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Tramboo SR
Division of Veterinary Parasitology, FVSc & AH, SKUAST-K, Shuhama (Alusteng), Srinagar, Jammu and Kashmir, India

Shahardar RA
Division of Veterinary Parasitology, FVSc & AH, SKUAST-K, Shuhama (Alusteng), Srinagar, Jammu and Kashmir, India

Allaie IM
Division of Veterinary Parasitology, FVSc & AH, SKUAST-K, Shuhama (Alusteng), Srinagar, Jammu and Kashmir, India

Bulbul KH
Division of Veterinary Parasitology, FVSc & AH, SKUAST-K, Shuhama (Alusteng), Srinagar, Jammu and Kashmir, India

Khan AA
Division of Livestock Production and Management, FVSc & AH, SKUAST-K, Shuhama (Alusteng), Srinagar, Jammu and Kashmir, India

Mir MS
Division of Veterinary Pathology, FVSc & AH, SKUAST-K, Shuhama (Alusteng), Srinagar, Jammu and Kashmir, India

Hussain I
Division of Veterinary Biochemistry, FVSc & AH, SKUAST-K, Shuhama (Alusteng), Srinagar, Jammu and Kashmir, India

Rather MA
Division of Veterinary Public Health, FVSc & AH, SKUAST-K, Shuhama (Alusteng), Srinagar, Jammu and Kashmir, India

Ahanger AA
Division of Veterinary Pharmacology and Toxicology, FVSc & AH, SKUAST-K, Shuhama (Alusteng), Srinagar, Jammu and Kashmir, India

Corresponding Author:
Allaie IM
Division of Veterinary Parasitology, FVSc & AH, SKUAST-K, Shuhama (Alusteng), Srinagar, Jammu and Kashmir, India

Prevalence of ectoparasites in equines of Kashmir valley

Tramboo SR, Shahardar RA, Allaie IM, Bulbul KH, khan AA, Mir MS, Hussain I, Rather MA and Ahanger AA

Abstract
In a study on prevalence of ectoparasites in equines, a total of 2008 equines (1288 males and 720 females), aged >3 years and <3 years were examined from different areas of Kashmir valley. Out of 2008 equines examined for ectoparasites, 423 (21.07%) were found positive for *Haemaphysalis bispinosa* and 347 (17.28%) were found positive for *Damalinia equi*. The prevalence of ticks was found to be highest on ponies (22.17%) followed by 20.85 % on mules, 12.5% on horses and 9.68% on donkeys. The prevalence of lice was also found to be highest on ponies (19.55%) followed by 10.00% on horses, 8.06% on mules and 0.00% on donkeys. Highest infection with ticks was encountered in summer season (41.93%) followed by 22.80% in spring, 19.00% in autumn and 0.20% in winter. The seasonal occurrence of lice on equines revealed highest infestation rate of 41.80% in winter followed by 20.60% in autumn, 4.40% in spring and 2.56% in summer. Average number of ticks found on donkeys was 4 followed by ponies (2.72), horses (2.5) and mules (1.98). Similarly, average number of lice found on ponies was 20.14 followed by horses (19.06) and mules (18.88). However, donkeys were not found to be infected with lice. Overall prevalence of ticks and lice was found higher on young equines (28.00% and 20.09%, respectively) as compared to adult equines (18.96% and 16.43%, respectively). Overall prevalence of ticks and lice was found to be higher on female equines (27.64% and 26.39%, respectively) as compared to male equines (17.39% and 12.19%, respectively). A total of two species of flies collected comprised of *Tabanus* spp. and *Chrysomyia* spp. *Gasterophilus intestinalis* eggs were also collected from ponies in Pahalgam area and the maggots belonging to *Chrysomyia* spp. were recovered from an injured pony in Srinagar.

Keywords: Ectoparasites, equines, Kashmir

Introduction
Equines which have been domesticated since 5000 years [1] have been used by humans in many different ways for travel, work, food and pleasure. They are used for riding and transport, carrying things or pulling carts, or to help plough farmer's fields in agriculture. Today, horses are mostly used for entertainment and sports. Horse/Pony riding in Kashmir is one of the best ways to explore the beautiful countryside and mountain passes of Kashmir. For trekking, these horses/ponies can be used for carrying luggage and trekking equipment up to the highlands of Kashmir. The tourist resorts of Gulmarg, Sonamarg, Doodhpathri, Pahalgam and Yousmarg in Kashmir are dominated with good horses/ponies for rides [2].
Like other parasitic diseases, ectoparasites of equines *viz* lice, flies, ticks and mites continue to cause a significant threat to the health of equines [3, 4]. They cause decreased performance, unthriftness and some can even help in proliferating the life cycle of some internal parasites [5]. Many of these ectoparasites (e.g. lice) are host specific where others (e.g. ticks) parasitize a wide range of hosts. The irritation caused by lice results in restlessness, improper feeding and self inflicted wounds. Among flies, the old world screw-worm fly, *Chrysomya bezziana* deposits eggs in the wounds, resulting from accidents which develop into maggots. The maggots penetrate into tissues, which they liquify and extend the lesion considerably. If not treated properly, death from screwworm infection is frequent. The adult flies of *Gasterophilus* spp. cause great annoyance due to their egg laying activity than their larvae inside the stomach (Gastric myiasis). Similarly, the bites of tabanid flies are painful and irritating and may give rise to weals in addition to transmitting other infectious agents including *Trypanosoma evansi*. Two general groups of ticks that attack equines are hard ticks and soft ticks. Hard ticks have long association with the host, feed slowly, take a large blood meal and transmit various in

fectious agents. The mites and their developmental stages cause lesions which are intensely pruritic and covered with thick scabs or crusts. Although the prevalence of ectoparasites in the general population is usually low, but can be high in vulnerable groups. Scientific knowledge on how to deal best with parasitic skin diseases in different settings is scanty, and evidence-based measures for control are not available [6]. Keeping all the factors in mind, the present study was planned to work out the prevalence of ectoparasites in equines of Kashmir valley for development of suitable control measures against them.

Materials and Methods

Study area: This study was conducted on locally reared equines of Kashmir valley at major tourist destinations *viz.* Pahalgam, Gulmarg and Sonamarg from March 2017 to February 2018. Pahalgam, located in south Kashmir is nearly 90 kms from Srinagar. It is a famous tourist town located at 34.01°N 75.19°E with temperate climate having long, cold winters with heavy snowfall and short and mild summers. Gulmarg, located in north Kashmir is a popular skiing destination in north Kashmir and is nearly 56 kms from Srinagar and is located at 34°15'N 74°25'E. It has a humid continental climate where the wet winter season sees heavy snowfall but summer is mild and pleasant. Sonmarg, also known as meadow of Gold is a hill station in central Kashmir about 80 km north-east of Srinagar located at 34.40°N 74.71°E. This area also receives heavy snowfall during winter months but summer is mild and pleasant [2].

Method of collection: A total of 2008 animals were examined for ectoparasites like ticks, lice and other periodic parasites, which were collected from various body parts and later on examined for identification in the Entomology laboratory of the Division. The lice were collected carefully by blunt forceps and ticks were detached carefully to avoid decapitation and bottled along with a label denoting the identity of the host, centre of collection, site of infestation, date of collection and sample number. The intensity of tick and lice infestation was carried out by counting number of ticks and lice / number of animals infested. Periodic parasites like flies were collected using fly net and the captured flies were transported to the laboratory in glass vials containing 30% ethanol. Fly larvae, extracted from wounds were also placed into a liquid preservative (30% ethanol and 5% glycerine) as quickly as possible after collection till transportation to the Entomology laboratory [7].

Fixation, preservation and examination: The ticks were fixed in 30% ethanol to which few drops of solvent ether was added, which facilitated the killing of ticks with their legs in stretched condition [8] and preserved in a mixture of 70% alcohol and glycerine (90ml of 70% alcohol + 10 ml glycerine). They were examined in laboratory grossly under stereozoom and dissection microscope. Permanent mounts were made by first washing the preserved specimen in tap water, then treating with 10% potassium hydroxide for twelve hours followed by washing in tap water, dehydrating in ascending grades of acetic acid or ethanol, clearing in xylene and mounting in Canada balsam or DPX mountant. Lice and their nits were brought to the laboratory in live condition along with some hairs from animal body in glass vials. In the laboratory, they were fixed in 30% ethanol to which few drops of solvent ether were added and permanently mounted on a microscopic slide. For a lice to be mounted, they were first dehydrated in ascending grades of alcohol i.e, 30%, 50%, 70%, 90% and absolute alcohol for at least 20-30 minutes each and then mounted in Canada balsam or DPX mountant for more detailed study [7]. Permanent mounts of different parts of flies were prepared in the laboratory in same way as in case of ticks. The wings, antennae, mouthparts and legs in case of flies, anterior spiracles and posterior spiracles in case of larvae were dissected under stereomicroscope after boiling in 10% KOH with the help of dissecting needle and examined under 10X, 40X for further structural details.

Statistical analysis: The results were subjected to standard statistical analysis as per Snedecor and Cochran [9]. The data on the prevalence between different groups was analyzed using 'z' test of proportions.

Results and Discussion

Out of 2008 equines examined for ectoparasites, 423 (21.07%) were found positive for ticks (Table 1). Our results are almost in agreement with findings of Soundararajan *et al.* [10] who reported an overall incidence of tick infestation in equines as 13.33% in Tamil Nadu but vary with Katoch *et al.* [11] who reported the overall incidence of tick infestation in equines as 61.05% in R.S. Pura, Jammu; Duell *et al.* [12] who reported 85% prevalence of ticks on equines in Central Oklahoma and Kumsa *et al.*[13] who recorded an overall prevalence of 39.04% of tick infestation on horses in central Oromia, Ethiopia. The tick identified in our study was found to be *Haemaphysalis bispinosa* (Fig.1), which is in agreement with the observation made by Greay *et al.* [14] who observed *H. longicornis* to be the most common species present on horses in Australia. The prevalence of ticks was found to be highest in ponies (22.17%) followed by mules (20.85%), horses (12.5%) and donkeys (9.68%), with non-significant statistical variation ($P>0.05$) between ponies, mules, horses and donkeys. In our study, 347 (17.28%) samples were found positive for lice (Table 1), which is in line with the studies of Tafese *et al.* [15] who found 18.3% prevalence of lice in equines of Modjo district in Central Oromia, however, varied from Payne *et al.* [16] who reported 5% prevalence of lice in equines in the western highlands of Cameroon and Egri *et al.* [17] who reported 100.00 % prevalence of lice in equines of Russia. The lice identified in our study was found to be *Damalinia equi* (Fig.1) and its prevalence was found to be highest in ponies (19.55%) followed by horses (10.00%), mules (8.06%) and donkeys (0.00%), with significant statistical variation ($P<0.05$) between ponies, mules and horses, but non-significant statistical variation ($P>0.05$) between donkeys and mules. Intensity of infection in our study was very low. Average number of ticks found on donkeys was 4 followed by ponies (2.72), horses (2.5) and mules (1.98) (Table 2). Our results are in line with Seo *et al.* [18] who reported the intensity of 4-5 ticks per horse in South Korea. Similarly, average number of lice found on ponies was 20.14 followed by horses (19.06) and mules (18.88). However, donkeys were not found to be infected with lice (Table 2). Highest infection with ticks was encountered in summer season (41.93%) followed by 22.80% in spring season, 19.00% in autumn season and 0.20% in winter season (Table 1). The highest prevalence rate encountered in summer in the present study can be due to the fact that in summer the environmental conditions like temperature and humidity are favourable for their multiplication. Ponies revealed an overall

43.54% prevalence of ticks in summer followed by 23.13% in spring, 22.22% in autumn and 0.25% in winter. Mules showed an overall 40.32% in summer followed by 27.74% in spring and 9.52% in autumn. Horses showed an overall prevalence of 30.23% in summer followed by 28.57% in spring and 2.00% in autumn. Donkeys revealed an overall 37.50% in summer only. The overall seasonal occurrence of lice in equines revealed highest infection rate of 41.80% in winter season followed by 20.60% in autumn season, 4.40% in spring season and 2.56% in summer season. The highest prevalence rate in winter can be due to the fact that in winter, the hair coat of animals is thick which provides optimal conditions for multiplication of lice and they can easily spread to other animals due to close contact during confinement. Ponies revealed an overall 46.03% prevalence of lice in winter followed by 23.46% in autumn, 5.22% in spring and 3.04% in summer. Mules showed an overall 31.58% in winter followed by 7.14% in autumn, 1.61% in summer and 1.45% in spring. Horses showed an overall prevalence of 23.91% in winter followed by 10.00% in autumn. Lice was not found in donkeys in any of the seasons.

Table 1: Overall and Seasonal Prevalence of ticks and lice in equines of Kashmir valley

Season	Type	No. of samples	Ticks	Lice
Spring	Pony	402	93 (23.13)	21 (5.22)
	Mule	69	15 (27.74)	1 (1.45)
	Horse	21	6 (28.57)	0 (0.00)
	Donkey	8	0 (0.00)	0 (0.00)
	Total	500	114 (22.80)^{C}	22 (4.40)^{A}
Summer	Pony	395	172 (43.54)	12 (3.04)
	Mule	62	25 (40.32)	1 (1.61)
	Horse	43	13 (30.23)	0 (0.00)
	Donkey	8	3 (37.50)	0 (0.00)
	Total	508	213 (41.93)^{D}	13 (2.56)^{A}
Autumn	Pony	405	90 (22.22)^{b}	95 (23.46)^{b}
	Mule	42	4 (9.52)^{a}	3 (7.14)^{a}
	Horse	50	1 (2.00)^{a}	5 (10.00)^{a}
	Donkey	3	0 (0.00)^{a}	0 (0.00)^{a}
	Total	500	95 (19.00)^{B}	103 (20.60)^{B}
Winter	Pony	404	1 (0.25)	186 (46.03)^{b}
	Mule	38	0 (0.00)	12 (31.58)^{a}
	Horse	46	0 (0.00)	11 (23.91)^{a}
	Donkey	12	0(0.00)	0 (0.00)^{a}
	Total	500	1 (0.20)^{A}	209 (41.80)^{B}
Grand Total		2008	423 (21.07)	347 (17.28)

Note: Percent values (in parenthesis) of prevalence across different hosts in a particular season that bear different small case superscript differ significantly. Percent values (in parenthesis) of overall prevalence in different seasons for a particular parasite/group of parasites that bear different upper case superscript differ significantly

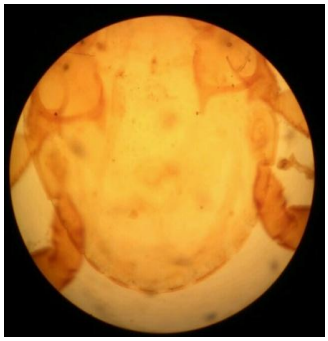
Table 2: Intensity of infestation of ectoparasites on equines

S. No	Species	Average number of ticks/animal	Average number of lice/animal
1.	Pony	2.72 (1-5)	20.14 (1-55)
2.	Mule	1.98 (1-5)	18.88 (1-35)
3.	Horse	2.50 (1-5)	19.06 (1-35)
4.	Donkey	4.00 (1-6)	0.00 (0)

Note: Intensity of infestation= Number of ticks or lice / number of equines infested

Overall prevalence of ticks was found to be significantly *(P*<0.05) higher in young equines (28%) as compared to adult equines (18.96%) and that of lice was found to be non-significantly (*P*>0.05) higher in young equines (20.09%) as compared to adult equines (16.43%) (Table 3). The prevalence of ticks was found significantly (*P*<0.05) higher in young ponies (30.53%) as compared to adult ponies (19.46%), non-significantly (*P*>0.05) higher in adult mules (21.43%), horses (13.28%) and donkeys (9.68%) as compared to young mules (18.60%), horses (9.38%) and donkeys (0.00%). The prevalence of lice was found non- significantly (*P*>0.05) higher in young ponies (21.37%), mules (13.95%) and horses (12.5%) as compared to adult ponies (18.96%), mules (6.55%) and horses (9.37%). Donkeys were found to be negative. Overall prevalence of ticks and lice was found to be significantly (*P*<0.05) higher in female equines (27.64% and 26.39%, respectively) as compared to male equines (17.39% and 12.19%, respectively) (Table 3). The prevalence of ticks was found significantly (*P*<0.05) higher in female ponies (30.25%) as compared to male ponies (17.82%), non-significantly (*P*>0.05) higher in female mules (25%), male horses (14.29%) and female donkeys (12.5%) as compared to male mules (17.89%), female horses (9.68%) and male donkeys (8.70%). The prevalence of lice was found significantly (*P*<0.05) higher in female ponies (29.89%) and horses (20.97%) as compared to male ponies (13.98%) and horses (3.06%) and non-significantly (*P*>0.05) higher in female mules (10.23%) as compared to male mules (6.5%). Donkeys were found to be negative.

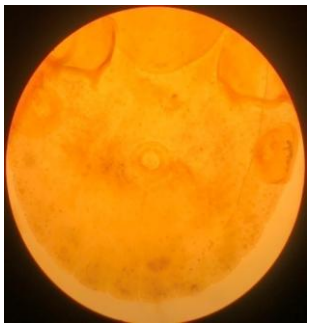
Anterior End



Posterior End

***Haemaphysalis* spp. (Male)**

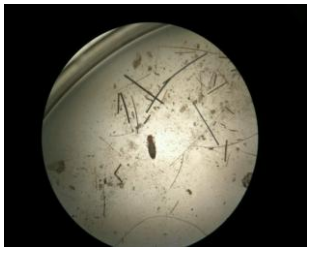
Anterior End



***Haemaphysalis* spp. (Female)**

Posterior End
***Haemaphysalis* spp. (Female)**

Damalinia equi



Adult *Damalinia* louse with egg cemented to the hair

Fig 1: Ticks (*Haemaphysalis* spp.) and lice (*Damalinia equi*) on microscopic examination

Table 3: Age and Sex wise prevalence of ticks and lice on equines of Kashmir valley

Type		No. of samples	Ticks	Lice
Pony	Adult	1213	236 (19.46)[a]	230 (18.96)
	Young	393	120 (30.53)[b]	84 (21.37)
	Total	1606	356 (22.17)	314 (19.55)[C]
	Male	1044	186 (17.82)[a]	146 (13.98)[a]
	Female	562	170 (30.25)[b]	168 (29.89)[b]
Mule	Adult	168	36 (21.43)	11 (6.55)
	Young	43	8 (18.60)	6 (13.95)
	Total	211	44 (20.85)	17 (8.06)[A]
	Male	123	22 (17.89)	8 (6.50)
	Female	88	22 (25.00)	9 (10.23)
Horse	Adult	128	17 (13.28)	12 (9.37)
	Young	32	3 (9.38)	4 (12.50)
	Total	160	20 (12.50)	16 (10.00)[B]
	Male	98	14 (14.29)	3 (3.06)[a]
	Female	62	6 (9.68)	13 (20.97)[b]
Donkey	Adult	31	3 (9.68)	0 (0.00)
	Young	0	0(0.00)	0 (0.00)
	Total	31	3 (9.68)	0 (0.00)[A]
	Male	23	2 (8.70)	0 (0.00)
	Female	8	1 (12.50)	0 (0.00)
Total	Adult Equines	1540	292 (18.96)[a]	253 (16.43)
	Young Equines	468	131 (28.00)[b]	94 (20.09)
	Male Equines	1288	224 (17.39)[a]	157 (12.19)[a]
	Female Equines	720	199 (27.64)[b]	190 (26.39)[b]

Note: Host wise and total prevalence between adult and young equines bearing different small case superscript differ significantly. Overall prevalence values (all ages) for different hosts bearing different upper case superscripts differ significantly. Host wise and total prevalence between male and female equines bearing different small case superscript differ significantly. Overall prevalence values (sexes combined) for different hosts bearing different upper case superscripts differ significantly.

A total of two species of flies were collected in the present study comprising of *Tabanus* spp. and *Chrysomyia* spp. (Fig 2). In south-western Nigeria, Adeyefa and Dipeolu [19] recorded flies like *Stomoxys, Tabanus, Glossina* and *Hippobosca* spp. on horses. A number of such studies are on record such as Barros *et al.* [20] who collected tabanids on horses in the Nhecolândia, Pantanal State of Mato Grosso do Sul, Brazil; Krčmar [21] who collected 13,439 specimens of horse flies in Eastern Croatia, where, most of the species belonged to the genus *Tabanus*, followed by the genera *Chrysops*; Zeghouma *et al.* [22] who collected horse flies belonging to two subfamilies i.e. *Chrysopsinae* and *Tabaninae* in El Tarf Province of northeastern Algeria and Suh *et al.* [23] who collected a total of 2,999 horse and deer flies (tabanids) in Korea, where, *Chrysops mlokosiewiczi* was the most frequently collected species.

***Tabanus* spp.**

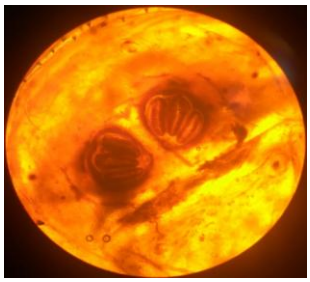
Antenna of *Tabanus*

***Chrysomyia* spp.**

Fig 2: *Tabanus* spp. and *Chrysomyia* spp. collected from equines of Kashmir valley

Gasterophilus intestinalis eggs were collected from hairs of forelimbs and shoulder region of ten ponies in Pahalgam area and larvae of the same species were collected from the stomach of a dead pony in the Baltal area of Sonamarg during postmortem examination (Fig 3). Agneessens *et al.* [24] reported *Gasterophilus intestinalis* in 58% of horse stomachs in Belgium and Studzińska and Wojcieszak [25] reported the prevalence of nearly 15% gasterophilosis in horses from the south-eastern part of Poland. Pandey *et al.* [26] who examined ninety four horses from the Settat region of Morocco for *Gasterophilus intestinalis* and *G. nasalis* larvae, found all the horses to be infected with *G. intestinalis* and Edwards *et al.* [27] examined the stomachs of 448 horses for *Gasterophilus intestinalis* larvae and found 237 (52.7%) to be infected with *G. intestinalis* in northern England and Wales. Kibebe and Hagos [28] recovered *Gasterophilus* spp. larvae from 50% of euthanised horses in Ethiopia.

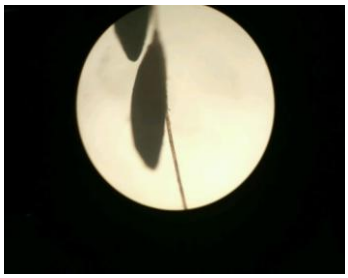
Screw worm fly (*Chrysomyia bezziana*) maggot



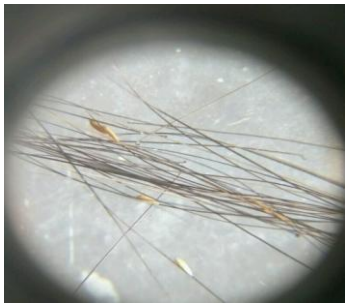
Posterior stigmal plate of *Chrysomyia bezziana* larva

***Gasterophilus* larva**

***Gasterophilus* spp. eggs attached to the body hairs**



***Gasterophilus* spp. eggs attached to the body hair**



***Gasterophilus* spp. eggs attached to the body hairs**

Fig 3: *Chrysomyia bezziana* maggot, *Gasterophilus* larva and its Eggs

An extensive procedure was also carried out in the present study with removal of at least 300 to 400 maggots from the wound of an abandoned pony on Nala Maar Road in Kawdara area of Srinagar city, which were found belonging to screw-worm fly of the genus *Chrysomyia* based on presence of band of spines on each segment (Fig 3). These flies deposit their eggs in wounds resulting from accidents etc. which develop into maggots. The maggots penetrate into the tissues, which they liquefy and extend the lesion considerably. The wound develops an evil odour and a foul-smelling liquid oozes out [29].

Conclusion

In the present study, out of 2008 equines examined in different areas of Kashmir valley, 21.07% and 17.28% were found to be infected with ticks and lice, respectively. The only species of tick found was *Haemaphysalis bispinosa* and the only species of lice found was *Damalinia equi* with very low intensity of infection. The incidence of infection with both ticks and lice was found to be more in females and young equines as compared to males and adult equines. The incidence of ticks was found to be highest in summer while those of lice in winter. The two species of flies collected from body of equines were *Tabanus* and *Chrysomyia* spp. The eggs of *Gasterophilus* spp. were collected from hairs of forelimbs and shoulders of ponies from Pahalgam area of Kashmir and larvae of *Gasterophilus intestinalis* were recovered from stomach of a dead pony during post-mortem examination. In addition to this, 300-400 larvae of *Chrysomya bezziana* were recovered from wound of an abandoned pony.

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