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Short Paper

Clinical, bacteriological and histopathological aspects of first-time pyoderma in a population of Iranian domestic dogs: a retrospective study

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Abstract

Background: Staphylococci are the most common cause of pyoderma in dogs. **Aims:** The purpose of the present study was to investigate clinical, bacteriological and histopathological aspects of bacterial skin infections in a population of Iranian domestic dogs with first-time pyoderma. **Methods:** The study animals were 61 clinical cases of Iranian domestic dogs with first-time pyoderma. The diagnosis of pyoderma was based on the history, the presence of variable gross cutaneous lesions, positive findings on microscopic examination of surface cytology and histopathological findings. **Results:** Detection of pyoderma amongst adult dogs was significantly higher than puppies (P=0.001). Large breed dogs were presented more frequently for pyoderma in comparison to small breeds (P=0.002). Bacterial species were recovered from 43 of the 61 (70.49%) studied animals. No isolates were recovered from 18 studied dogs. The most frequently recovered bacterial genus was *Staphylococcus* (32/43 isolates, 74.41%) including: *S. epidermidis* (22/43 isolates, 51.16%), *S. aureus* (7/43 isolates, 16.27%), and *S. pseudintermedius* (3/43 isolates, 6.97%). Staphylococci species resistance was most commonly seen against amoxicillin (94.11%), penicillin (83.35%), and ampicillin (76.47%). Resistant to cephalexin and cefoxitin was 5.88% and 2.94%, respectively. A total of 27 of the staphylococci isolated (84.37%) were resistant to at least one antimicrobial agent and 19 isolates (59.37%) were resistant to three or more antimicrobial drugs. **Conclusion:** A better understanding of this microbial population is critical for clarification of the pathophysiology of bacterial skin diseases.

Key words: Dog, Pyoderma, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus pseudintermedius*

Introduction

Staphylococci are the most common cause of bacterial skin infection in humans and dogs (von Eiff *et al*., 2002; Pinchbeck *et al*., 2006). They can be differentiated by their ability to produce coagulase, with coagulase positive staphylococci characterized more pathogenic than coagulase negative species (von Eiff *et al*., 2002; Devriese *et al*., 2005; Rich, 2005). In cases of canine pyoderma the most commonly isolated coagulase-positive staphylococci are *Staphylococcus pseudinter-medius*, *Staphylococcus aureus* and *Staphylococcus schleiferi* (Shimizu *et al*., 2001; Morris *et al*., 2006; Fazakerley *et al*., 2009). Authors reported the isolation of different other kinds of coagulase-positive and coagulase-negative staphylococci from dogs with pyoderma (Zdovc *et al*., 2004; May, 2006; Hauschild and Wójcik, 2007).

Treatment of staphylococci skin infections fully depend on the case; true first time pyoderma cases, that is, cases that have not previously been treated for pyoderma, with no risk factors, are usually treated with empirically certain antimicrobial agents. Otherwise, culture is indicated. Therefore, assessment of changes in antimicrobial susceptibility patterns over time is important to determine whether the organism remains susceptible to empirically selected antimicrobial agents.

To the best of our knowledge, the present study is the first assessment of antimicrobial susceptibility of staphylococci isolated from dogs with first-time pyoderma carried out in Iran. The objectives were:

1) To study the prevalence of staphylococci isolation from dogs with first time pyoderma

2) To investigate the number of cultures that were positive for a bacterial genus from dogs presenting to the Veterinary Teaching Hospital of Ferdowsi University of Mashhad.

This is important in understanding changes in staphylococcal populations and their antimicrobial susceptibility patterns in dogs with first time pyoderma.

Materials and Methods

Dogs and clinical presentation

This research proposal has received ethical approval from Ferdowsi University of Mashhad Research Office. The study animals were 61 clinical cases of Iranian domestic dogs with first-time pyoderma admitted to

Veterinary Teaching Hospital of Ferdowsi University of Mashhad, Mashhad, Iran from September 2012 to October 2017.

The dogs' breeds (pure and mixed breed dogs; small [<9 kg] or large [≥9 kg]; according to the official breed standard from the American Kennel Club (www.akc.org/breeds)), sex (male or female), and age (puppy [0-6 months]; juvenile [7 months-2 years]; adult [more than 2 years]), were recorded to determine whether they were associated with the likelihood of dogs exhibiting pyoderma.

The diagnosis of pyoderma was based on the history, the presence of variable gross cutaneous lesions (papules, pustules, epidermal collarettes, erythema, crusting, lichenification, and/or hyperpigmentation), positive findings on microscopic examination of surface cytology (glass slide impression smears revealing neutrophils with intracellular bacteria) and, in most of the cases, histopathological findings. More information including anatomic site and depth of bacterial skin diseases were recorded. The pyoderma was classified as superficial when only small pustules or papules were found, and as deep when there were noduli and/or fistulating tracts (Holm *et al*., 2002).

Underlying diseases were excluded via the following tests performed on all dogs: coat brushings, skin scrapings, impression smears, fungal culture, skin biopsies, complete blood count and in a limited number of cases, biochemical profiles and endocrine function tests. In order to diagnose allergic dermatitis, a detailed interpretation of historical and clinical signs and a restriction-provocation dietary method were used.

Specimen collection and bacterial isolation

Swabs for bacterial culture were taken from pustule or papule without previous sterilization. All swabs were grown on Columbia Blood agar (Merck, USA) and Mannitol Salt agar (Merck, USA). All plates were incubated aerobically and anaerobically at 37°C for 18-24 h. Isolates were recognized on the basis of colony morphology, Gram-staining, pigment production and hemolysis. All samples were subject to further analysis and biochemical testing. For all Gram-positive, catalase-positive, oxidase negative cocci with colony morphology fitting with *Staphylococcus* species, coagulase activity was determined via the tube coagulase test using rabbit plasma.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of the isolates was determined on Mueller-Hinton agar (Merck, USA) using the disk diffusion method (CLSI, 2013). Eighteen different antimicrobial agents were studied: ampicillin (10 µg/disk), amoxicillin (25 µg/disk), penicillin (10 units/disk), cephalexin (30 µg/disk), cefoxitin (30 µg/disk), cloxacillin (5 µg/disk), oxytetracycline (30 µg/disk), doxycycline (30 µg/disk), gentamicin (10 µg/disk), kanamycin (30 µg/disk), streptomycin (10 µg/disk), erythromycin (15 µg/disk), enrofloxacin (5 µg/disk), norfloxacin (10 µg/disk), difloxacin (10 µg/disk), chloramphenicol (30 µg/disk), novobiocin (30 µg/disk), and Sulphamethoxazole/Trimethoprin (25 µg/disk). For quality control *S. aureus* reference strain ATCC 25923 was used.

Dermatohistopathology

According to the permission given by the owners, a minimum of two 6 mm punch biopsy samples of affected skin were obtained from 42 dogs of the 61 studied dogs. 19 owners did not accept the terms of agreement to perform this test. All biopsies were assessed by one pathologist who was blind to the results of all other tests. Histopathologically (using haematoxylin and eosin staining), superficial pyodermas were classified as bacterial infections that involve the epidermis and follicular epithelium and when only small pustules or papules were found. Deep pyodermas were diagnosed as follicular infection that breaks through the hair follicle and when there were noduli and/or fistulating tracts (Holm *et al*., 2002; Miller *et al*., 2013).

Statistical analysis

In order to report the association of independent variables (breed, age, sex) with the occurrence of pyoderma, χ^2 test was used. Statistical significance was set at $P<0.05$. All statistical analyses were performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Demographic information

Included in the study were 61 dogs with first time pyoderma that had been treated at Veterinary Teaching Hospital of Ferdowsi University of Mashhad, Mashhad, Iran. Overall, 36 (59.01%) studied dogs were male and 28 dogs (40.98%) were female. The majority of studied dogs (41; 67.21%) were large breeds compared to 20 (32, 79%) small breed dogs. The median age was 34.2 months (minimum value-maximum value: 1-108 months; nine studied dogs (9.83%) were puppies, 19 (39.34%) were juveniles, and 33 (50.81%) were adults. The diagnosis of first time pyoderma amongst adult dogs was significantly higher than puppies ($P=0.001$) and in large breeds in comparison to small breeds ($P=0.002$).

Anatomic distribution of skin lesions in dogs with pyoderma

Table 1 displays the sites where pyoderma skin lesions occurred in the studied dogs. According to the data, two anatomic sites (the "head, face, and pinna" and the "trunk") were the most affected lesion regions, each with 19 cases (26.76%).

Underlying diseases in dogs with pyoderma

An identifiable underlying disease was present in 52 (85.24%) of the 61 studied dogs. Twenty-three dogs had allergic skin disease (including: 10 dogs with flea bite hypersensitivity, 8 dogs with atopic dermatitis, and 5

Table 1: Anatomic site predilection to canine pyoderma in 61 dogs with first-time pyoderma

Anatomic site	Surface pyoderma	Superficial pyoderma	Deep pyoderma	Total
Head, face, pinna	1	3	15	19
Fore limbs	1	3	6	10
Hind limbs	1	4	5	10
Paws	0	0	1	1
Dorsum	0	3	2	5
Trunk	1	5	13	19
Ventrum	1	3	2	6
Perineal	1	0	0	1
Total	6	21	44	71

Total number is greater than 61 (No. of studied dogs), since some of them had more than one anatomic site of pyoderma involvement

dogs with food hypersensitivity), 11 dogs had cutaneous manifestations of visceral leishmaniosis, 9 dogs had parasitic skin diseases, 3 dogs had neoplasia (including: basal cell tumor, fibroblastoma, and hemangiosarcoma), 4 dogs had endocrine disease, 1 dog had seborrhetic dermatitis, 1 dog had *Malassezia* dermatitis, and for 9 dogs an underlying disease was not identified.

Bacterial species distribution

Bacterial species were recovered from 43 of the 61 (70.49%) studied animals. No isolates were recovered from 18 studied dogs. The most frequently recovered bacterial genus was *Staphylococcus* (32/43 isolates, 74.41%). *Staphylococcus epidermidis* (22/43 isolates, 51.16%) represents the most frequent recovered bacterial isolates in the studied dogs. Two other staphylococci species recovered from the studied animals were *S. Aureus* (7/43 isolates, 16.27%), and *S. pseudintermedius* (3/43 isolates, 6.97%) (Table 2).

Table 2: Bacterial species were recovered from 43 (70.49%) of 61 of the studied animals

Species	No. of dogs
Staphylococcus epidermidis	22
Staphylococcus aureus	7
Staphylococcus pseudointermedius	3
Streptococcus dysgalactiae	1
Streptococcus equi	1
Proteus	5
Escherichia coli	2
Glucose non fermentative	1
Pseudomonas	1
Total	43

Antimicrobial susceptibility

Staphylococci species resistance was mostly seen against amoxicillin (94.11%), penicillin (83.35%), and ampicillin (76.47%). Resistance to cephalexin and cefoxitin was 5.88% and 2.94%, respectively. A total of 27 of the staphylococci isolated (84.37%) were resistant to at least one antimicrobial agent. 55% (19/32 staphylococci isolates) of the isolates were resistant to three or more antimicrobial drugs (multi resistance).

Histopathologic results

In the present study, 44 cases were categorized as deep and 27 as superficial pyoderma. The dog with superficial lesions had a combination of perivascular to periadnexal to interface dermatitis, epidermal hyperplasia with superficial pustulation and crusting. Neutrophils are also prominent in the inflamed dermis. The histological changes in dogs with deep pyoderma included a suppurative folliculitis and furunculosis, with pyogranulomatous (and eosinophilic in three dogs) perivascular and periadnexal dermatitis and pyogranulomatous panniculitis. Intra- and subcorneal pustules (neutrophilic) without bacteria were seen in two dogs. A complete signalment and histopathological features of 42 dogs with first time pyoderma are summarized in Table 3.

Discussion

The results of the present study indicate that most of the domestic dogs admitted to Veterinary Teaching Hospital of Ferdowsi University of Mashhad (Northeast of Iran) with clinical first time pyoderma were infected with bacterial species (70.49%), as described by other authors (Futagawa-Saito *et al*., 2004; Hartmann *et al*., 2005; Huerta *et al*., 2011). To date, there is only one reported study (Huerta *et al*., 2011) in which all samples obtained from dogs with first time pyoderma were positive for staphylococci.

In the present study, 44 of the culture positive cases were categorized as deep pyoderma. It is very surprising that the majority of the dogs had deep infection as first time pyoderma. In contrast to the present study in which the prevalence of deep pyoderma is higher than superficial and surface pyoderma, Holm *et al*. (2002) reported higher prevalence of superficial pyoderma among first time pyoderma-affected dogs (153 of 201 culture positive cases). However, they reported higher prevalence of deep pyoderma among recurrent cases (109 of 201 culture positive cases).

The nine bacterial species isolated in the present study (Table 2) are known opportunistic pathogens that can be found both in healthy dogs as part of the normal skin microbiota and in various canine infections including pyoderma (May, 2006; Gortel, 2013; Weese, 2013).

In the present study, the most frequently recovered bacterial genus was *Staphylococcus* (32/43 isolates, 74.41%). Holm *et al*. (2002) reported the isolation of staphylococci from mucosal sites (and not lesional sites) in 92% of the 201 cases of first-time pyoderma in

Table 3: Characteristics and histopathological features of 42 dogs with first time pyoderma

Dog No.	Breed	Age (month)	Sex	Category of main clinical presentation	Gross (macroscopic) lesions	Microscopic features
1	Terrier	21	M	Pruritic	Pruritus and multiple erythematous papulopustular lesions of varying size on abdomen	Moderate hyperkeratosis, superficial dermatitis (mixed type inflammatory cells), epidermal spongiosis
2	Mixed	30	F	Pruritic	Mild pruritus and erythematous macular on abdomen and hind limbs	Skin containing epithelial layer with mature collagen bundles in dermis without dermal adnexa (hair follicules or sebaceous glands)
3	Terrier	60	F		An inflammation around the neck with pruritus and crust	Severe hyperkeratosis, suppurative dermatitis
4	Mixed	24	M	Pruritic	Pruritus, alopecia and primary erythema	Mild infiltration of inflammatory cells
5	Terrier	18	M	Pruritic	Pruritus, and alopecia	Mild hyperkeratosis, mild infiltration of inflammatory cells in the dermis, eosinophilic intracytoplasmic inclusion bodies in follicular epithelium
6	Doberman	6	M	Pruritic	Diffuse erythematous pruritic lesions in addition to crusts and exudative ulcers	Spongiosis, intraepidermal vesicle, acute dermatitis, hemorrhage, vasculitis
7	Mixed	7	M	Pruritic	Pruritus and erythematous lesions	Acute exudative dermatitis
8	GSH	18	M	Alopecia	Alopecia, ulcer and erythema on the flank	Acute exudative dermatitis, intraepidermal vesicle and pustule
9	Terrier	42	F	Alopecia	Bilaterally symmetrical alopecia, pruritus, and erythematous lesions	Epidermal spongiosis, diffused severe infiltration of neutrophils and eosinophils, crust formation
10	Asian shepherd	24	M	Alopecia	Extensive scaling and alopecia with facial involving	Sebaceous adenitis, Hidradenitis
11	Spitz	24	F	Alopecia	Bilateral symmetrical flank alopecia and multiple comedones on the ventrum	Hyperkeratosis, granulation tissue
12	GSH	30	F	Alopecia	Crusting, alopecia, and hyperpigmentation of neck, nose and ear pinna	Severe hyperkeratosis, crust formation, acute hemorrhagic dermatitis, fungal microorganisms, severe hemorrhage
13	GSH	6	M	Facial dermatitis	Scale and crust on the ear pinna, periocular and perioral ulcerated nodules	Acute dermatitis, epithelial necrosis, granulation tissue, crust formation
14	GSH	6	F	Facial dermatitis	Periocular and perioral ulcerated nodules	Acute dermatitis, crust formation
15	Terrier	48	F	Facial dermatitis	Nasal depigmentation and pustular dermatitis	Skin hydropic degeneration, intracytoplasmic inclusion body
16	Mixed	7	F	Facial dermatitis	Perioral ulcerative dermatitis and a large, non-pigmented, ulcerated lesion of the nasal planum	Acute suppurative dermatitis
17	GSH	18	F	Facial dermatitis	Large crusty lesions on nasal planum	Hyperkeratosis, spongiosis, hydropic degeneration, fibrinous exudates, severe hyperemia, neutrophilic infiltration, crust formation
18	GSH	108	M	Facial dermatitis	Nasal planum ulcerative dermatitis, periocular ulcerative dermatitis	Severe spongiosis, severe inflammatory cell infiltration (mixed type), crust formation, suprabacillary clefts, vesicle formation
19	Mixed	12	F	Facial dermatitis	Scale and crust on the ear pinna, periocular, and perioral ulcerated nodules	Epithelial spongiosis, superficial necrosis, crust formation, infiltration of mononuclear cells in dermis
20	GSH	30	M	Facial dermatitis	Severe facial dermatitis	Acute lymphadenitis, hemorrhage, ulcer formation
21	GSH	12	F	Facial dermatitis	Periocular and perioral ulcerated nodules	Epithelial necrosis, ulcer formation, acute dermatitis, leishman bodies in macrophages, infiltration of macrophages
22	Doberman	36	F	Facial dermatitis	Periocular and perioral ulcerated nodules	Ulcer formation, severe infiltration of macrophages containing Leishman bodies
23	Mixed	5	M	Facial dermatitis	Large ulcerative lesions around mouth and on ventral part of muzzle	Infiltration of macrophages containing Leishman bodies - ulcer formation
24	Terrier	12	M	Facial dermatitis	Ulcerative nasal planum	Leishman bodies
25	Asian shepherd	24	M	Facial dermatitis	Severe chronic crusty lesion and ulcers on head and face	Crust formation, acute suppurative dermatitis, epithelial necrosis
26	GSH	6	F	Facial dermatitis	Severe ulcerative dermatitis of the margin of both pinna	Ulcer formation, necrotic debris, infiltration of neutrophils, microbial colonies, granulation tissue, hyaline degeneration of collagen bundles
27	Spitz	7	F	Facial dermatitis	Ulcer on the nasal planum	Severe infiltration of neutrophils and macrophages, hyperemia, pyogranulomatous dermatitis
28	Mixed	18	M	Facial dermatitis	Ulcer on the nasal planum	Crust formation, diffused pyogranulomatous dermatitis, Leishman bodies
29	Terrier	24	M	Facial dermatitis	Severe bilateral mucopurulent discharge and crusts on the nose	Severe hyperkeratosis, crust formation, acute dermatitis, Leishman bodies
30	GSH	42	M	Facial dermatitis	Perioral pustular dermatitis	Crust formation, infiltration of mononuclear cells in dermis, ulcer
31	GSH	18	F	Facial dermatitis	Crusty lesions on nasal planum	Hyperkrtatosis, spongiosis, hydropic degeneration, fibrinous exudate, severe hyperemia, neutrophilic infiltration and crust formation
32	Husky	18	M	Pododermatitis	Pododermatitis and hyperkeratosis of foot pads	Epithelial necrosis, granulomatous dermatitis
33	Terrier	48	M	Dermatitis of hind limbs	Ulcerated nodule on rear leg	Mild hyperkeratosis, lymphocytic dermatitis
34	Doberman	12	M	Pyoderma of trunk, ventrum an genital regions	Superficial pyoderma of testicular scrotum	Epithelial necrosis, ulcer formation, acute suppurative dermatitis, crust formation, proliferation of fibroblasts and healing process
35	Doberman	3	M	Pyoderma of trunk, ventrum an genital regions	Papulopostular and exudative lesions on ventrum	Acute fibrinopulerant dermatitis, ulcer formation
36	Mixed	36	M	Pyoderma of trunk, ventrum an genital regions	Vesicles on prepuce and an ulcer on pinna	Ulcer formation, hyperkeratosis, spongiosis
37	Mixed	18	M	Pyoderma of trunk, ventrum an genital regions	Large inflamed and erosive testes	Epithelial hyperplasia, spongiosis
38	Doberman	1	M	Pyoderma of trunk, ventrum an genital regions	Vesiculopustular on the ventrum	Hyperemia, acute dermatitis, spongiosis
39	Shi Tzu	60	F	Pyoderma of trunk, ventrum an genital regions	Large pustules on the flank area	Keratinic tissue without cellular component
40	Mixed	84	M	Pyoderma of trunk, ventrum an genital regions	Severe traumatic ulcers	Dissociation of keratinocytes, intracellular edema and hydropic degeneration with dermatitis
41	Terrier	168	M	Pyoderma of trunk, ventrum an genital regions	Ulcerative and erythematous lesions on forelimb	Epithelial necrosis, ulcer formation, necrotic debris and many infiltration of neutrophils
42	Terrier	60	F	Tumor	A 3 × 3 mass near the pinna	Basal cell tumor and epithelial necrosis and dermatitis

GSH: German Shepherd dog, M: Male, and F: Female

Sweden. Also, Huerta *et al*. (2011) reported a higher frequency of isolation of staphylococci in the pyoderma-affected dogs than in the healthy dogs. In their study, staphylococci were recovered from 24 (100%) dogs with first time pyoderma and from 27 (100%) of the dogs with recurrent pyoderma.

According to the results of the present study, *S. epidermidis* was the most prevalent bacterial isolate. Similarly, Schmidt *et al*. (2014) reported this species as the most common staphylococci species in healthy Labrador retrievers in the UK. *Staphylococcus epidermidis* is regularly isolated from the skin and haircoat of normal dogs (Miller *et al*., 2013). This organism is not considered pathogenic unless isolated from lesioned skin. Recent studies indicate the outset of these species' involvement in canine pyoderma and otitis (Hauschild and Wójcik, 2007).

The second most common *Staphylococcus* in the current study was *S. aureus*. This bacterium is a CoPS and is commonly isolated from normal skin and should be considered resident. Reports of human infection or colonization from companion animals (Simoons-Smit *et al*., 2000; van Duijkeren *et al*., 2004) have shown the potential of animals to act as reservoirs for transmission of *S. aureus*.

According to the present study, *S. pseudintermedius* was covered from three (6.97%) canine pyoderma cases. In contrast, other studies have found that *S. pseudintermedius* is a prevalent isolate among dogs with pyoderma (Morris *et al*., 2006; Fazakerley *et al*., 2009). The reason for our finding is not clear. In a recent study in South Korea (Han *et al*., 2016) the overall prevalence of *S. pseudintermedius* in healthy dogs (29.4%) was comparatively lower than in previous studies in that area (35.5% and 74.7%) (Yoo *et al*., 2010; Moon *et al*., 2012).

In the present study the most efficient antibiotics against *Staphylococcus* spp. were cephalosporins; resistant to cephalexin and cefoxitin was 5.88% and 2.94%, respectively. However, we did not use oxacillin for the detection of methicillin resistance. CLSI (2013) recommends usage of cefoxitin instead of oxacillin when using the disk diffusion method to determine resistance against methicillin for *S. aureus*.

The results of the present study showed the effectiveness of the different groups of antimicrobial agents examined, and confirmed the results of previous authors who report cephalosporins, fluoroquinolones, amoxicillin/clavulanic acid, gentamicin and rifampin to be first-line choices in *Staphylococcus*-induced canine pyoderma (Morris *et al*., 2006; Vanni *et al*., 2009). A number of authors report differences in the resistance patterns between isolates (Futagawa-Saito *et al*., 2004; Hartmann *et al*., 2005). According to the results of *in vitro* susceptibility studies it would not be meaningful to use antibiotics such as amoxicillin, penicillin and ampicillin to treat canine pyoderma since there is high resistance against these antibiotics. Based on findings of the present study, empirical treatment of canine superficial pyoderma caused by *Staphylococcus* spp. with cephalosporins appears appropriate. However, bacterial culture and antimicrobial susceptibility testing should always be pursued in recurrent or refractory infections.

In conclusion, we found that the most frequently recovered bacterial genus from a population of Iranian domestic dogs with first time clinical pyoderma was *Staphylococcus* including: *S. epidermidis*, *S. aureus*, and *S. pseudintermedius*. Staphylococci species resistance was most commonly seen against amoxicillin, penicillin, and ampicillin. According to results of the present study, a total of 27 of the staphylococci isolated (84.37%) were resistant to at least one antimicrobial agent. This result should be interpreted with caution because of the small population of staphylococci. A large-scale study is necessary to explain the association of epidemiological variables affecting bacterial skin infections in dogs.

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