis is largely determined by the extent of neuronal degeneration in the enteric nervous system [3].

- Although disease phenotype is sub-classified as acute (severe), sub-acute (moderate) and chronic (mild), these represent different categories within a continuum of disease severities [5].

- Phenotypic sub-classification is clinically justified as it offers valuable prognostic information; acute and sub-acute cases are invariably fatal while approximately half of chronic cases survive with appropriate nursing care [6].

- The ante-mortem diagnostic approach relies on consideration of disease-associated risk factors, the clinical presentation of the case, exclusion of other differential diagnoses, the adoption of appropriate ancillary diagnostic techniques and, on occasion, histopathological confirmation of enteric neuronal loss [7].

- The post-mortem diagnostic approach relies on histological confirmation of neuronal degeneration/loss within the enteric plexuses and autonomic ganglia [8].

- Following clinical suspicion or diagnostic confirmation of ED, short- and long-term risk avoidance strategies should be considered to minimize disease probability in other grazing equids on the same premises [5]

Introduction

Equine Dysautonomia (ED, *syn* Equine Grass Sickness) is a polyneuropathy affecting both the central and peripheral nervous systems of, almost exclusively, grazing horses. Following the original report of ED in 1907 [9], it has subsequently been recognized throughout most of

Northern Europe [10-19]. Suspected cases have also been reported in the Falkland Islands [20] and Australia [21]. Although North America has largely been considered free of the disease, ED was recently described in a mule in the USA [22]. A clinically and pathologically identical disease, Mal Seco, is well recognized in South America [23-26].

Despite the reported widespread neuroanatomical distribution of degenerative neuronal lesions in ED, the autonomic (ANS) and enteric nervous systems (ENS) remain the most consistently and severely affected regions [1]. The spectrum of clinical signs which define ED largely, but not exclusively, reflect involvement of the ANS and ENS and the severity of disease and gross pathological findings can largely be attributed to the extent of enteric neuronal loss (see *physical examination findings and pathological findings*).

Despite extensive research efforts over the past century, the etiology of ED remains elusive. Current research efforts are primarily focused on the potential role of either *Clostridium botulinum* neurotoxins (via toxicoinfection) [27-29] or ingested pasture-derived mycotoxins [3;4]. The *Clostridium botulinum* hypothesis was extensively investigated during the 1920s [2] and, and has recently received renewed interest. Table 1 summarizes the factors which either support or refute the involvement of *C. botulinum* in ED. In addition to the key association with grazing, the hypothesized role of pasture-derived mycotoxins in ED is largely justified by the seasonality of the disease [30;31] and the geographic and temporal clustering of cases [32], which likely reflect climatic influences on etiological agent exposure [30;33].

Table 1: Factors which support and refute involvement of *C. botulinum* in ED.

Supportive Factors	Refutative Factors
• Reportedly successful historic botulinum vaccine trial (1922 and 1923) [2]	• Disease phenotypic differences between ED and neuroparalytic botulism [4]

• Significantly greater prevalence of intestinal *C. botulinum* bacteria and/or toxin in ED cases versus control animals [27-29]	• Greater prevalence of other (non-*botulinum*) clostridial species in intestinal tract of ED cases *versus* controls (possibly reflecting generalized clostridial overgrowth) [34;35]
• Risk factors supportive of involvement of a soil-borne agent [36;37]	• Neuropathology apparently inconsistent with action of *C. botulinum* neurotoxins [38]
• Inverse association between disease risk and systemic concentration of antibodies against *C. botulinum* bacteria and toxin [39]	• SNARE protein expression in EGS ganglion and enteric neurons inconsistent with action of *C. botulinum* neurotoxins [38]
• Higher mucosal IgA against BoNT/C and D in acute ED cases *versus* control animals [40]	• Lack of evidence of temporal and geographic clustering of ED and neuroparalytic botulism cases

Patient History

Consideration of the history of both the patient and the premises is beneficial in ED diagnosis. Appropriate questioning of the owner will allow the clinician to ascertain which of the published risk factors apply to the case and consider the diagnostic value of any relevant associations. A summary of the various published horse-, premises, management- and climate-level factors associated with either an increased or decreased risk of ED occurrence, or recurrence on a previously affected premises, is presented in Table 2. It is worth noting that, despite a general agreement between studies, occasional inconsistencies exist with regard to some of these reported associations.

Table 2: Factors associated with increased (↑) or decreased (↓) risk of ED occurring (occ), or recurring on the same premise (rec).

Level	Factor	↑ or ↓ risk
Horse-level	Age (2-7 years) [30;31]	↑ (Occ)
	Low serum antibodies to *C. botulinum* type C and BoNT/C [39]	↑ (Occ)
	Good body condition [31]	↑ (Occ)
	Contact with a previous ED case [30]	↓ (Occ)

Premises-level	Previous ED occurrence on premises [30;36]	↑ (Occ)
	High soil nitrogen content [36;41]	↑ (Occ)
	Pasture/soil disturbance [36]	↑ (Occ)
	High number of horses [31]	↑ (Occ)
	Presence of younger animals [37]	↑ (Occ/Rec)
	Stud farms/livery and riding establishments [37]	↑ (Rec)
	Loam and sandy soils [37]	↑ (Rec)
	Rearing of domestic birds [37]	↑ (Rec)
	High herbage iron, lead, arsenic and chromium [41]	Association
	Abundance of Ranunculus spp (buttercups) [41]	Association
	High soil titanium [42]	↑ (Occ)
	Low soil zinc and chromium [42]	↑ (Occ)
	Chalk soil [37]	↓ (Rec)
Management-level	Grazing [30;31]	↑ (Occ)
	Recent movement (especially within preceding 2 weeks) [30;31]	↑ (Occ)
	Change in feed type/quantity within preceding 2 weeks [39]	↑ (Occ)
	Ultimate and penultimate use of an ivermectin-based anthelmintic [39]	↑ (Occ)
	Mechanical removal of feces from pasture [37]	↑ (Rec)
	Manual removal of feces from pasture [37]	↓ (Rec)
	Grass cutting [37]	↓ (Rec)
	Co-grazing with ruminants [37]	↓ (Rec)
	Feeding of supplementary hay/haylage [39]	↓ (Occ)
Climate-level	Recent (within 10-14 days) cool, dry weather and irregular ground frosts [33]	↑ (Occ)
	Increased sun hours and frost days [33;43]	↑ (Occ)
	Higher average maximum temperature [43]	↓ (Occ)

As well as carefully considering the applicability of some of the risk factors in each case, a careful clinical history should also be obtained. Typically, severe (acute) cases of ED will have a sudden onset, with little prior indication of impending disease. In contrast, mild (chronic) cases will typically have an insidious onset, where retrospective consideration will often reveal a more protracted, yet recent change in demeanor, reduction in feed intake and/or weight loss.

Physical Examination

The clinical phenotype of the case is largely determined by the severity of neuronal loss [44]; partly within the ANS, but predominantly within the ENS. Consequently, the clinical presentation of a severely affected case will differ quite markedly from that of a mildly affected case, despite an identical neuroanatomical distribution of lesions. Similarly, physical examination findings and ancillary diagnostic test results derived from ED cases of differing severities can often reveal quite disparate results. Consequently, the terms acute, subacute and chronic are generally applied to reflect severely, moderately and mildly affected cases, respectively; although one should remain aware that these are relatively arbitrary terms applied to a continuum of disease severities, with inevitable overlap between each. However, adoption of this terminology can be clinically beneficial from a prognostic viewpoint. Since a proportion of chronic cases may survive, their differentiation from acute and sub-acute cases, which are invariably fatal, is important. Table 3, which summarizes the clinical signs associated with the acute/sub-acute (combined) and chronic ED sub-categories, highlights the fact that, although certain clinical findings are common to all 3 sub-categories, others are more typical of a specific sub-category. The reader is referred to other publications which cover the clinicopathologic correlates associated with each individual clinical sign in more detail [3;5;45].

Table 3: Clinical signs associated with the 3 sub-categories of ED (acute and sub-acute ED [combined] and chronic ED).

Clinical Sign	Acute/Sub-acute ED	Chronic ED
Dull demeanour	Present – generally more prevalent sign than moderate/severe colic	Present - generally less severe than acute/sub-acute ED
Reduced appetite	Acute - usually profoundly anorexic Sub-acute – variable	Common, but variable severity and often progressive in the early stages of disease
Clinical dehydration	Often apparent in acute ED, less so in sub-acute ED	Mild in some cases, dependent on degree of dysphagia

Tachycardia	Acute: typically 80 - 120 bpm Sub-acute: typically 60 – 80 bpm	Typically 45 – 60 bpm
Bilateral ptosis	Almost invariably present	Almost invariably present
Hypersalivation	Acute: Often marked intermittent drooling evident. No nasal return of saliva Sub-acute: rarely present	Not a feature of chronic ED
Dysphagia	Often profoundly dysphagic, although can be difficult to recognise if anorexic	Varying degrees of dysphagia; valuable prognostic indicator
Rhinitis sicca	Acute: rarely appreciable Sub-acute: may detect slight drying and hyperemia of the nasal mucosa	Variable severity Over time, large casts develop within nasal cavities resulting in stertor Inverse relationship with appetite
Sweating	Varies from patchy to generalised	Patchy sweating May worsen during periods of excitement / anxiety
Abdominal pain	Inconsistently present; if present – usually mild to moderate Not consistent with degree of tachycardia	Usually absent; if present – usually intermittent (e.g. post-prandial) and mild
Intestinal sounds	Often complete absence of intestinal sounds	Usually reduced, rarely absent
Abdominal shape	Occasional abdominal distension due to large intestinal tympany	Rapid development of characteristic "tucked up" abdominal silhouette ("greyhound-like" appearance)
Muscle tremors	Muscle tremors over triceps, flanks and quadriceps	Muscle tremors over triceps, flanks and quadriceps
Rectal temperature	Normal or elevated rectal temperature (up to 40ºC)	Normal or mild elevation in rectal temperature
Stance	Acute: Normal stance Sub-acute: May develop base-narrow stance over a few days	Base-narrow ("elephant on a tub") stance
Other clinical signs	---------	Paraphimosis in entire males (less common in geldings) Oesophageal choke and aspiration pneumonia may develop as secondary complication of dysphagia

Ancillary Diagnostic Tests

A variety of ancillary diagnostic tests can significantly improve the diagnostic accuracy when presented with a suspected case of ED. These can be considered as either (a) reflecting non-specific hematological, biochemical, bacteriological and/or physiological consequences of the disease, (b) reflecting the degree of ANS and/or ENS dysfunction, (c) reflecting other neurological deficits not attributable to ANS/ENS involvement and (d) providing histopathological evidence of neuronal degeneration. These are summarized in Table 4 a-d.

Table 4: Ancillary diagnostic tests which can be utilized in the diagnostic approach to suspected cases of ED.

Diagnostic test objective	Diagnostic test	Comment
Identification of non-specific ED-associated hematological, biochemical, physiological and/or bacteriological abnormalities	Increased hematocrit [46]	Reflective of total body water loss (e.g. small intestinal sequestration [acute ED], neurogenic impairment of drinking)
	Elevated serum urea concentration [46]	Reflective of total body water loss
	Elevate serum acute phase protein concentration [47;48	Reflective of systemic inflammatory response
	Elevated urine specific gravity, protein and creatinine concentration [49]	Reflective of attempted water retention (see above)
	Elevate peritoneal fluid specific gravity and protein concentration [50;51]	
	Fecal *C. perfringens* detection (ELISA) [34]	Considered to reflect non-specific clostridial overgrowth in dysmotile bowel
Identification of ANS and/or ENS dysfunction	Temporary reversal of ptosis following topical application of 0.5% phenylephrine ophthalmic solution to the conjunctival sac [52;53]	Confirms smooth muscle paralysis (Müller's superior tarsal muscle) as the mechanism underlying the ptosis
	Barium swallow study [54]	Retrograde flow reflecting abnormal / uncoordinated esophageal motility

	Esophagoscopy [5]	Evidence of retrograde flow of gastric fluid – either visualized or reflected by the presence of distal esophageal linear ulceration
	Nasogastric intubation [5]	Voluminous foul smelling gastric fluid reflux, potentially reflective of severe gastrointestinal ileus (acute ED)
	Abdominal ultrasonography [5]	Distended small intestinal loops visible, potentially reflective of generalized gastrointestinal ileus (acute ED)
	Transrectal palpation [5]	Distended small intestinal loops palpable, potentially reflective of generalized gastrointestinal ileus (acute ED) Firm corrugated colonic and cecal contents, potentially reflective of large intestinal ileus and desiccation of fibrous ingesta
Identification of non-ANS/ENS neurological dysfunction	Electromyography [55]	Reported findings considered consistent with skeletal muscle neuropathy
Histopathological evidence of neuropathology at appropriate anatomical sites	Histopathology of ileal biopsy (standard H&E staining) [7]	Evidence of neuronal loss and chromatolysis within myenteric and submucous plexuses Formalin fixed – 100% sensitive and specific [56] Cryostat sections – sub-optimal (73%) specificity (false positives resulting from processing artefacts)[56]
	Histopathology of ileal biopsy (synaptophysin immunostaining) [57]	Combined assessment of neuronal density and intensity of synaptophysin immunostaining – reported 100% sensitivity and specificity
	Histopathology of rectal biopsy (standard H&E staining) [58]	Poor sensitivity (21%) when applied to partial thickness biopsies due to low neuronal density [59]
	Histopathology of rectal biopsy (BAPP immunostaining) (R. Jago *pers. comm.*)	Retrospective application of this approach yielded 100% sensitivity and specificity Requires prospective validation
	Histopathology of gustatory papillae [60]	Retrospective application of this approach yielded 100% sensitivity and high specificity (98%) Requires prospective validation

Ancillary diagnostic tests can also be applied to specifically exclude other differential diagnoses. With regard to chronic ED, such tests may focus on the elimination of other diseases which present with rapid weight loss. With regard to acute ED cases, which present with small intestinal ileus and gastric reflux, the principal aim is to rule out other conditions with a similar presentation; namely strangulating and non-strangulating small intestinal obstructive lesions. Therefore, when presented with acute ED cases, there is a greater degree of investigative urgency to enable identification of other conditions which may require immediate surgical intervention. Consequently, exploratory laparotomy may be indicated in some acute ED cases, the findings of which will determine the need for further diagnostic approaches.

Given that acute ED carries a hopeless prognosis for survival, the clinician must decide whether the absence of a surgically correctable lesion at laparotomy is sufficient grounds for euthanasia on the basis of ruling out physical obstructions of the small intestine and failing to provide any beneficial surgical intervention. Alternatively, more confirmatory diagnostic evidence may be warranted before making this decision. Although histopathological examination of an appropriately sized formalin-fixed ileal biopsy is 100% diagnostically sensitive and specific [56], this requires a sufficient period of time for tissue fixing, thus necessitating recovery from anesthesia pending results of the examination. Unfortunately, histopathological examination of cryostat sections is not 100% specific (due to processing artefacts), thus risking an erroneous diagnosis and potentially an inappropriate euthanasia [56].

Post-mortem diagnostic confirmation

Post-mortem diagnostic confirmation is warranted given the potential risk to other grazing horses on the premises. Implementing short- and long-term risk avoidance strategies (see later) can be expensive and labor intensive and understandably more justifiable when ED is confirmed. Gross pathological findings generally reflect the extent of involvement of the ENS. For example, fluid distension of the small intestine and stomach and linear ulceration of the distal esophagus are typical findings in acute ED, whereas sub-acute cases will have firm corrugated impactions of the large colon and cecum, typically with a black coating on the surface of the firm ingesta [3;5]. Chronic ED cases are typically emaciated with scant intestinal contents, depending on the appetite and degree of dysphagia at the time of death [3;5]. Bilateral *rhinitis sicca* is commonly seen in chronic ED.

A definitive diagnosis is achieved by histopathological examination of autonomic (prevertebral and paravertebral) ganglia and intestinal ENS plexuses (see before) [61;62]. Autonomic ganglia most routinely collected for histopathological examination include the cranial cervical ganglia, the cranial and caudal mesenteric ganglia and the abdominal and thoracic sympathetic chain.

Typical histological features include chromatolysis with loss of Nissl substance, eccentricity or pyknosis of the nuclei, neuronal swelling and vacuolation, accumulation of intracytoplasmic eosinophilic spheroids and axonal dystrophy [8;61-63].

Treatment of chronic ED; selection of candidates

As previously stated, the majority of ED cases have a hopeless prognosis. Overall ED is associated with an approximately 80% mortality; comprising of 100% mortality in acute and sub-acute ED and approximately 50% mortality in chronic ED [6;64]. Therefore, the selection of

candidates for treatment relies firstly on sub-categorizing the case (see Table 2) and secondly, if the signs are consistent with chronic ED, selecting the appropriate cases for which treatment is indicated. Acute and sub-acute ED cases are invariably fatal [3]; however, supportive care (not covered in this review) in the form of intravenous fluid infusion, analgesic administration and regular gastric decompression may be initiated to stabilize the patient until a more definitive diagnosis is achieved, at which point euthanasia is indicated.

The criteria used for the selection of appropriate chronic cases are relatively arbitrary; however, the following list is a useful guide:

- Ability to swallow, albeit compromised

- Some remaining appetite, albeit markedly reduced

- Absence of continuous moderate to severe abdominal pain (rarely will chronic ED cases exhibit severe colic)

Additionally, in light of the prolonged period of convalescence required in many cases [6;64], an appropriate level of commitment should be declared by the owner; either time commitment if they opt for home nursing or financial commitment if they opt to refer to a clinical facility for nursing care.

Non-pharmacologic Treatment Options for chronic ED

Treatment of chronic ED is predominantly intensive nursing care. Assuming the above selection criteria are met, the greatest obstacle to survival is typically profound inappetance [3]. Consequently, the main aim of the nursing care is to attain an adequate level of voluntary feed

intake. This generally involves the provision of a selection of highly palatable feeds (ideally high in energy and protein). As individual preferences change regularly, it is often necessary to offer a variety of feed components and consistencies [5;65]. Although the addition of succulents (e.g. carrots, apples) to the diet may improve voluntary feed intake, these alone are insufficient to meet the animal's energy and protein requirements. Feeding small amounts regularly reduces waste and is more likely to result in a greater overall feed intake over a 24h period. At the authors' institute, newly hospitalized chronic cases receive 2 hourly small feeds, reducing to 4 hourly feeds after approximately 1 week of hospitalization. Both continuous flow enteral feeding and total and partial parenteral nutrition has been used in selected cases, with little evidence of a positive influence on case outcome [66]. However, via a reduction in the initial rate of weight loss, their use may extend the period of time available for a spontaneous improvement in appetite to occur. Additional non-pharmacologic approaches include regular hand grazing and light exercise in the form of in-hand walking and/or short periods of turn out to pasture.

Pharmacologic Treatment Options for chronic ED

Pharmacological treatment options in chronic ED can be sub-categorized as follows:

Appetite stimulants: unfortunately, attempts to pharmacologically improve the appetite of chronic ED cases have been unrewarding, as determined by both anecdotal evidence and placebo controlled trials [67]. Evaluated drugs include glucocorticoids, brotizalam, diazepam and cyproheptadine.

Pro-kinetics: Administration of the pro-kinetic drug cisapride has been shown to increase dry matter intake and decrease transit time [68]. However, as the principal reason for treatment failure is a lack of voluntary feed intake and not intestinal dysmotility, the use of such motility

enhancing drugs is rarely indicated. Furthermore, by definition, chronic cases have sufficiently coordinated intestinal motility to maintain an aboral flow of ingesta.

Analgesics: The judicious use of non-steroidal anti-inflammatory drugs is often beneficial. The analgesic properties of these drugs can be useful to combat low grade, intermittent (often post-prandial) abdominal pain. Furthermore, as ED cases have evidence of low grade systemic inflammation (e.g. elevated acute phase proteins) [47;48], the anti-inflammatory properties of this class of drugs is theoretically beneficial. Appropriate NSAID therapeutic options includes flunixin meglumine (0.5mg/kg PO or IV BID) or phenylbutazone (2.2mg/kg PO or IV BID).

Antimicrobials: The dysphagia present in most chronic ED cases may result in varying degrees of feed aspiration. In such cases, broad spectrum antimicrobial administration is indicated to minimize the development of inhalation pneumonia. Commonly adopted empirical antimicrobial treatments include oxytetracycline (10mg/kg IV SID), combined gentamicin (6.6mg/kg IV SID) and procaine penicillin (12mg/kg IM BID) and trimethoprim sulphonamide (15-30mg/kg PO BID). Occasionally, metronidazole (20mg/kg PO TID) may also be indicated when anaerobic infection is suspected.

Treatment Complications and Prognostic Indicators

Although it can be difficult to predict the response to treatment/nursing early in the disease process, a number of factors can be used as potential prognostic indicators. These are itemized below:

Appetite: Although the voluntary feed intake of cases can fluctuate markedly over a 24h or 48h period, persistence of complete anorexia for several (e.g. 5) consecutive days generally is a poor prognostic indicator, provided that several feed options have been offered. Persistent anorexia

and associated weight loss remain the principal reasons for euthanasia in cases receiving nursing care.

Magnitude and rate of weight loss: A recent retrospective study of hospitalized chronic ED cases demonstrated the prognostic value of considering the magnitude and rate of weight loss over specific periods of treatment. Survival prediction curves have the potential to offer useful prospective prognostic information on individual cases [64].

Rhinitis sicca: Studies have identified an inverse association between survival and the severity of rhinitis sicca [69]. It is hypothesized that severe rhinitis sicca will directly and negatively influence appetite, potentially via an effect on olfaction.

Inhalation pneumonia: Some chronic ED cases with severe dysphagia may develop inhalation pneumonia as a consequence of feed aspiration. It is worth noting that coughing appears to be a poor indicator of this complication; rather, cases become pyrexic and increasingly dull and inappetant. Consequently, recognition of this complication is often dependent on regular ultrasonographic examinations of the cranioventral thorax, assessing for the presence of lung consolidation and occasionally pleural fluid accumulation.

Fulminant diarrhea: Severe diarrhea has been recognized in a proportion of chronic ED cases, usually those receiving supplementary liquid enteral feeding via a nasogastric tube [66]. This is a recognized complication of enteral feeding in horses, thought to partly reflect the low fiber content of commercially available liquid feeds [70]. However, this complication has also been recorded in cases receiving alfalfa slurries, whereby a bacterial overgrowth in the cecum and large colon was suspected. Consequently, when adopted in the treatment of ED cases, enteral feeding should be introduced gradually and the liquid feed should contain as high a fiber content as feasible.

Long-term complications

Clinical recovery is generally assumed if cases regain appetite and body weight. Although most recovered cases will return to their previous level of performance, some residual problems have been reported. These include poor exercise tolerance, persistent low grade dysphagia, recurrent esophageal obstruction, occasional mild colic episodes and coat changes (piloerection at the site of previous sweating) [6;65;71]. Considering that even clinically recovered cases have evidence of extensive neuronal loss in the ENS, it has been hypothesized that clinical recovery may partly be associated with compensatory mechanisms which have restored and maintained an adequate degree of intestinal motility [72]. Interstitial cells of Cajal have been proposed as compensatory cellular candidates.

Summary

ED is a multi-system neuropathy of equids characterized by damage to autonomic, enteric and somatic neurons. It has an extremely high mortality rate and significant welfare, emotional and financial consequences. The etiology is unknown.

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